Anatomical, haemodynamic, biochemical and imaging characteristics of the coronary collateral circulation in acute and chronic atherosclerotic disease processes

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Thesis by published works

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DECLARATION

This work was undertaken at Royal North Shore Hospital & The Kolling Institute of Medical Research under the supervision of Professor Ravinay Bhindi.

Unless otherwise acknowledged in the text, this is the sole work of the author Dr Usaid Allahwala, and has not been submitted elsewhere in any form for the purpose of obtaining another degree.

Dr Usaid Allahwala is the first author in all published manuscripts presented in this thesis.

In each publication he contributed primarily to study design, patient recruitment, performance of investigations, data analysis, writing and correction of the manuscripts.

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ABSTRACT

Aim: Although frequently identified during coronary angiography, the mechanisms by which coronary collaterals develop, and their prognostic implications are, to date, unknown. This body of work aims to determine the prevalence and predictors of coronary collateral recruitment in the setting of ST elevation myocardial infarction (STEMI) and chronic total occlusion (CTO) and to determine their prognostic impact. Furthermore, to identify biochemical, cellular and haemodynamic processes by which collaterals are recruited and mature, and influence haemodynamics in the coronary circulation.

Methods: Anatomical grading of collaterals using the Rentrop classification was performed in a large cohorts of patients with STEMI and CTO to determine predictors, reproducibility and prognostic implications of collaterals. Data linkage with other health parameters including a history of obstructive sleep apnoea (OSA) and prior coronary artery bypass grafting (CABG) was performed to determine impact of comorbidities and haemodynamic modulation on collateral recruitment. Subsequent systematic reviews and meta-analyses were performed. Invasive haemodynamic assessment of coronary blood flow and pressure in the presence and absence of collaterals was correlated with endothelial, haematological, biochemical and proteomic markers in both human and animal studies.

Results: The important and novel findings are;

- The presence of acutely recruited robust collaterals in the setting of STEMI are associated with a reduction in mortality and improved left ventricular function.
- In the setting of a CTO, robust collaterals do not reduce mortality or risk of future ischaemic events, but do increase likelihood of successful percutaneous revascularisation.

- Collateral maturation is driven by an elevation in shear stress, alterations in blood flow and tissue ischaemia.
- The presence of collaterals results in a consistent increase in coronary blood flow in the donor vessel, with resultant effect on both pressure and flow derived indices of ischaemia assessment commonly used in clinical practice.
- Recruitment and maturation of coronary collaterals are associated with upregulation of endothelial derived chemoattractant proteins, growth factors and transcription factors.
- Coronary artery bypass grafting to a donor vessel, results in poorer collateral recruitment, likely driven by alterations in coronary blood flow and endothelial shear stress
- The presence of OSA is associated with more robust coronary collaterals in both the setting of STEMI and CTO, however in more severe forms of OSA, characterised by severe and prolonged hypoxia, collateral recruitment is attenuated.

Conclusions: Coronary collaterals impart significant prognostic implications in the setting of acute and chronic coronary artery disease, recruited as a result of alterations in coronary haemodynamics and tissue ischaemia with resultant downstream activation of growth factors, chemokines and transcription factors. Ongoing research is necessary to determine whether this prognostic advantage can be translated into meaningful therapeutic targets along with a greater understanding of clinical implications of collaterals.

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2. **Allahwala UK,** Khachigian LM, Nour D, Ridiandres A, Billah M, Ward M, Weaver J, Bhindi R. Recruitment and maturation of the coronary collateral circulation: Current understanding and perspectives in arteriogenesis. *Microvascular Research*. 132:104058. doi: 10.1016/j.mvr.2020.104058.

3. **Allahwala UK,** Weaver JC, Nelson GI, Nour D, Ray M, Ciofani JL, Ward M, Figtree G, Hansen P, Bhindi R. (2020). Effect of Recruitment of Acute Coronary Collaterals on In-Hospital Mortality and on Left Ventricular Function in Patients Presenting With ST Elevation Myocardial Infarction. *American Journal of Cardiology. 125(10): 1455-1460.* doi: 10.1016/j.amjcard.2020.02.023.

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5. **Allahwala UK**, Nour D, Alsanjari O, Bhatia K, Nagaraja V, Khatri JJ, Cockburn J, Hildick-Smith D, Sakata Y, Ward M, Weaver JC, Bhindi R. Prognostic implications of the rapid recruitment of coronary collaterals during ST elevation myocardial infarction (STEMI): a meta-analysis of over 14,000 patients. *Journal of Thrombosis and Thrombolysis.* doi: 10.1007/s11239-020-02282-6

6. **Allahwala UK,** Kott K, Bland A, Ward M, Bhindi R. (2020). Predictors and Prognostic Implications of Well-Matured Coronary Collateral Circulation in Patients with a Chronic Total Occlusion (CTO). *International Heart Journal*. 61(2): 223-230. doi: 10.1536/ihj.19-456

7. **Allahwala UK**, Nour D, Bhatia K, Ward M, Lo S, Weaver JC, Bhindi R. (2020). Prognostic impact of collaterals in patients with a coronary chronic total occlusion: A meta-analysis of over 3,000 patients. *Catheterization & Cardiovascular Interventions*. DOI:10.1002/ccd.29348

8. **Allahwala UK,** Brilakis ES, Byrne J, Davies JE, Ward MR, Weaver JC, Bhindi R. (2019). Applicability and Interpretation of Coronary Physiology in the Setting of a Chronic Total Occlusion. *Circulation: Cardiovascular Interventions*. 12(7): e007813. DOI: 10.1161/CIRCINTERVENTIONS.119.007813.

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I confirm that the above authorship attribution statements are true and accurate

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As supervisor for the candidature upon which this thesis is based, I can confirm that the authorship attribution statements above are correct.

Professor Ravinay Bhindi

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TABLE OF CONTENTS

List of Abbreviations

ACE-I	Angiotensin Converting Enzyme Inhibitor
ACS	Acute Coronary Syndrome
AHI	Apnoea-Hypopnoea Index
AMI	Acute Myocardial Infarction
ANOVA	A One-Way Analysis of Variance
APV	Average Peak Velocity
ARB	Angiotensin Receptor Blocker
BMI	Body Mass Index
CABG	Coronary Artery Bypass Graft
CAD	Coronary Artery Disease
CBF	Coronary Blood Flow
CC grade	Collateral Connection Grade
CCB	Calcium Channel Blocker
CCS Class	Canadian Cardiovascular Society Class
CFI	Collateral Flow Index
CFR	Coronary Flow Reserve
CMR	Cardiac Magnetic Resonance Imaging
CPI	Collateral Pressure Index
CRP	C reactive protein
CTO	Chronic Total Occlusion
DES	Drug Eluting Stent
DFR	Diastolic Hyperaemia-Free Ratio
ECG	Electrocardiogram
ECM	Extracellular Matrix
EDTA	Ethylenediaminetetraacetic acid
EEG	Electroencephalogram
EgR-1	Early Growth Response-1
ELISA	Enzyme Linked Immunosorbent Assay
EMG	Electromyogram
eNOS	Endothelial Nitric Oxide Synthase
FFR	Fractional Flow Reserve
FGF-B or FGF-2	Fibroblast Growth Factor-B or 2
FSS	Fluid Shear Stress
G-CSF	Granulocyte Colony Stimulating Factor
GM-CSF	Granulocyte Macrophage Colony Stimulating Factor
HDL	High density lipoprotein
HIF-1α	Hypoxia Inducible Factor-1 alpha
HMR	Hyperaemic Microvascular Resistance
HSR	Hyperaemic Stenosis Resistance
ICAM-1	Intracellular Adhesion Molecule 1
iFR	Instantaneous Wave Free Ratio
IL	Interleukin

IMR	Index of Microvascular Resistance
IQR	Interquartile Range
IRA	Infarct Related Artery
LAD	Left Anterior Descending Artery
LCx	Left Circumflex Artery
LDL	Low density lipoprotein
LIMA	Left Internal Mammary Artery
LV	Left Ventricular
LVEDV	Left Ventricular End Diastolic Volume
LVEF	Left Ventricular Ejection Fraction
MCP-1	Monocyte Chemoattractant Protein 1
mRNA	Messenger Ribonucleic Acid
miRNA	Micro Ribonucleic Acid
MMP	Matrix Metalloproteinases
NLR	Neutrophil to Lymphocyte Ratio
NOS	Newcastle-Ottawa Scale
NT Pro-BNP	N Terminal pro B-type Natriuretic Peptide
NYHA	New York Heart Association
ODI	Oxygen Desaturation Index
OMT	Optimal Medical Therapy
OR	Odds Ratio
OSA	Obstructive Sleep Apnoea
PCI	Percutaneous Coronary Intervention
pPCI	Primary Percutaneous Coronary Intervention
PRISMA	Preferred Reporting Items for Systematic reviews and Meta-Analysis
QCA	Quantitative Coronary Angiography
QPCR	Quantitative real time polymerase chain reaction
RCA	Right Coronary Artery
RFR	Resting Full Cycle Ratio
RIMA	Right Internal Mammary Artery
ROS	Reactive Oxygen Species
SAQ	Seattle Angina Questionnaire
SCAD	Spontaneous Coronary Artery Dissection
SMC	Smooth Muscle Cell
SMD	Standard Mean Difference
STEMI	ST Elevation Myocardial Infarction
SVG	Saphenous Vein Graft
TGF-β	Transforming Growth Factor Beta
TIMI	Thrombolysis in Myocardial Infarction
TNF-α	Tumour Necrosis Factor-α
VCAM-1	Vascular Cell Adhesion Molecule 1
VEGF	Vascular Endothelial Growth Factor
VF	Ventricular Fibrillation
VSMC	Vascular Smooth Muscle Cell

SECTION I: INTRODUCTION

Chapter 1: Introduction, Aims & Scope

This chapter includes material published as: Allahwala UK, Ward M, Brieger D, Weaver J, Bhindi RIndications for Percutaneous Coronary Intervention (PCI) in Chronic Total Occlusion (CTO): Have We Reached a DECISION or Do We Continue to EXPLORE After EURO-CTO?. *Heart, Lung & Circulation*. 28: 1484-1489. https://doi.org/10.1016/j.hlc.2019.03.014 along with supplementary data for this thesis.

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REVIEW

Indications for Percutaneous Coronary Intervention (PCI) in Chronic Total Occlusion (CTO): Have We Reached a DECISION or Do We Continue to EXPLORE After EURO-CTO?

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A coronary chronic total occlusion (CTO) is a common finding during coronary angiography and is associated with increased mortality and poorer cardiovascular prognosis. Technological developments in percutaneous strategies for revascularisation have resulted in increased interest in this area. However, until recently, there has been a paucity of robust scientific randomised data comparing the efficacy of medical management with percutaneous strategies. Both observational data, and to a lesser extent, randomised data, suggest that CTO percutaneous coronary intervention (PCI) should be considered in symptomatic patients. However, in the absence of any randomised data, CTO PCI should not be performed for prognostic benefit. Ongoing trials are needed to confirm these findings as well as to identify the optimal timing and strategy of such interventions.

Keywords Chronic total occlusion • Percutaneous Coronary Intervention • Interventional cardiology • Coronary artery disease • Revascularisation

Introduction

Coronary chronic total occlusions (CTO) are often referred to as the final frontier in interventional cardiology owing to their relative frequency and prognostic importance coupled with the paradoxical low adoption of revascularisation. Having been previously consigned to specialised operators in a few select centres, CTO percutaneous coronary intervention (PCI) is becoming increasingly adopted and is of mounting relevance to the wider cardiology community with advances in wire types, device technology and treatment approaches [1]. With this increasing interest comes increasing scientific scrutiny. As a result, patient advocates, cardiologists and hospital administrators increasingly demand robust evidence for this often resource intense procedure. In the last 2 years, three trials: Percutaneous intervention for concurrent Chronic Total Occlusion in patients with ST-Elevation Myocardial Infarction (EXPLORE) [2]; A randomised multicentre trial to compare revascularisation with optimal medical therapy for the treatment of chronic occlusions (Euro-CTO) [3]; and DECISION-

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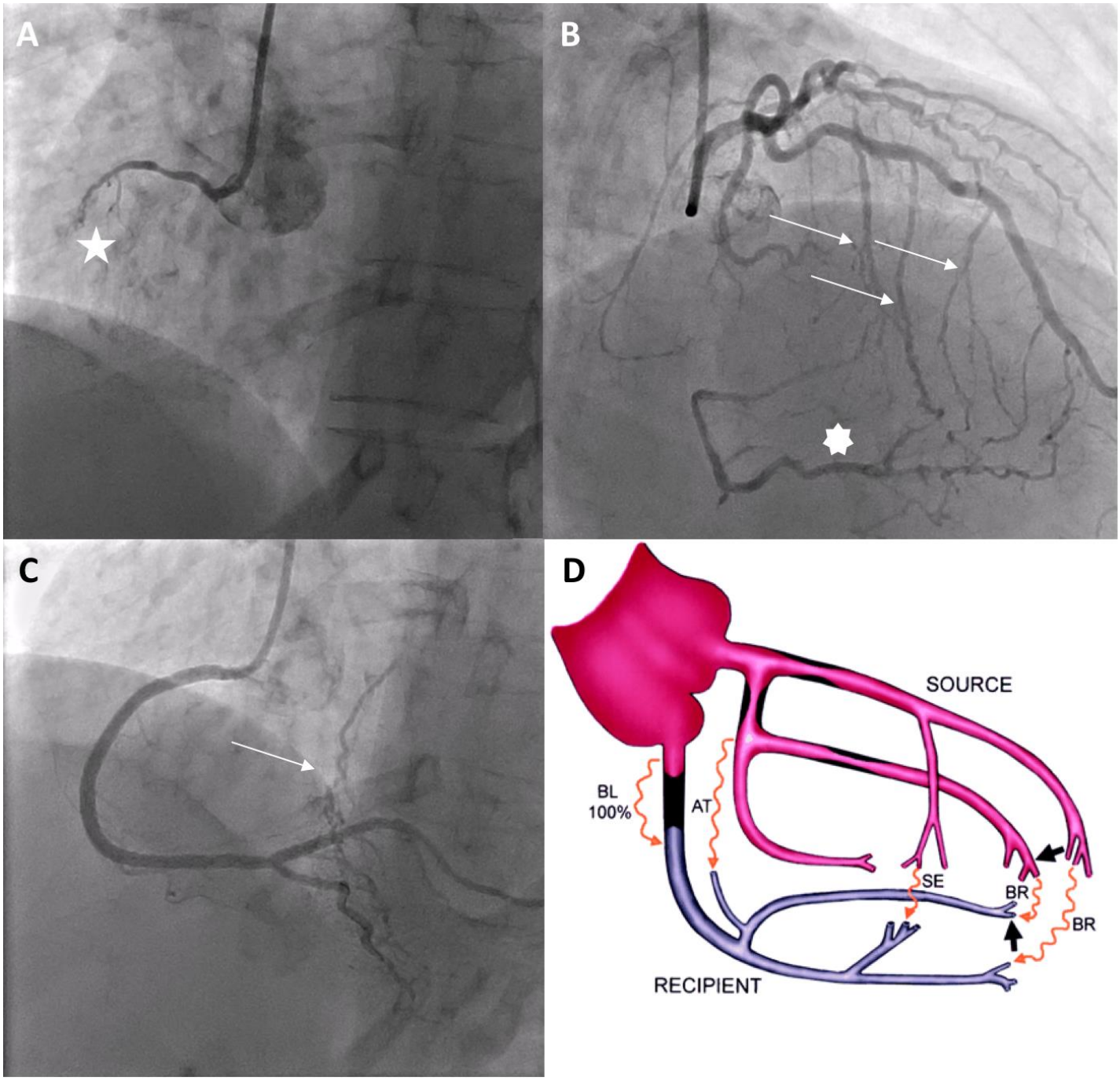
1.1 Coronary artery disease and collaterals in acute coronary syndromes and chronic total occlusion

Coronary artery disease (CAD) is a pathological process characterised by atherosclerotic plaque accumulation in the epicardial arteries, both obstructive or non-obstructive (1). CAD remains the leading cause of morbidity and mortality in Australia, affecting 15% of people over the age of 75 (2). The process often has long stable periods, but can also become unstable, typically due to an acute thromboembolic event caused by plaque rupture or erosion. This dynamic nature allows classification of the disease into either acute coronary syndrome (ACS) or stable coronary disease, more recently termed chronic coronary syndrome.

Coronary collaterals are preformed primitive anastomotic channels connecting a myocardial territory supplied by 1 epicardial coronary artery, with that supplied by another vessel (3), thereby providing an alternative source of blood supply to myocardium, in the setting of an occluded coronary artery (4). Although often considered the hallmark of CAD, collaterals are present in patients with normal coronary arteries (4,5), and have the ability to increase capacitance following an increase in myocardial oxygen demand. Collaterals can be seen in the setting of an ACS, where there is an abrupt epicardial coronary artery occlusion or in stable coronary disease where there is more gradual luminal stenosis prior to eventual occlusion. Although some previous studies have suggested that the presence of collaterals in are associated with smaller infarct size and less ventricular aneurysm formation, improved ventricular function (6), lower rates of future cardiovascular events (7) and improved survival (8,9), not all studies have found a similar prognostic benefit (10,11). Therefore a considerable controversy remains with respect to whether the presence of collaterals confers prognostic benefit in the setting of an ACS.

One of the other manifestations of severe CAD is a chronic total occlusion (CTO), which is the complete or near-complete occlusion of a coronary artery, defined as Thrombolysis in Myocardial Infarction (TIMI) grade 0 flow, present for greater than 3 months (12). Angiographically this is appreciated as filling of the occluded vessel through collaterals from either the ipsilateral vessel, or contralateral vessel (Figure 1.1). Whilst the precise timing of an occlusion is difficult to determine, often the distinction is based upon the onset of symptoms or a history of acute myocardial infarction (AMI) in the target vessel territory (13). Coronary CTOs are commonly identified during angiography, with rates between 20-52% (14,15) dependent on the indication for angiography, particularly higher in those patients with previous coronary artery bypass grafting (CABG) (16).

Figure 1.1: Coronary CTOs and Collaterals



(A) A chronic total occlusion (CTO) of the right coronary artery (RCA) with no contrast opacification of the vessel distal to the point of occlusion (5-point star). (B) Contrast injection of the left coronary artery in the same patient shows contrast opacification of the distal RCA (7 point star) via septal collaterals (white arrows) from the left anterior descending artery (LAD). (C) Following RCA revascularisation there is contrast opacification of the RCA, with filling of the septal collateral (white arrow) from the RCA, suggesting bi-directional flow potential in collaterals. (D) Common types of collaterals; septal (SE), atrial (AT), branch to branch within ventricular free walls (BR) and bridging across the lesion from donor to recipient vessels (BL). Adapted from (17).

The presence of a CTO is associated with higher mortality (16), greater recurrence of ventricular arrhythmia (18) and ongoing anginal symptoms compared with other forms of stable CAD. Similar to the situation with ACS, there remains considerable controversy with respect to whether presence of robust coronary collaterals is associated with a prognostic benefit. Previous studies have shown that despite collaterals perfusing the territory subtended by an occluded vessel, these are insufficient to prevent ischaemia during increased demand (19). Whilst it may be expected that more robust angiographically assessed coronary collaterals would be associated with a lower degree of ischaemic myocardium, previous studies have shown no correlation between angiographic assessment of collaterals and invasively determined myocardial perfusion (20). Furthermore, well developed coronary collaterals do not correlate with positron emitting tomography (PET) derived myocardial blood flow (21), suggesting robust collaterals do not prevent ischaemia, and hence may not sufficiently obviate the risk of subsequent mortality. Another paradoxical finding with respect to angiographic collateral maturity is the finding of an increased risk of ventricular arrhythmias in those patients with robust collaterals as compared to those with poorer developed collaterals (18,22). This has been attributed to greater hibernating myocardium in the peri-infarct zone with electrical instability especially in the border zone of partially necrotic areas, and may explain the lack of benefit with robust collaterals.

Along with the significant controversy with respect to prognostic implications of the presence and robustness of coronary collaterals, the mechanisms by which they are recruited and develop are uncertain and remain incompletely understood. This process, termed arteriogenesis, refers to the maturation and remodelling of pre-existing arterioles into functional vessels. These arterioles of 30-50μm internal diameter, can grow into small arteries over 25-fold their original size (23). Although often attributed to hypoxia stimulating

this growth, certain territories where arteriogenesis occurs, such as in the peripheral vasculature (24), skeletal muscle (25) and the mesentery (26) are not hypoxic, other territories where collaterals are also found, such as coronary arteries, are hypoxic, making a clear distinction difficult. Furthermore, analogous to a CTO, stenoses which slowly progress toward complete occlusion are often only minimally ischaemic, and yet this is the condition where collaterals show maximal adaptation (27,28). Consequently, it appears that the increase in fluid shear stress through arterioles, as a consequence of vessel occlusion and resultant activation of endothelial cells is the predominant driver of arteriogenesis. However, the mechanisms and mediators through which this mechanical input is transcribed into vascular adaptation remains uncertain.

1.1.1 Revascularisation of CTOs

Despite the association between CTO and poorer clinical outcomes, whether patients with a CTO should be revascularised, is an area of ongoing conjecture. Although a collateral circulation is the angiographic hallmark of a CTO, >90% of patients with a CTO have significant anginal symptoms of at least moderate severity, suggesting insufficient potential to increase coronary flow with stress, and consequently, viable myocardium (16,19,29). Observational data also suggests that the presence of a CTO is associated with higher mortality (16) and greater occurrence of ventricular arrhythmia (18) above that of patients with other forms of CAD.

Despite these apparent negative prognostic effects of the presence of a CTO, there is significant geographic variation with respect to rates of revascularisation, with percutaneous coronary intervention (PCI) for a CTO attempted in 6-9% of cases in North America (30), while this number is significantly greater in Japan at 61.2% (31). Similarly, rates of surgical

revascularisation of patients with a CTO remains low at 23% (16), with the vast majority of patients (65%) being treated with guideline directed medical therapy (16). The hesitation to adopt a more ubiquitous approach to revascularisation may be attributable to the neutral results of recent randomised control trials - EXPLORE (32), Euro-CTO (33) and DECISION-CTO (34), which compared PCI with optimal medical therapy (OMT).

In the Evaluating Xience V and left ventricular function in PCI on occLusiOns afteR STEMI (EXPLORE) trial (32), patients undergoing PCI for ST elevation myocardial infarction (STEMI), who were found to have a concurrent CTO in a non-infarct related artery were randomised to either CTO PCI with OMT (n=150) or OMT alone (n=154) within 7 days of the index presentation. The primary endpoint was cardiac MRI determined left ventricular ejection fraction (LVEF) and left ventricular end diastolic volume (LVEDV) at 4 months. Procedural success was 77% by operator adjudication but 73% by core laboratory assessment. At 4 months, there was no difference in LVEF or LVEDV between the 2 groups, although in a sub-group analysis, in patients with a CTO of the LAD, CTO PCI resulted in a higher LVEF compared to OMT alone (47.2% vs 40.4%, $p<0.05$). Similarly, the longer term follow up (median follow up 3.9 years) showed no difference in LVEF, cardiac death or MACE, although freedom from angina was greater in the CTO PCI group (35).

The Euro-CTO trial (33), was a prospective, randomised, multicentre, open-labelled control trial comparing treatment between CTO PCI with OMT against OMT alone in a 2:1 randomised ratio in 396 patients. The primary endpoint was the change in health status as assessed by the Seattle angina questionnaire (SAQ) at 12 months. OMT was defined as the use of aspirin, an angiotensin converting enzyme inhibitor and a statin, along with at least 2 anti-anginals. Of note, complete revascularisation was mandated in the study, with 52.2% of

patients undergoing DES to other vessels prior to randomisation of the CTO. Of the 259 patients randomised to PCI, 220 patients (86.6%) had procedural success. At 12 months, in the intention to treat analysis, a greater improvement in SAQ subscales was seen in patients undergoing PCI as compared to OMT. This corresponded with significantly lower angina frequency, higher rates of freedom from angina and higher quality of life scores. Whilst not powered for hard endpoints, there was a lower rate of ischaemia driven revascularisation in the CTO PCI with OMT group compared to OMT alone (2.9% vs 6.7%, $p<0.05$), suggesting a role for CTO PCI with respect to both symptoms, quality of life and clinically relevant endpoints. However a criticism of this study was that patients and clinicians were not blinded to the treatment type which may have impacted the treatment effect.

The Drug-Eluting Stent Implantation Versus Optimal Medical Treatment in Patients With Chronic Total Occlusion **(**DECISION-CTO) trial (34) was also a prospective, open-labelled randomised trial comparing CTO PCI with OMT against OMT alone (in a 1:1 fashion) with respect to clinical endpoints. Inclusion criteria were patients with stable angina, acute coronary syndrome (ACS) and silent ischaemia with the primary endpoint the composite of all-cause mortality, AMI, stroke or repeat revascularisation. The study was stopped prematurely after enrolment of 834 patients. Of the 459 patients randomised to PCI, the success rate was 91.1%. In the intention to treat analysis, at 3 years they reported no difference between the CTO PCI with OMT group compared to the OMT alone group with respect to the cumulative primary endpoint (20.6% vs 19.6%, $p=0.54$). Similarly, no difference in quality of life scores was observed between the groups.

However this study has had significant criticisms with respect to its design, with patients having revascularisation of non CTO lesions following randomisation, meaning patients

within the OMT alone group had revascularisation of other lesions performed. Furthermore, there was significant cross over within groups (18.1% in the OMT group underwent PCI whilst 15.6% of the PCI group were only treated with OMT). When analysed based upon treatment received OMT did not meet the pre-specified non-inferiority margin, suggesting a symptomatic benefit with CTO PCI.

While these studies suggested symptomatic benefit with CTO PCI, no mortality or morbidity advantage with revascularisation was observed. Importantly, no distinction was made in these trials on the robustness, functionality and interaction with myocardium of the coronary collaterals supplying the CTO. Specifically no distinction was made between those patients who had apparently robust collaterals compared with those who had poorer collaterals. It is thus imperative to have a detailed understanding of the hallmark of CTOs – coronary collaterals, to be able to determine their prognostic effect and implications to further guide treatment strategies in a cohort of patients in whom evidence based management is thus far, lacking.

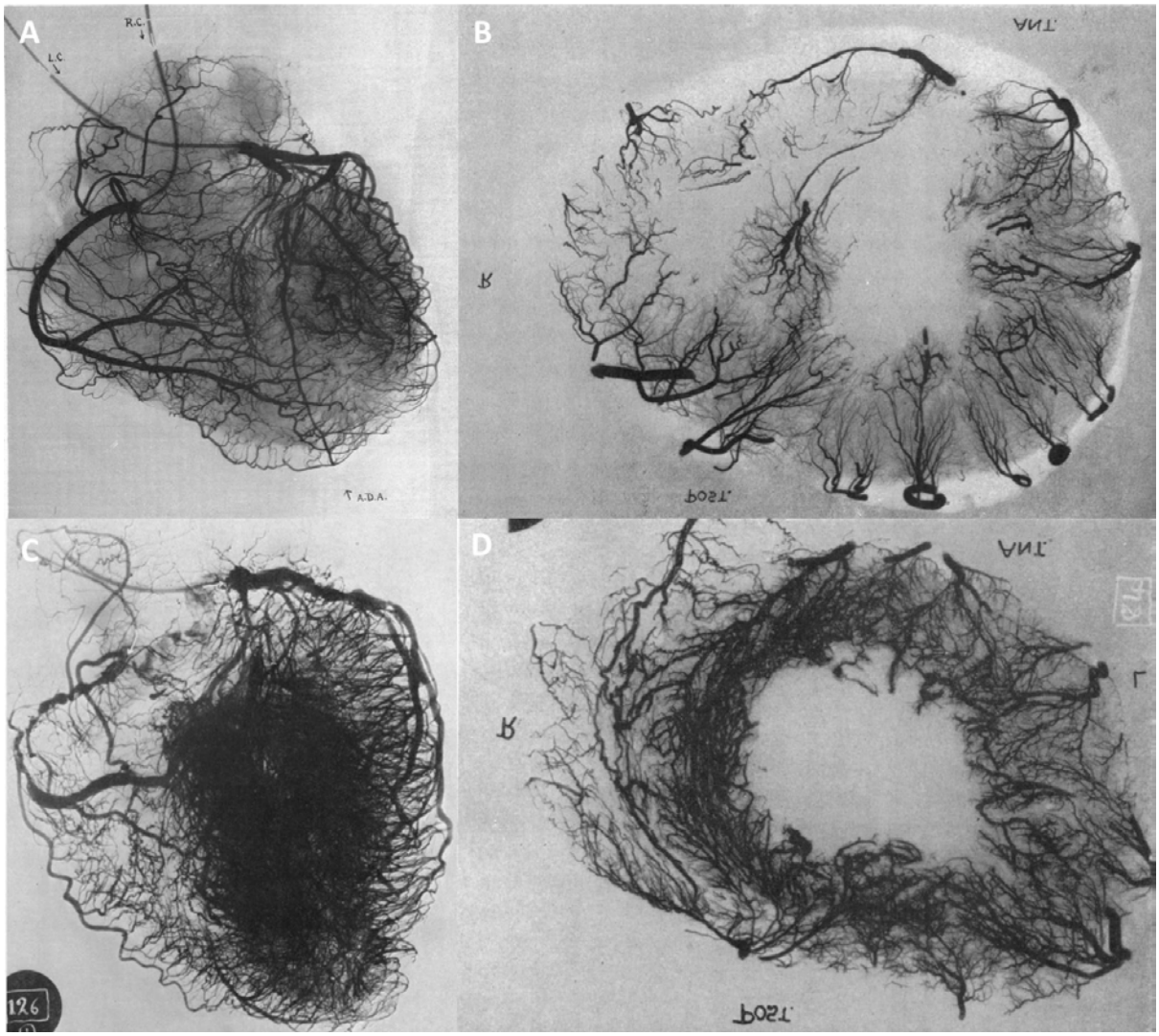
1.2 The Coronary Collateral Circulation

The coronary collateral circulation which is a preformed network of primitive anastomotic connections between coronary arteries as detailed above, was first described in the 17th and 18th centuries (36). Collateral arteries or connections have also been identified in different species, with considerable variation noted (36). Despite these early anatomical findings, considerable controversy initially remained about whether these collateral connections were found only in patients with pathological coronary disease, or were inherent and present in all humans with the ability to be recruited to functional vascular conduits. However, this was

largely put to rest by the seminal work of Fulton in the 1960s (37,38) using bismuth-oxychloride-gelatin radiographic contrast medium containing uniformly sized particles of 0.5-2.0μm, who found numerous anastomoses in normal hearts and those with CAD, with greater number and size of collaterals in those with CAD, suggesting collaterals are recruitable and mature in response to changes in the vasculature (Figure 1.2).

Since this pioneering anatomical work, the focus has shifted towards understanding the functionality of these collaterals as well as to determine their prognostic effects, and more recently therapeutic approaches to stimulate their development for clinically meaningful outcomes.

Figure 1.2: Post Mortem Anatomical Assessment of Coronary Collateral Connections.



(A): Post mortem coronary angiogram of a normal heart with ligation of the LAD performed prior to injection of contrast. The anastomotic connection was demonstrated by filling of the LAD distal to the occlusion. (B) Subendocardial coronary anastomotic connections shown in short axis of a patients with normal coronary arteries. (C&D) Coronary Angiogram and subendocardial connections in a patient with severe CAD and post mortem LAD ligation. Significantly greater numbers and size of connections noted compared with the patient with normal coronary arteries. Adapted from (36-38)

1.3 The Importance of understanding the Coronary Collateral Circulation in Coronary Artery Disease.

The relatively high incidence and prevalence of functional coronary collaterals in patients with CAD lends weight to the importance of understanding the interplay between these vessels on coronary haemodynamics, perfusion effects, symptoms and outcomes. In particular these vessels are not simple passive conduits, but capable of exerting effects on multiple facets of coronary physiology. It is further important to understand the mechanisms by which collaterals are recruited, mature and develop to consider potential future therapeutic targets and options for patients with CAD, where current revascularisation strategies are not suitable in approximately 20 - 30% (39).

1.4 Aims, Objectives and Scope of this Thesis

The ultimate aims of this body of work is to identify, summarise and synthesise, the mechanisms by which coronary collaterals are recruited and transition from primitive connections to larger functional vascular systems in both the acute setting, such as during an ACS, as well as in the chronic setting. Furthermore, specifically identifying the biochemical, mechanical and physiological changes required for collaterals to become functional, and conversely the effect the collateral circulation has on coronary, endothelial and cardiac function, particular the way it affects prognosis and clinical outcomes.

The specific aims of this work are;

- To determine the current understanding of arteriogenesis and recruitment and maturation of the coronary collateral circulation.

- To identify the prevalence, predictors and prognostic implications of functional coronary collaterals in patients presenting with an ACS.
- To identify the prevalence, predictors and prognostic implications of functional coronary collaterals in patients with a CTO.
- To identify the effect of coronary collaterals on coronary haemodynamics with respect to pressure and flow perturbations and how these correlate with symptoms of ischaemia and angina in patients with native coronary circulation and prior CABG.
- To identify endothelial, haematological, biochemical and proteomic markers which correlate with recruitment of the coronary collateral circulation in human and animal studies .
- To identify the impact of intermittent hypoxia and intrathoracic cavity pressure swings in the setting of obstructive sleep apnoea (OSA) on the coronary collateral circulation in both the acute and chronic setting.

The experimental and research work includes epidemiological population studies, observational cohort studies, invasive longitudinal clinical assessment as well as basic cellular and biochemical assessment from both human as well as animals. This provides a comprehensive body of work, which assesses many facets of the coronary collateral circulation and its implications.

1.5 Significance of Research

As detailed above, there remains significant knowledge gaps with respect to the mechanisms by which collaterals mature, and indeed what prognostic implications the presence or absence of collaterals imparts on clinical outcomes. To date, the prognostic implications of the presence and robustness of coronary collaterals in patients presenting with an ACS have not

been definitively determined. In particular this has implications for prognostication and use of health care resources, as well as determining future potential therapeutic options. Similarly, whilst the poor prognosis in patients with a CTO has been well described, whether the robustness of collaterals impacts on outcomes is uncertain. This has dramatic implications for identifying which patients may benefit from conventional revascularisation, which has consistently not been shown in randomised control trials including all-comers irrespective of degree of coronary collaterals. By understanding the effects of flow and pressure changes in the coronary arteries in patients with coronary collaterals, a greater understanding of the process by which they mature will be gained, along with determination of which chemokines, growth factors, transcription factors and others are implicated in this process. There has been little research into the effects of OSA, which is known to be associated with cardiovascular disease, on the coronary collateral circulation. This may be a key to understanding the manner in which OSA can have effects systemically.

1.6 Ethics & Methodology

1.6.1 Collateral Robustness Assessment

A number of chapters in the subsequent thesis are based upon anatomical grading of coronary collaterals as determined on coronary angiography with subsequent correlation with clinical endpoints, physiological indices of coronary pressure and blood flow and systemic biomarkers. Collateral robustness was assessed using the Rentrop classification(40) and the Collateral Connection grade (CC)(20). The Rentrop classification grades collaterals into 4 categories; where grade 0 = no filling of any collateral channel; Grade 1 = filling of the side branches of the infarct related artery; Grade 2 = Partial filling of the epicardial vessel of the infarct related artery' Grade 3 = complete filling of the epicardial vessel. For the analyses,

unless otherwise states, patients were divided into those with poor collateral recruitment (Rentrop grade 0 or 1) and robust collateral recruitment (Rentrop grade 2 or 3) as has been done in previous studies (11,41-47). The CC grade is based on the size of collaterals, rather than their ability to opacify the epicardial vessel, was also assessed, whereby grade 0 = No continuous connection between donor and recipient artery, grade 1 = Continuous, threadlike connections (diameter ≤ 0.3mm) and grade 2 = Continuous, small, side-branch-like size of the collaterals throughout its course (diameter ≥0.4mm).

All coronary angiograms were assessed by the author of this body of work with a subset of patients having 2 blinded senior clinicians assessing collateral robustness to ensure a high degree of interobserver correlation. This methodology was chosen as previous studies have shown very low rates of interobserver or intraobserver variability for Rentrop grading (20,48).

Ethics approval was gained for all data that was obtained at the patient level. A number of different ethics approval was obtained for the purposes of the study. The following ethics approval was gained for each of the published chapters.

1.6.2 Chapter 3, 4 & 14 Ethics Approval

For these chapters, patients included in the *Angiographic Predictors of Outcomes in Patients presenting with an ST Elevation Myocardial Infarction: The IMAGE MI Registry* were included. The IMAGE MI study was a single centre, non-randomised, retrospective registry to evaluate clinical, biochemical and imaging outcomes in patients undergoing primary percutaneous coronary intervention (p-PCI) for the treatment of an ST elevation myocardial infarction (STEMI). Institutional ethics approval was obtained from the Northern Sydney

Local Health District Human Research Ethics Committee (NSLHD HREC) prior to commencing the project [Appendix I & II].

1.6.3 Chapter 6,13, 15 & 16 Ethics Approval

Patients in the *"Coronary Angiographic predictors of outcomes in patients presenting with cardiovascular based symptoms: The CATH LAB Study"*, were included in the study. The CATH LAB study was a single centre non-randomised, retrospective registry to evaluate clinical, biochemical and imaging outcomes in patients undergoing coronary angiography and percutaneous coronary intervention (PCI). Institutional ethics approval was obtained from the Northern Sydney Local Health District Human Research Ethics Committee (NSLHD HREC) prior to commencing the project [Appendix III & IV]. Concurrently, patients were also included if they were recruited from the "*A multicentre registry of percutaneous coronary intervention (PCI) for chronic total occlusions (CTO) in patients with stable angina - The AUSNZ CTO Registry".* The AUSNZ CTO Registry is a multi-centre, non-randomised, single-arm, prospective registry to evaluate clinical and patient-centred outcomes, in patients undergoing CTO PCI. Institutional ethics approval was obtained from the Northern Sydney Local Health District Human Research Ethics Committee (NSLHD HREC) prior to commencing the project [Appendix V & VI].

1.6.4 Chapter 9 and 10 Ethics Approval

Consecutive patients presenting for elective CTO PCI between June 2018 and October 2020 were approached to be included in the "A comprehensive evaluation of biochemical, haemodynamic, structural and cellular of collateral function in human coronary arteries in chronic total occlusions and coronary artery disease – The COLLATERAL CTO Study". This is a single centre, prospective observational study including patients presenting for

elective CTO PCI as well as patients undergoing diagnostic angiography for stable coronary artery disease. Institutional ethics approval was obtained from the Northern Sydney Local Health District Human Research Ethics Committee (NSLHD HREC) prior to commencing the project [Appendix VII & VIII].

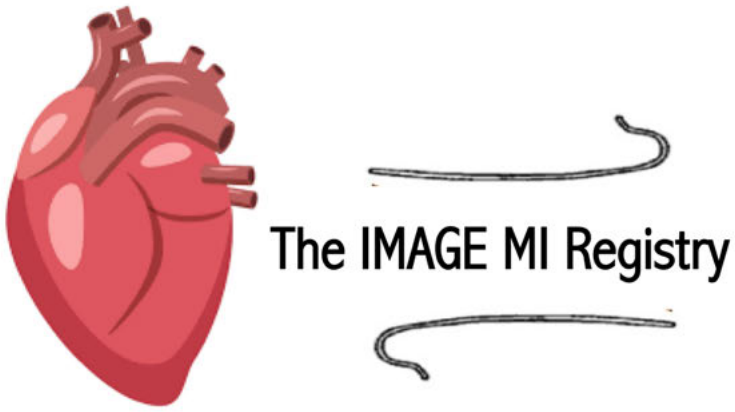
1.6.5 Chapter 12 Ethics Approval

Ethics approval to use animals was obtained from the Northern Sydney Local Health District Animal Ethics Committee (NSLHD-AEC), which conformed to the *Australian code for the care and use of animals for scientific purposes (49) (Appendix IX).*

Appendix I: IMAGE MI Study Protocol Cover Sheet

AngIographic Predictors of OutcoMes in PAtients presentinG with an ST Elevation Myocardial Infarction

– The IMAGE MI Registry –



STATEMENT OF COMPLIANCE
This document is a protocol for a clinical research study. The study will be conducted in compliance with all stipulations of this protocol, the conditions of ethics committee approval, the NHMRC National Statement on Ethical Conduct in Human Research (2007) and the Note for Guidance on Good Clinical Practice (CPMP/ICH---135/95).

Appendix II: Northern Sydney Local Health District (NSLHD) Human Ethics Research Committee Approval for IMAGE MI Study

Research Office
Kolling Building, Level 13
Royal North Shore Hospital
St Leonards NSW 2065
Tel (02) 9926 4590 Fax (02) 9926 6179

NSW GOVERNMENT Health
Northern Sydney
Local Health District

5 July 2018

Dr Ravinay Bhindi
Department of Cardiology, Level 5 Acute Services Building
Royal North Shore Hospital, Reserve Road
St Leonards, NSW, 2065

Dear Dr Bhindi,

NSLHD reference: RESP/18/155
Study Title: Aniographic Predictors of Outcomes in Patients presenting with an ST Elevation Myocardial Infarction – The Image MI Registry -

Thank you for submitting a response, dated **22/06/2018** to the Northern Sydney Local Health District HREC Executive Committee's request for additional information/modification of the above study, which was first considered at a meeting of the HREC Executive held **20/06/2018.** Based on the information you have provided and in accordance with the NHMRC National Statement 2007 and NSW Health Policy Directive PD2010_055 Ethical and Scientific Review of Human Research in NSW Public Health Organisations, this project has been assessed as low/negligible risk and is therefore exempt from full HREC review.

This HREC has been accredited by NSW Ministry of Health as a Lead HREC under the model for single ethical and scientific review and Certified by the NHMRC under the National model for Harmonisation of Multicentre Ethical Review (HoMER).This lead HREC is constituted and operates in accordance with the National Health and Medical Research Council's *National Statement on Ethical Conduct in Human Research* and the *CPMP/ICH Note for Guidance on Good Clinical Practice*. No HREC members with a conflict of interest were present for review of this project.

I am pleased to advise that the HREC, at a meeting of its Executive Committee held on **04/07/2018** has granted ethical and scientific approval of the above **single centre** project. The HREC have determined that this project meets the requirements of the National Statement.

You are reminded that this letter constitutes *ETHICAL* and *SCIENTIFIC* approval only. You must not commence this research project at a site until a completed Site Specific Assessment Form and associated documentation have been submitted to the site Research Governance Officer and Authorised. A copy of this letter must be forwarded to all site investigators for submission to the relevant Research Governance Officer.

The project is approved to be conducted at
- **Royal North Shore Hospital**

If a new site(s) is to be added please inform the HREC in writing and submit a Site Specific Assessment Form (SSA) to the Research Governance Officer at the new site.

The following documents have been approved:

Document	Version	Date
Protocol of Study	2.0	22/06/2018
HREA Form	-	-

The following documents have been noted:
- Method of Payment Form

The Human Research Ethics Application reviewed by the HREC was **HREA AU/1/DBD6312.**

Please note the following conditions of approval:

- HREC approval is valid for **5 years** from the date of the HREC Executive Committee meeting and expires on **04/07/2023.** The Co-ordinating Investigator is required to notify the HREC 6 months prior to this date if the project is expected to extend beyond the original approval date at which time the HREC will advise of the requirements for ongoing approval of the study.
- The Co-ordinating Investigator will provide an annual progress report to the Institution beginning in **August 2019** as well as a final study report at the completion of the project using the template available on the Research Office website. An annual report is due **every year on 30 August**.
- The Coordinating Investigator will immediately report anything which might warrant review of ethical approval of the project in the specified format, including unforeseen events that might affect continued ethical acceptability of the project and any complaints made by participants regarding the conduct of the project.
- Proposed changes to the research protocol, conduct of the research, or length of approval will be provided to the HREC Executive for review, in the specified format.
- The HREC Executive will be notified, giving reasons, if the project is discontinued before the expected date of completion.
- Investigators holding an academic appointment (including conjoint appointments) and students undertaking a project as part of a university course are advised to contact the relevant university HREC regarding any additional requirements for the project.

Should you have any queries about your project please contact the Research Office, ph: 9926 4590, email NSLHD-Research@health.nsw.gov.au .

Please quote **NSLHD reference RESP/18/155** in all correspondence.

The HREC wishes you every success in your research.

Yours sincerely

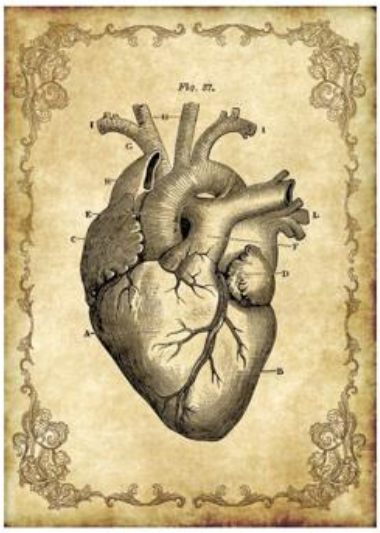
[redacted]

Jodi Humphries
Research Ethics Manager
Northern Sydney Local Health District

Appendix III: The CATH LAB Study Protocol Cover Sheet

Coronary Angiographic predicTors of outcomes in patients presenting witH cardiovascuLAr Based symptoms

– The CATH LAB Study –



– The CATH LAB Study –

CONFIDENTIAL

STATEMENT OF COMPLIANCE

This document is a protocol for a clinical research study. The study will be conducted in compliance with all stipulations of this protocol, the conditions of ethics committee approval, the NHMRC National Statement on Ethical Conduct in Human Research (2007) and the Note for Guidance on Good Clinical Practice (CPMP/ICH---135/95).

Appendix IV: Northern Sydney Local Health District (NSLHD) Human Ethics Research Committee Approval for The CATH LAB Study

☆ **no_reply@regis.health.nsw.gov.au** 9 April 2020 at 5:58 pm

2020/ETH00525: Application HREA - Approved

To: Usaid Allahwala

Date of Decision Notification: **09 April 2020**

Dear Usaid Allahwala

2020/ETH00525: Coronary Angiographic predicTors of outcomes in patients presenting witH cardiovascuLAr Based symptoms – The CATH LAB Study –

Thank you for your letter, dated 23 March 2020 responding to the Northern Sydney Local Health District HREC's request for additional information/modification for the above project, which was first considered by the HREC Executive on 11 March 2020.

The application was assessed as a **Low and Negligible Risk**.

I am pleased to advise that the Full HREC at its meeting has granted ethical and scientific approval of the above **single centre project on 6 April 2020**. The Full Committee were satisfied that this project meets the requirements of the *National Statement on Ethical Conduct in Human Research, 2007 (updated 2018).*

This project has been Approved to be conducted at the following sites:
Royal North Shore Hospital

The following documentation was reviewed and is included in this approval:

- Protocol, Version 2, Dated 23 March 2020

The Human Research Ethics Application reviewed by the HREC was:
Version: 2
Date: 21 March 2020

Application Documents - (**Please note : Due to security reasons, this link will only be active for 14 days**.)

Waiver of Consent
A Waiver of Consent has been granted for this project. It was approved at a meeting of the full HREC held on 06 April 2020. Please note, the approval is subject to fulfilling conditions a) to i) of Section 2.3.10 of the National Statement on Ethical Conduct in Human Research (NHMRC, 2007) and is only valid for as long as these conditions can be met.

This email constitutes ethical and scientific approval only.
This project cannot proceed at any site until separate research governance authorisation has been obtained from the Institution under whose auspices the research will be conducted at that site.

This HREC is constituted and operates in accordance with the *National Statement on Ethical Conduct in Human Research 2007 (updated 2018).* The processes used by this HREC to review multi-centre research proposals have been certified by the National Health and Medical Research Council. No HREC members with a conflict of interest were present for review of this project.

Please note the following conditions of approval:

- HREC approval is valid for **5 years** from the date of approval and expires on **06 April 2025.** The Co-ordinating Investigator is required to notify the HREC 6 months prior to this date if the project is expected to extend beyond the original approval date at which time the HREC will advise of the requirements for ongoing approval of the study.
- The Co-ordinating Investigator will provide an annual progress report **at the anniversary date of the project** as well as a final study report at the completion of the project within the Research Ethics and Governance Information System (REGIS).
- The Co-ordinating Investigator will immediately report anything which might warrant review of ethical approval of the project in the specified format, including unforeseen events that might affect continued ethical acceptability of the project and any complaints made by study participants regarding the conduct of the study.
- Proposed changes to the research protocol, conduct of the research, or length of HREC approval will be provided to the HREC for review, in the specified format.
- The HREC will be notified, giving reasons, if the project is discontinued before the expected date of completion.
- Investigators holding an academic appointment (including conjoint appointments) and students undertaking a project as part of a university course are advised to contact the relevant university HREC regarding any additional requirements for the project.

Please note it is the responsibility of the sponsor or the co-ordinating investigator of the project to register this study on a publicly available online registry (eg Australian New Zealand Clinical Trial Registry www.anzctr.org.au) if applicable.

Please contact us if you would like to discuss any aspects of this process further, as per the contact details below. We look forward to managing this application with you throughout the project lifecycle.

Regards,

Vanessa Cooper
Research Ethics Manager
Research Office
Level 13, Kolling Building
Tel (02) 9926 7825 I vanessa.cooper@health.nsw.gov.au
NSLHD-Research@health.nsw.gov.au
http://www.nslhd.health.nsw.gov.au/AboutUs/Research/Office

Appendix V: The AUSNZ CTO Registry Protocol Cover Sheet

A multicentre registry of percUtaneouS coronary interveNtion (PCI) for chronic total occlusions (CTO) in patients with stable angina.

The AUSNZ CTO Registry

STATEMENT OF COMPLIANCE
This document is a protocol for a clinical research study. The study will be conducted in compliance with all stipulations of this protocol, the conditions of ethics committee approval, the NHMRC National Statement on Ethical Conduct in Human Research (2007) and the Note for Guidance on Good Clinical Practice (CPMP/ICH---135/95).

Appendix VI: Northern Sydney Local Health District (NSLHD) Human Ethics Research Committee Approval for The AUSNZ CTO Registry

Research Office
Kolling Building, Level 13
Royal North Shore Hospital
St Leonards NSW 2065
Tel (02) 9926 4590 Fax (02) 9926 6179

Health
Northern Sydney
Local Health District

24 May 2018

Dr Usaid Allahwala

Dear Usaid,

NSLHD reference: RESP/17/364
Study Title: A multicentre registry of percutaneous coronary intervention (PCI) for chronic total occlusions (CTO) in patients with stable angina
HREC reference: LNR/17/HAWKE/502

Thank you for submitting a response, dated 25 March 2018 to the Northern Sydney Local Health District HREC Executive Committee's request for additional information/modification of the above study, which was first considered at a meeting of the HREC Executive held 18 December 2017. Based on the information you have provided and in accordance with the NHMRC National Statement 2007 and NSW Health Policy Directive PD2010_055 Ethical and Scientific Review of Human Research in NSW Public Health Organisations, this project has been assessed as low/negligible risk and is therefore exempt from full HREC review.

This HREC has been accredited by NSW Ministry of Health as a Lead HREC under the model for single ethical and scientific review and Certified by the NHMRC under the National model for Harmonisation of Multicentre Ethical Review (HoMER).This lead HREC is constituted and operates in accordance with the National Health and Medical Research Council's *National Statement on Ethical Conduct in Human Research* and the *CPMP/ICH Note for Guidance on Good Clinical Practice*. No HREC members with a conflict of interest were present for review of this project.

I am pleased to advise that the HREC, at a meeting of its Executive Committee held on 23 May 2018 has granted ethical and scientific approval of the above **single centre** project. The HREC have determined that this project meets the requirements of the National Statement.

You are reminded that this letter constitutes *ETHICAL* and *SCIENTIFIC* approval only. You must not commence this research project until you receive notification of Site Specific Assessment Authorisation from the Research Governance Officer. The LNRSSA submitted with this LNR application has been forwarded to the NSLHD Research Governance Officer for review.

The project is approved to be conducted at
Royal North Shore Hospital
If a new site(s) is to be added please inform the HREC in writing and submit a Site Specific Assessment Form (SSA) to the Research Governance Officer at the new site.

The following documents have been approved:

Document	Version	Date
HREA (AU/1/670333)	1.3	14/12/2017
CTO Registry Protocol – clean and tracked	2	20/03/2018
Participant Information Sheet and Consent Form – clean and tracked	3	23/03/2018

The following documents have been noted:

CTO Registry Cover letter

The Human Research Ethics Application reviewed by the HREC was **HREA AU/1/6700333**.

Please note the following conditions of approval:

- HREC approval is valid for **5 years** from the date of the HREC Executive Committee meeting and expires on **23 May 2023**. The Co-ordinating Investigator is required to notify the HREC 6 months prior to this date if the project is expected to extend beyond the original approval date at which time the HREC will advise of the requirements for ongoing approval of the study.
- The Co-ordinating Investigator will provide an annual progress report to the Institution beginning in **August 2019** as well as a final study report at the completion of the project using the template available on the Research Office website. An annual report is due **every year on 30 August**.
- The Coordinating Investigator will immediately report anything which might warrant review of ethical approval of the project in the specified format, including unforeseen events that might affect continued ethical acceptability of the project and any complaints made by participants regarding the conduct of the project.
- Proposed changes to the research protocol, conduct of the research, or length of approval will be provided to the HREC Executive for review, in the specified format.
- The HREC Executive will be notified, giving reasons, if the project is discontinued before the expected date of completion.
- Investigators holding an academic appointment (including conjoint appointments) and students undertaking a project as part of a university course are advised to contact the relevant university HREC regarding any additional requirements for the project.

Should you have any queries about your project please contact the Research Office, ph: 9926 4590, email NSLHD-Research@health.nsw.gov.au .

Please quote **NSLHD reference RESP/17/364** in all correspondence.

The HREC wishes you every success in your research.

Yours sincerely

Jodi Humphries
Research Ethics Manager
Northern Sydney Local Health District

Appendix VII: COLLATERAL CTO Study Protocol Cover Sheet

A COmprehensive evaluation of biochemical, haemodynamic, structural and cellular predictors of collateral function in human coronary arteries in Chronic total Occlusions and coronary artery disease

– The Collateral CTO Study –

STATEMENT OF COMPLIANCE
This document is a protocol for a clinical research study. The study will be conducted in compliance with all stipulations of this protocol, the conditions of ethics committee approval, the NHMRC National Statement on Ethical Conduct in Human Research (2007) and the Note for Guidance on Good Clinical Practice (CPMP/ICH---135/95).

Appendix VIII: Northern Sydney Local Health District (NSLHD) Human Ethics Research Committee Approval for COLLATERAL CTO Study

Research Office
Kolling Building, Level 13
Royal North Shore Hospital
St Leonards NSW 2065
Tel (02) 9926 4590 Fax (02) 9926 6179

Health
Northern Sydney
Local Health District

27 August 2019

Professor Ravinay Bhindi
Department of Cardiology
Royal North Shore Hospital
St Leonards
NSW 2065

Dear Professor Bhindi,

NSLHD reference: RESP/18/086
Title: A comprehensive evaluation of biochemical, haemodynamic, structural and cellular of collateral function in human coronary arteries in chronic total occlusions and coronary artery disease

Thank you for submitting an application for authorisation of this project. I am pleased to advise that the delegate of the Chief Executive for Northern Sydney Local Health District has granted authorisation for the above project to commence at **Royal North Shore Hospital.**

The version of the SSA reviewed by NSLHD RGO was: **AU/2/8C2536**

Ethical approval for this study was granted by the **Northern Sydney Local Health District HREC** at a meeting of the Executive Committee **held** on **23/05//2018.**

The documents authorised for use at this site are:

Document	Version	Date
Human Research Ethics Application (AU/1/4C25310)	1.3.01	23/05/2018
Protocol	3	25/02/2019
Participant Information Sheet and Consent Form – Master	3	25/02/2019
Participant Information Sheet and Consent Form – RNSH	1	19/08/2019
The Seattle Angina Questionnaire-7	No Version	No Date

The NSLHD RGO Notes:

- Risk Assessment
- Budget
- Professor Figtree emailed dated 18 June 2019- Confirmation of access to Bio-heart.

Site authorisation will cease on the date of HREC expiry **23/05/2023**

To comply with the NSLHD reporting requirements to the NHMRC Good Practice Program please forward an email to NLSHD-Research@health.nsw.gov.au with the date of the first participant recruited.

It is the responsibility of researchers from University of Sydney to ensure they comply with the University's insurance policy to undertake clinical trials. For further details please contact the Clinical Trial Governance Team on 02 8627 5078.

You are reminded that, in order to comply with the Guidelines for Good Clinical Research Practice (GCRP) in Australia, and in accordance with additional requirements of NSLHD, the Chief Investigator is responsible for ensuring the following:

1. The HREC is notified of anything that might warrant review of the ethical approval of the project, including unforeseen events that might affect the ethical acceptability of the project.
2. The HREC is notified of all Serious Adverse Events (SAEs) or Serious Unexpected Suspected Adverse Reactions (SUSARs) in accordance with the Serious Adverse Event Reporting Guidelines.
3. Proposed amendments to the research protocol or conduct of the research which may affect the ethical acceptability of the project, and are submitted to the lead HREC for review, are copied to the Research Governance Officer.
4. Proposed amendments to the research protocol or conduct of the research which may affect the ongoing site acceptability of the project are to be submitted to the Research Governance Officer.
5. The Institutional annual report for all Human Research is due to the NSLHD Research Office on the 30 August. In addition, annual report acknowledgment from the Lead HREC should be submitted to the Research Governance Officer.

Standard forms and additional guidance documents are available on the Research Office Website:
http://www.nslhd.health.nsw.gov.au/AboutUs/Research/Office

Yours sincerely

Natanya Hunt
Research Governance Officer
Research Office
Northern Sydney Local Health District

Appendix IX: Northern Sydney Local Health District (NSLHD) Animal Ethics Research Committee Approval for Study

Northern Sydney Local Health District
Animal Ethics Committee

Research Office
Kolling Building, Level 13
Royal North Shore Hospital
St Leonards NSW 2065
Tel (02) 9926 4590 Fax (02) 9926 6179

NSW GOVERNMENT Health
Northern Sydney
Local Health District

3 April 2019

Professor Ravinay Bhindi
Kolling Institute, Level 12
Royal North Shore Hospital
St Leonards, NSW, 2065

Dear Professor Bhindi,

NSLHD reference: RESP/18/209
Study Title: Coronary Chronic Total Occlusion Model In A Rat Using Repetitive Occlusive Ischaemia

Species	**Rattus Norvegicus**
Strain	**Sprague Dawley**
Sex, Age and Weight	**Male, 11 weeks**
Total number animals approved for use	**40**

Thank you for submitting a response, dated 11 March 2019 to the Northern Sydney Local Health District Animal Ethics Committee's (AEC) request for additional information/modification of the above study, which was first considered at an AEC meeting held 19 July 2018. I am pleased to inform you that the AEC, at its Executive meeting on 21 March 2019 has granted ethical approval for the above study.

In order to comply with the *Australian Code of Practice for the Care and Use of Animals for Scientific Purposes*, and in line with AEC requirements, you are reminded of the following conditions of approval:

1. AEC approval is valid for **3 years** from the date of the AEC meeting and expires on **21 March 2022.**
2. The Animal Research Authority will be granted yearly; subject to receipt of an annual progress report submitted using the AEC standard reporting template. Note that the ethics approval dates and ARA dates are not the same. Your ARA expires on **1 February 2020**.
3. The Coordinating Investigator will immediately report, using the AEC standard reporting template, anything which might warrant review of ethical approval of the project in the specified format, including unforeseen events that might affect continued ethical acceptability of the project and welfare of animals
4. All named investigators listed on the project application form must complete an acceptable animal ethics course (either through the University of Sydney, or University of New South Wales) within 12 months of this approval. On completing the course, or if investigators have already completed the course, please forward a copy of the course completion certificate to the Research Office.
5. Any cages/tanks/pens used by you in the Animal Houses are clearly labelled with the NSLHD study reference number.
6. All monitoring records are kept in a location accessible to animal house personnel and AEC members.
7. Any proposed changes to the AEC-approved protocol must be submitted for review prior to implementation. The AEC is notified as soon as possible of any changes to the protocol. This includes notifying AEC of any changes to the staff involved with the handling of animals for this protocol, procedures, animal numbers or source of animals.

Please quote **NSLHD reference RESP/18/209** in all correspondence.

The AEC wishes you every success in your research.

Yours sincerely

[redacted]

Mr Ed Līdums
Chairperson
NSLHD AEC

Northern Sydney Local Health District
Animal Ethics Committee

Research Office
Kolling Building, Level 13
Royal North Shore Hospital
St Leonards NSW 2065
Tel (02) 9926 4590 Fax (02) 9926 6179

NSW GOVERNMENT Health Northern Sydney Local Health District

ANIMAL RESEARCH AUTHORITY

Name(s) of Authority Holder(s) (list *all* persons involved in protocol including investigators and animal handlers): Professor Ravinay Bhindi Dr Usaid Allahwala Dr Anisyah Ridiandries
Project Title : Coronary Chronic Total Occlusion Model In A Rat Using Repetitive Occlusive Ischaemia
Project Summary (In Lay Terms) : The aim of this study is to induce new blood vessel (collateral vessel) formation in the rat heart in the presence of a blocked coronary artery. In the heart, collateral vessels grow from pre-existing vessels in the event a blocked coronary artery. The collaterals form as an alternate route to supply blood and oxygen to the blocked areas of the heart, preventing or minimising the effects a heart attack. To achieve collateral formation in the rat heart, we will achieve this by the process of repetitive occlusive ischaemia (ROI), whereby a balloon like apparatus (pneumatic snare) is inflated intermittently atop the left anterior descending artery (LAD) of the rat. The balloon is inflated intermittently to cause momentary occlusion of the vessel. This cause reduced blood flow to the heart making it ischaemic (low oxygen), this momentary ischaemia promotes formation of collaterals. After the protocol of 10 days, the LAD will be blocked with a suture for 30 minutes. The suture will be removed after 30 minutes and animals will be allowed to recover. After regular analgesia and close monitoring for 24 hours, animals will be euthanised.
Location at which the research is to be conducted: Kolling Institute, Royal North Shore Hospital
For Office Use Only: The NSLHD Animal Ethics Committee hereby approves the conduct of the above animal research application.
Application/Protocol No: RESP/18/209
Date of Project Approval by AEC: 21 March 2019 **Dates between which the Authority remains in force:** 21 March 2019 to ARA expiry 1 February 2020
Conditions: In order to comply with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes, and in line with AEC requirements, you are reminded of the following conditions of approval: 1. AEC approval is valid for 3 years from the date of the AEC meeting and expires on **21 March 2022.** 2. The Animal Research Authority will be granted yearly; subject to receipt of an annual progress report submitted using the AEC standard reporting template. Note that the ethics approval dates and ARA dates are not the same. Your ARA expires on **1 February 2020.** 3. The Coordinating Investigator will immediately report, using the AEC standard reporting template, anything which might warrant review of ethical approval of the project in the specified format, including unforeseen events that might affect continued ethical acceptability of the project and welfare of animals 4. All named investigators listed on the project application form must complete an acceptable animal ethics course (either through the University of Sydney, or University of New South Wales) within 12 months of this approval. On completing the course, or if investigators have already completed the course, please forward a copy of the course completion certificate to

the Research Office. 5. Any cages/tanks/pens used by you in the Animal Houses are clearly labelled with the NSLHD study reference number. 6. All monitoring records are kept in a location accessible to animal house personnel and AEC members. 7. Any proposed changes to the AEC-approved protocol must be submitted for review prior to implementation. The AEC is notified as soon as possible of any changes to the protocol. This includes notifying AEC of any changes to the staff involved with the handling of animals for this protocol, procedures, animal numbers or source of animals.
Approved Duration of the Project : Three (3) years from initial approval
Establishment issuing ARA: Northern Sydney Local Health District Animal Ethics Committee

Yours sincerely

[redacted]

Mr Ed Līdums
Chairperson
NSLHD AEC

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SECTION II: ARTERIOGENESIS

Chapter 2: Recruitment and maturation of the coronary collateral circulation: Current understanding and perspectives in arteriogenesis

This chapter is presented as the published work: Allahwala UK, Khachigian LM, Nour D, Ridiandres A, Billah M, Ward M, Weaver J, Bhindi R. Recruitment and maturation of the coronary collateral circulation: Current understanding and perspectives in arteriogenesis. *Microvascular Research*. 132:104058. doi: 10.1016/j.mvr.2020.104058

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Recruitment and maturation of the coronary collateral circulation: Current understanding and perspectives in arteriogenesis☆

Usaid K. Allahwala[a,b,*], Levon M. Khachigian[c], Daniel Nour[a], Anisyah Ridiandres[b], Muntasir Billah[b], Michael Ward[a], James Weaver[d,e], Ravinay Bhindi[a,b]

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ARTICLE INFO

Keywords:
Chronic total occlusion
Arteriogenesis
Collateral circulation
Collaterals

ABSTRACT

The coronary collateral circulation is a rich anastomotic network of primitive vessels which have the ability to augment in size and function through the process of arteriogenesis. In this review, we evaluate the current understandings of the molecular and cellular mechanisms by which this process occurs, specifically focussing on elevated fluid shear stress (FSS), inflammation, the redox state and gene expression along with the integrative, parallel and simultaneous process by which this occurs. The initiating step of arteriogenesis occurs following occlusion of an epicardial coronary artery, with an increase in FSS detected by mechanoreceptors within the endothelium. This must occur within a 'redox window' where an equilibrium of oxidative and reductive factors are present. These factors initially result in an inflammatory milieu, mediated by neutrophils as well as lymphocytes, with resultant activation of a number of downstream molecular pathways resulting in increased expression of proteins involved in monocyte attraction and adherence; namely vascular cell adhesion molecule 1 (VCAM-1), monocyte chemoattractant protein 1 (MCP-1) and transforming growth factor beta (TGF-β). Once monocytes and other inflammatory cells adhere to the endothelium they enter the extracellular matrix and differentiate into macrophages in an effort to create a favourable environment for vessel growth and development. Activated macrophages secrete inflammatory cytokines such as tumour necrosis factor-α (TNF-α), growth factors such as fibroblast growth factor-2 (FGF-2) and matrix metalloproteinases. Finally, vascular smooth muscle cells proliferate and switch to a contractile phenotype, resulting in an increased diameter and functionality of the collateral vessel, thereby allowing improved perfusion of the distal myocardium subtended by the occluded vessel. This simultaneously reduces FSS within the collateral vessel, inhibiting further vessel growth.

1. Introduction

Despite advances in operative and percutaneous strategies for revascularisation for coronary artery disease (CAD), these strategies are not suitable in approximately 20–30% of patients (Zbinden et al., 2005). A chronic total occlusion (CTO) is the complete, or near-complete occlusion of an epicardial coronary vessel, present for at least 3 months, and is associated with poorer clinical outcomes compared to other forms of stable CAD (Allahwala et al., 2019a). 50–60% of symptomatic patients with a CTO are treated with medical management rather than percutaneous coronary intervention (PCI) or coronary artery bypass grafting (CABG) (Ramunddal et al., 2014), which is associated with poorer symptomatic relief (Werner et al., 2018).

The coronary collateral circulation is a preformed network of immature anastomoses which connect the territory supplied by one epicardial coronary artery with that supplied by another (Pitt, 1959). This network has the potential to provide an alternative source of blood supply to a myocardium subtended by an occluded vessel [Fig. 1]. An understanding of the pathophysiological basis for the maturation of the coronary collateral circulation is essential. In this review, we describe the current understanding of the development of the coronary collateral circulation through the process of arteriogenesis.

☆ UA has received an unconditional research grant from Heart Research Australia. RB has received an unconditional future Leader Fellowship from the Heart Foundation of Australia.

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E-mail address: usaid.allahwala@sydney.edu.au (U.K. Allahwala).

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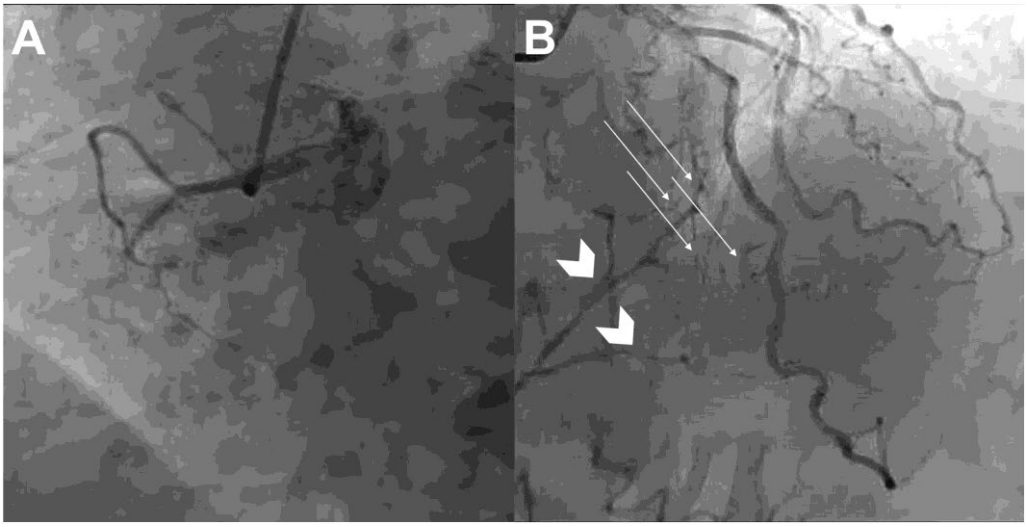

Fig. 1. Chronic total occlusion of the right coronary artery.
(A) Coronary angiogram identifying CTO of the right coronary artery (RCA) (B) Angiogram of the left coronary artery showing retrograde filling of the distal RCA (block arrows) through collateral arteries (narrow arrows) originating from the left anterior descending artery (LAD).

2. Development of the vasculature

The formation of blood vessels in the mature cardiovascular system occurs through 3 distinct dynamic processes; vasculogenesis, angiogenesis and arteriogenesis. These systems are influenced by a range of factors including signalling and transcriptional control, soluble mediators and their receptors, biomechanical forces and hypoxia.

2.1. Vasculogenesis

Vasculogenesis, largely restricted to the embryonic phase, is the initial formation of the immature vascular system, whereby mesodermal cells differentiate into hemangioblasts, which aggregate to form primitive blood islands. Within this network, the inner cell population differentiates into hematopoietic precursors and the outer cell population gives rise to the primitive endothelial cells, which generate blood vessels (Carmeliet, 2000). This vascular plexus subsequently develops into a complex organised and interconnecting network. There is an abundance of data that suggests that collateral arterioles are preformed, and present at birth, with anatomical studies in neonatal hearts finding the presence of coronary collaterals in 33–78% of hearts, more common in those with longer gestational age and younger maternal age (Bloor et al., 1966; Reiner et al., 1961). Similarly, in patients with angiographically normal coronary arteries, 20–25% of patients have sufficient collateral flow to prevent ischemia following balloon occlusion of an epicardial vessel (Wustmann et al., 2003), suggesting the innate presence of a functional, yet dormant coronary collateral circulation. Whilst there is evidence that vasculogenesis may occur in adults, facilitated via local or bone marrow-derived stem cell populations (Luttun and Carmeliet, 2003), this is not believed to play a significant role in the maturation of the coronary collateral circulation (Koerselman et al., 2003).

2.2. Angiogenesis

Angiogenesis is the process by which pre-existing capillaries proliferate and form new capillary networks. Angiogenesis occurs naturally during the menstrual cycle, and in pregnancy, and is the mainstay of pathological processes, including malignancy and rheumatoid arthritis (Carmeliet, 2000).

Hypoxia is an important stimulus driving angiogenesis, with decreased local oxygen tension initiating the expression of hypoxia-inducible genes, including the transcription factor hypoxia inducible factor-1 alpha (HIF-1α) (Kotch et al., 1999; Zelzer et al., 1998). Several genes are under the transcriptional control of HIF-1α, most notably, vascular endothelial growth factor (VEGF). VEGF is a potent mitogen for endothelial cells, and binds to one of 2 main tyrosine kinase receptors; VEGFR1 (Flt-1) and VEGFR 2 (KDR/Flk-1) on endothelial cells. This binding induces a complex response in endothelial cells including the expression of endothelial nitric oxide synthase (eNOS) and matrix metalloproteinases (MMPs) necessary for permeability of basal membranes and surrounding matrix (Tammela et al., 2005). Endothelial cells proliferate as solid sprouts and form lumina with neighbouring cells (sprouted angiogenesis) or the capillary wall protrudes into the capillary lumen to split the vessel in two (intussusceptive or splitting angiogenesis) (Carmeliet, 2000). Given the timing of collateral vessel appearance, particularly in the coronary system where visible collaterals are seen within hours of occlusion (Allahwala et al., 2020a), angiogenesis, which relies on hypoxia, is believed to be too slow and unlikely to contribute significantly to the process of coronary collateral recruitment and maturation (Scholz et al., 2002).

2.3. Arteriogenesis

Arteriogenesis, refers to the maturation and remodelling of pre-existing arterioles into functional vessels. These vessels which are pre-existing arterioles of 30-50 μm internal diameter that can grow into small arteries over 25-fold their original size (Scholz et al., 2001). Initial evidence from the surgical literature demonstrated that in response to arterial occlusion, arteriolar anastomoses matured into larger vessels (Fulton, 1965; Baroldi and Scomazzoni, 1967). Alterations in fluid shear stress (FSS) are believed to be the predominant initial stimulus for arteriogenesis (Carmeliet, 2000). However, there is a concurrent role of inflammation and homeostatic oxidative and reductive environments, which is required simultaneously with elevated FSS to create a favourable “arteriogenic environment”. The role of hypoxia on arteriogenesis, however, remains controversial, particularly whether it is necessary as an initiating factor, or augments the process.

3. Fluid shear stress

In the setting of normal coronary anatomy, there is minimal flow

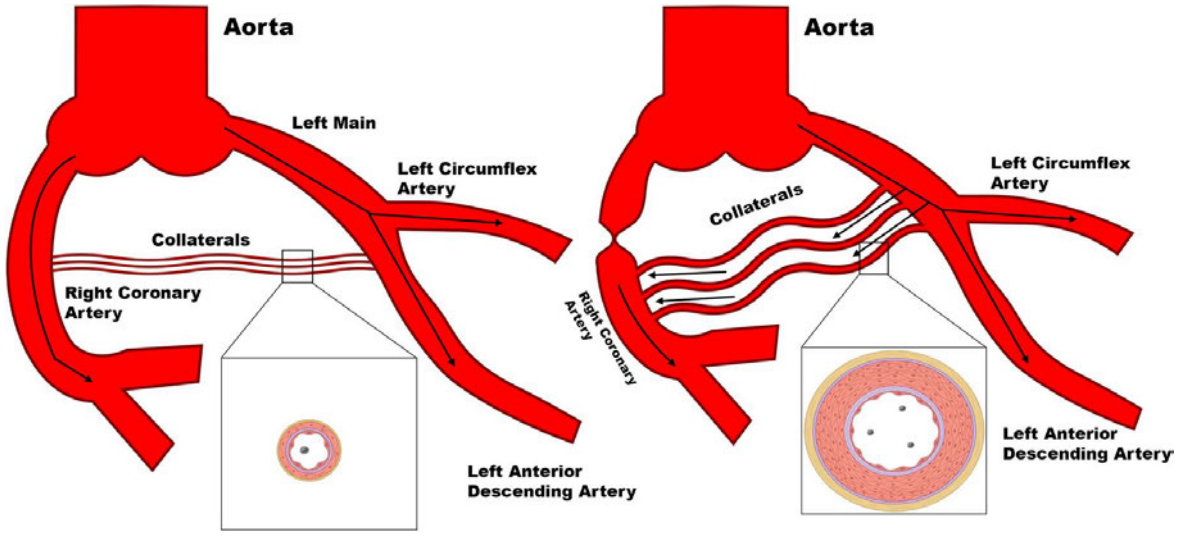

Fig. 2. Effect of occluded epicardial vessel on collaterals.
In the resting state without significant epicardial stenosis, the primitive coronary collaterals are non-functional and not visible on coronary angiography. Flow (indicated with arrows) is anterograde down the right coronary artery, left anterior descending artery and the left circumflex artery. With progressive epicardial stenosis, as in the setting of a CTO or sub-total occlusion, there is increased flow from the donor vessel through these collaterals (in this case from the left anterior descending artery to the right coronary artery through these collaterals as shown in arrows), resulting in elevation of FSS and downstream molecular and cellular alterations. This results in increased collateral size, SMC proliferation and increased capacitance.

through these pre-existing anastomoses (primitive collaterals), due to the high pressure in both distal circulations. However, following complete occlusion of a vessel, there is a dramatic reduction in pressure distal to the occlusion, and a resultant increased pressure gradient between the distal vessel and the donor vessel from which these primitive collaterals originate. Consequently, there is increased flow along these vessels, which results in an increase in FSS, the force per unit areal and may be considered as pressure, frictional wall shear at the cell surface, and tensile or compensatory forces acting to counter an externally applied force [Fig. 2]. Early studies using in vitro model systems defined FSS as a modulator of endothelial structure, function and gene expression (Resnick and Gimbrone, 1995; Gimbrone et al., 1997).

Numerous animal studies have since implicated an elevated FSS as a key driving stimulus for collateral maturation and arteriogenesis. In peripheral porcine and rabbit models of a CTO (Pipp et al., 2004), following occlusion and an increase in FSS, there is augmentation in the size, function and number of collaterals, above and beyond that of an occluded vessel without elevated FSS. In a similar study by Eitenmuller et al. (Eitenmuller et al., 2006), an elevation in FSS was associated with increased collateral blood flow, suggesting an elevated FSS is vital as an initiator of arteriogenesis, although not the only factor.

4. Mechanoreceptors

A potent, although not solitary, stimulus for arteriogenesis is the elevation in FSS as detected by endothelial cells. Whilst the precise mechanisms by which this change in physical stimulus is firstly detected, and then subsequently converted to downstream changes in protein expression remain to be fully elicited, a number of cell-matrix molecules, cell junction molecules, membranal structures and the endothelial cell cytoskeleton have been proposed to function as FSS sensors, or so-called “mechano-receptors”.

4.1. Cell-matrix and cell-cell junction molecules

4.1.1. Integrins

Integrins are the main receptor proteins that cells use to bind to and respond to the extracellular matrix (ECM) (Alberts et al., 2002). These molecules act as signal transducers and modulators of transcriptional regulation, capable of transducing mechanical stimuli into biochemical signals (Schwartz and Ginsberg, 2002; Ingber, 1998). In vitro studies have demonstrated the involvement of integrins in activation of IκB (proteins which act to inhibit Rel/NF-κB transcription factors) when exposed to shear stress (Bhullar et al., 1998), along with phosphorylation of Flk-1 and its binding to the adaptor protein Shc by elevated FSS (Chen et al., 2001). In vitro studies using human umbilical vein endothelial cells, suggests that in response to FSS, the integrin beta 2 chain is involved in extracellular signal-regulated kinase (ERK1/2) activation (Takahashi and Berk, 1996).

4.1.2. Cell-cell junction molecules

Platelet endothelial cell adhesion molecule-1 (PECAM-1) is expressed as a junctional adhesion molecule on endothelial cells (Tzima et al., 2005). In response to elevated shear stress, there is rapid phosphorylation of PECAM-1, with associated binding of PECAM-1 cytoplasmic tail to the phosphatase SHP-2, as well as direct ERK activation (Tzima et al., 2005; Osawa et al., 2002). There is subsequent downstream activation of NF-κB and NF-κB dependent genes. Conversely, inhibition of PECAM-1 or SHP-2 expression abrogates activation of ERK1/2 by shear stress (Fujiwara et al., 2001; Kano et al., 2000).

4.1.3. Adherens junctions

Adherens junctions provide important adhesive contacts between neighbouring endothelial cells with its specialised transmembrane proteins forming extracellular adhesive contacts between cells, and intracellular links to the actin cytoskeleton and signalling pathways including the regulation of gene transcription (Hartsock and Nelson, 1778). VE-cadherin is the major adhesive protein of the adherens junction and is specific for vascular endothelial cells. It can transfer information by interacting with the cytoskeleton via several anchoring molecules including β-catenin (Dejana et al., 2001). Deletion or truncation of VE cadherin impairs remodelling and maturation of the vascular network and on the cellular level abolishes transmission of intracellular signalling via VEGFR2 (Carmeliet, 1999). In endothelial cells lacking VE-cadherin, shear-stress-mediated signalling events such as the protein phosphorylation and gene induction are abolished, suggesting

the involvement of the adherens junctions and VEGFR2 in FSS transduction (Shay-Salit et al., 2002).

4.2. Membranous structures

4.2.1. Ion channels

Endothelial cells are not excitable, so they do not express high levels of voltage-gated channels and do not show a propagated response to depolarization or hyperpolarization like neurons or myocytes. However, the ionic conductance regulated by shear stress could influence a broad range of endothelial cell and vascular functions. The electrical coupling between endothelial cells and smooth muscle cells (SMC) also means that endothelial cell ion channels could directly control vascular tone (Emerson and Segal, 2000). A number of calcium, chloride and potassium and sodium transporters as well as unknown cation transporters have been implicated as critical mechanoreceptors to elevated sheer stress (Gerhold and Schwartz, 2016; Ziegelhoeffer et al., 2003).

4.2.2. Tyrosine kinase receptors

Elevated shear stress results in rapid, and transient tyrosine phosphorylation of Flk-1 and its concomitant association with the adaptor protein Shc, along with clustering of Flk-1 (Chen et al., 1999). These alterations of Flk-1 result in downstream chemical signalling via association with Shc, and gene transcription. Other studies have similarly shown rapid translocation of Flk-1 in response to flow (Shay-Salit et al., 2002).

4.2.3. Caveolae

Caveolae are flask-shaped invaginated structures present at the surface of many cell types including endothelium (Bruns and Palade, 1968), which has been shown in animal studies to play an important role in acute and longer term mechanotransduction (Yu et al., 2006) through its effects on increasing protein-tyrosine phosphorylation, eNOS activation, and coupling to the Ras/Raf/ERK MAPK pathway in a cholesterol-dependent manner (Rizzo et al., 1998a; Schnitzer et al., 1995; Rizzo et al., 1998b).

4.2.4. G-proteins

G-proteins are transmembrane cell signalling proteins comprising a receptor, a heterotrimeric G protein, and an effector (Wettschureck and Offermanns, 2005). In vitro studies (Chachisvilis et al., 2006) have suggested that when exposed to elevated shear stress, G protein-coupled receptor activity increases with greater proportion of activated conformation. Regulator of G-protein signalling 5 (RGS5) has been implicated in SMC activation (Arnold et al., 2014) whilst angiotensin II type 1 receptor is also activated in response to mechanical stretch (Wang et al., 2018).

4.3. The endothelial cytoskeleton

The endothelial cytoskeleton undergoes rapid changes in response to flow, and is capable of binding directly, or indirectly to the shear stress receptors mentioned above. Shear stress initiates a cytoplasmic actin-remodelling response that is used for endothelial cell shape change (Osborn et al., 2006). Computational modelling in Ingber's Tensegrity model (Ingber, 2003) suggests physical deformation which occurs through fluid shear stress, results in differing distribution of force in the cytoskeleton, initiating gene transcription. Furthermore, there is conformational change of the cytoskeleton in the activated and proliferating endothelial cell phenotype (Cai et al., 2004a).

5. Intracellular effects following elevation in FSS

Signalling from the activated mechanoreceptors is coupled with transcription factors, which regulate gene expression, through a number of pathways. One of the major signalling cascades downstream of cell surface receptors is the ERK pathway (Eblen, 2018). Activation of tyrosine kinase receptors results in receptor dimerization and autophosphorylation on multiple tyrosine residues, generating binding sites for adaptor and signalling proteins, which can recruit RAS proteins, which in turn signal to the ERK pathway (Eitenmuller et al., 2006). Similarly activation of cell surface receptors activities the Rho-pathway (Wojciak-Stothard and Ridley, 2003) and the nitric oxide pathway (Sriram et al., 2016). The early growth response-1 (EGR-1) transcription factor, which is expressed in human and murine atherosclerosis (McCaffrey et al., 2000), is rapidly induced following endothelial cell activation or stress (Khachigian, 2016). EGR-1 overexpression via adenoviral gene transfer results in a significant increase in arteriolar density in the ischemic rat hind limb, whilst EGR-1 knockout mice have decreased arteriogenesis (Sarateanu et al., 2006). Similarly, in a peripheral mouse model (Thulasingam et al., 2019), diabetic mice had smaller calibre collaterals, with a reduction in EGR-1 expression, which could be reversed with insulin treatment, highlighting the important role of this master regulator on arteriogenesis.

In-vivo animal studies, have suggested that protein kinase B (Akt), p38 and Src play an important role in collateral growth (Reed et al., 2009). Interestingly, Src appears to be redox dependent, resulting in upstream effects on p38 and Akt activation, with differing results in wild type or healthy animals as compared to metabolic syndrome models. The tumour suppressor protein p53 has been shown to negatively impact on collateral growth in in-vivo animal models (Pfaff et al., 2018).

6. Mediators of arteriogenesis

In a favourable arteriogenic environment, mechanoreceptor activation and subsequent upregulation of intracellular regulatory pathways consequently results in increased expression of a number of endothelial cell derived proteins, including vascular cell adhesion molecule 1 (VCAM-1), intracellular adhesion molecule 1 (ICAM-1), transforming growth factor beta (TGF-β) as well as cytokines such as monocyte chemotactic protein 1 (MCP-1), and granulocyte macrophage colony stimulating factor (GM-CSF). [Table 1].

6.1. Vascular cell adhesion molecule 1 (VCAM-1)

VCAM-1 is a protein expressed by endothelial cells, inducible by a number of mechanisms including elevated FSS, inflammation and reactive oxygen species (ROS) (Cook-Mills et al., 2011), all three of which play a crucial role in arteriogenesis. A number of effects have been associated with VCAM-1 expression on endothelial cells, with a primary role of regulating inflammation associated vascular adhesion and the transendothelial migration of leukocytes, such as macrophages and T-cells (Kong et al., 2018). In a pig hindlimb occlusion model, elevated FSS resulted in increased collateral flow and associated upregulation of VCAM-1 levels (Pipp et al., 2004). In vitro experiments of rabbit endothelial cells exposed to elevated FSS was associated with induction of VCAM-1 within 2–6 h (Scholz et al., 2000).

6.2. Intracellular adhesion molecule 1 (ICAM-1)

ICAM-1 is a surface glycoprotein which is expressed on endothelial cells, predominantly in response to inflammation, rather than by increased FSS (Hubbard and Rothlein, 2000), which promotes the adhesion of monocytes, neutrophils and lymphocytes (Nie et al., 1997; Steeber et al., 1998). The role of ICAM-1 in arteriogenesis has been illustrated in animal models. In a rabbit hindlimb model, administration of antibodies to endothelial ICAM-1 resulted in inhibition of arteriogenesis, suggesting the adhesion, invasion and infiltration of monocytes is essential in arteriogenesis (Hoefer et al., 2004). Another mechanism by which ICAM-1 may be associated with the initiation of

Table 1
Transcription factors, cytokines and growth factors associated with arteriogenesis.

	Source	Induction	Role
Chemoattractant proteins			
VCAM-1 (Pipp et al., 2004; Cook-Mills et al., 2011; Kong et al., 2018; Scholz et al., 2000)	EC	FSS, inflammation and ROS	Monocyte attraction and adhesion to EC, activation of mechanosensory complexes which activate intracellular changes in response to FSS
ICAM-1 (Hubbard and Rothlein, 2000; Nie et al., 1997; Steeber et al., 1998; Hoefer et al., 2004; Allahwala et al., 2019b)	EC	Inflammation and elevated FSS	Monocyte adhesion to EC, activate intracellular pathways
MCP-1 (Hoefer et al., 2004; Allahwala et al., 2019b; Deshmane et al., 2009; Buschmann et al., 2003; Heil et al., 2004; Ito et al., 1997; Voskuil et al., 2004; Heil et al., 2002; van Royen et al., 2003)	EC	Elevated FSS	Monocyte binding to EC, activating intracellular pathway
TGF-β (Negishi et al., 2001; van Royen et al., 2002; Wahl et al., 1987; Wiseman et al., 1988; McCartney-Francis et al., 1990)	Numerous cells	Elevated FSS	Expression of cytokines (IL-1, TNF-α and FGF-β)
Cytokines			
TNF-α (Breese et al., 1994; Nilsen et al., 1998; Hoefer et al., 2002; Grundmann et al., 2005)	EC, Macrophages, VSMCs	Macrophage stimulation from EC from FSS	Activates and adhesion of monocytes, secretion of chemokines, direct arteriogenic effect
Interleukins (Stabile et al., 2006; Latkovskis et al., 2012)	Inflammatory cells, ECs, VSMCs	?Elevated FSS	Uncertain
Growth factors			
FGF-2 (Ziegler et al., 2010; Schaper, 2009; Poling et al., 2011; Deindl et al., 2003; Yanagisawa-Miwa et al., 1992; Unger et al., 1994; Yang et al., 1996; Quinkler et al., 1989; Schaper and Scholz, 2003; Werner et al., 2004; Lazarous et al., 2000; Lederman et al., 2002; Simons et al., 2002)	Macrophages, VSMCs	FSS, EC activation	Proliferation of EC and SMCs, EC migration and differentiation
VEGF (Ishida et al., 2001; Heil et al., 2000; Clayton et al., 2008)	Multiple cell types	Hypoxia and ?FSS	SMC migration, monocyte chemotaxis
Colony Stimulating Factors (GM-CSF, G-CSF) (Becher et al., 2016; Buschmann et al., 2001; Grundmann et al., 2006; Sugiyama et al., 2011; Ripa et al., 2006; Seiler et al., 2001)	Macrophages and myeloid cells	FSS	Prolongation of monocyte and macrophage life cycle, amplifies effect of MCP-1
MMP (Cai et al., 2003; Dodd et al., 2011; Haas et al., 2007; Heil and Schaper, 2004)	Macrophages, EC, VSMCs	FSS, Hypoxia	Proteolytic enzyme resulting in ECM remodelling, stimulate EC proliferation

FGF-2 = fibroblast growth factor; EC = endothelial cell; FSS = fluid shear stress; ICAM-1 = intracellular adhesion molecule 1; IL-1 = interleukin 1; MCP-1: monocyte chemoattractant protein 1; ROS = reactive oxygen species; SMC = smooth muscle cell; TGF-β = transforming growth factor beta; TNF-α = tumour necrosis Factor-alpha; VCAM-1 = vascular cell adhesion molecule 1; VEGF = vascular endothelial growth factor; VSMC; vascular smooth muscle cells.

arteriogenesis, during its inflammatory stage, is through neutrophil chemo-attraction (Lautz et al., 2018; Ohki et al., 2005). In the setting of metabolic syndrome, ICAM-1 is associated with excessive neutrophil attraction, which may impair collateral function (Joseph et al., 2017). In human studies, we have shown that ICAM-1 levels are elevated in the presence of collateral vessels, which reduces following regression of collaterals (Allahwala et al., 2019b).

6.3. Monocyte chemoattractant protein-1 (MCP-1)

MCP-1 is a member of the CC-chemokine family, which is a potent chemotactic factor for monocytes (Deshmane et al., 2009). MCP-1 is induced in the vascular endothelium by increase in fluid shear stress (Shyy et al., 1994) and is involved in attracting and binding monocytes (via β_2 integrins like Mac-1) that invade the intimal space (Buschmann et al., 2003). Numerous animal studies have implicated MCP-1 in the recruitment of collaterals. MCP-1 is upregulated in collateral arteries with arteriogenesis significantly hampered in genetically altered mice which lack this CC-chemokine receptor (Heil et al., 2004). Ito et al. (Ito et al., 1997), infused MCP-1 into the growing collateral circulation following femoral artery ligation in the rabbit, resulting in a significant enhancement of collateral conductance. In MCP-1 deficient mice (Voskuil et al., 2004) and mice deficient of CC-chemokine receptor-2 (CCR2), the major receptor for MCP-1 (Heil et al., 2002), there was a reduction in monocyte accumulation as well as reduced flow restoration following arterial ligation. Intravenous infusions of 10- and 100-fold higher amounts of MCP-1 did not affect the growth of femoral collaterals. This may be due to rapid binding of MCP-1 to erythrocytes which prevents spill-over effects (Buschmann et al., 2003). Furthermore, genetic knockout or blockade of Mac-1 via antibody infusion significantly reduces collateral artery formation (Hoefer et al., 2004). In human studies, we have previously shown that MCP-1 is associated with the presence of coronary collaterals (Allahwala et al., 2019b).

6.4. Ephrins

The Ephrins comprise a family of membrane associated ligands that bind to the largest family of receptor tyrosine kinases, the Eph receptors, with Ephrin B2 exclusive expressed by arterial endothelial cells (Kullander and Klein, 2002). In vivo animal studies have illustrated that an increase in shear stress results in increased expression of ephrin B2 which limits SMC migration and controls monocyte extravasation (Korff et al., 2008).

6.5. Transforming growth factor beta (TGF-β)

Transforming growth factor beta (TGF-β) is expressed by different cells types under conditions of increased FSS (Negishi et al., 2001). It is associated with arteriogenic properties, increasing the capacity of the collateral vasculature (van Royen et al., 2002) and inducing MCP-1 expression (Wahl et al., 1987; Wiseman et al., 1988). TGF-β also increases expression of IL-1, TNF-α and FGF-2 (McCartney-Francis et al., 1990), as well as promoting the contractile phenotype of VSMC and MMP production (Risinger et al., 2010). In an animal peripheral artery disease model, TGF-β delivered via an endovascular drug delivery device was associated with an improvement in arteriogenesis (Grundmann et al., 2007).

7. Monocyte activation

Following attraction, adherence and activation of monocytes, they differentiate into macrophages which stimulate arterial growth, through production of inflammatory cytokines (Buschmann et al., 2003), growth factors, and MMP (Cai et al., 2000).

7.1. Cytokines

7.1.1. Tumour necrosis Factor-alpha (TNF-α)

TNF-α is a proinflammatory cytokine that plays a crucial role in chronic inflammatory diseases, such as rheumatoid/psoriatic arthritis and Crohn's disease. TNF-α, secreted in particular by cells of the monocyte/macrophage lineage (Breese et al., 1994), evokes pleiotropic immunomodulatory functions, including upregulation of cellular adhesion molecules and secretion of chemokines, such as IL-8 and MCP-1 (Nilsen et al., 1998). A number of animal studies in peripheral occlusive models suggests that TNF-α, directly upregulates arteriogenesis (Hoefer et al., 2002), whilst inhibition with biological agents attenuates collateral size and maturation (Grundmann et al., 2005).

7.1.2. Interleukins

Interleukins are inflammatory cytokines expressed by monocytes, epithelial and endothelial cells (Hsing et al., 2006), which exert their biological functions on multiple cells through activation of IL-20R1/IL-20R2 or IL-22R1/IL-20R2 complexes (Blumberg et al., 2001). IL-20 appears to upregulate collateral formation through increased phosphorylation levels of JAK2/STAT5, Erk1/2, and Akt; activation of small GTP-binding proteins Rac and Rho; and intracellular release of calcium and subsequent endothelial migration and tubular formation (Tritsaris et al., 2007). Whist animal studies have suggested IL-16 is implicated in recruitment of CD4+ by CD8+ cells into the adventitia of collateral vessels (Stabile et al., 2006), to date, there has been little research looking at the role of interleukins in arteriogenesis. In human studies, a specific haplotype of the interleukin cytokine was associated with the presence of a CTO (Latkovskis et al., 2012).

7.2. Growth factors

7.2.1. FGF

Activated macrophages express FGF-2 (Ziegler et al., 2010) which promotes proliferation of endothelial and vascular SMCs (Schaper, 2009; Poling et al., 2011) via activation of the FGF-receptor 1 (Deindl et al., 2003). In a canine model of myocardial infarction (Yanagisawa-Miwa et al., 1992), intracoronary infusion of FGF-2 after ligation of the left anterior descending artery was associated with an increase in the number of collateral arteries. Other animal studies have similarly suggested an association with FGF-2 and arteriogenesis (Unger et al., 1994; Yang et al., 1996). Contrastingly however, other studies have suggested that it is the FGF receptor which is more important in arteriogenesis, with a study in the pig heart suggesting no change in FGF expression following progressive coronary artery occlusion (Quinkler et al., 1989). However in a peripheral rabbit model, with occlusion of the femoral artery, there was no change in transcription of FGF ligands 1 or 2, but the FGF receptor 1 was upregulated at a critical stage of arteriogenesis, although only for a brief window of time (Deindl et al., 2003; Schaper and Scholz, 2003). In human studies (Werner et al., 2004), plasma concentrations of FGF2 is strongly associated with collateral function as higher concentrations in those with relatively “young” coronary CTOs. Intra-arterial administration of FGF-2 in patients with peripheral artery disease (Lazarous et al., 2000; Lederman et al., 2002), was associated with an increase in walking time, although this did not translate into improved outcomes in patients with coronary artery disease (Simons et al., 2002).

7.2.2. VEGF

Whilst VEGF is one of the most potent mediators of angiogenesis, there has remained controversy with respect to its role in arteriogenesis. VEGF has the ability to induce SMC migration (Ishida et al., 2001) and induce monocyte chemotaxis (Heil et al., 2000). Furthermore, knock-out mice to VEGF receptor or VEGF have decreased peripheral collateral formation compared to wild type mice suggesting VEGF may be a determinant of arteriogenesis (Clayton et al., 2008). Matsunaga et al. (Matsunaga et al., 2000) showed in a canine coronary model that VEGF expression peaked early in the process of collateral recruitment, but decreased in the later stages of maturation, suggesting VEGF (and ischemia's) role in the initiation of collateralisation. Toyota et al. (Toyota et al., 2005) demonstrated the causal role of VEGF in this manner. Specifically, neutralizing antibodies to VEGF prevented coronary collateral growth. Thus, if the initiation of collateral development is prevented, then shear-induced remodelling is of no consequence.

7.3. Colony stimulating factors

Granulocyte-monocyte colony stimulating factor (GM-CSF) and granulocyte colony stimulating factor (G-CSF) are predominantly expressed by macrophages and other myeloid cells (Becher et al., 2016). They appear to be involved in arteriogenesis, likely through prolongation of monocytes and macrophages (Buschmann et al., 2001; Grundmann et al., 2006). The arteriogenic effect of G-CSF is suppressed by monocyte depletion, suggesting G-CSF might share the same mechanism involved in monocyte mobilisation (Sugiyama et al., 2011). Both intravascular (Buschmann et al., 2001) as well as subcutaneous (Ripa et al., 2006) administration of GM-CSF have been shown to be effective in stimulating arteriogenesis in animal models. In human studies, Seiler et al. (Seiler et al., 2001), have suggested a correlation between TNF-α levels and pharmacological stimulation of collateral artery GM-CSF.

7.3.1. Matrix metalloproteinases

MMPs, secreted by macrophages are the primary proteolytic enzymes responsible for extracellular matrix remodelling. MMPs are present in and around developing collateral arteries (Cai et al., 2003) and are essential for collateral artery grown in a repetitive coronary occlusion model (Dodd et al., 2011), as well as mesenteric arterial ligation model (Haas et al., 2007). A number of MMPs are known to be expressed by macrophages, with experimental data suggesting that MCP-1 locally augments MMP-1, MMP-2, MMP-3 and MMP-9 expression and activity (Heil and Schaper, 2004). These MMPs participate in the digestion of the extracellular matrix and even of the skeletal muscle cells to create additional space for the growing collateral vessel.

MMPs may also play a role in collateral formation through its effects on anti-angiogenic peptides, including angiostatin and endostatin. Angiostatin is produced by the effects of MMP on plasminogen, whilst endostatin is a cleavage product of collagen, both of which are associated with poorer coronary collateral formation in human studies (Matsunaga et al., 2005; Panchal et al., 2004). In the setting of metabolic syndrome, the increased expression of MMP-12 increases the concentration of angiostatin and endostatin and may explain the poorer degree of collateral formation observed (Dodd et al., 2013).

7.3.2. Nitric oxide

Endothelial nitric oxide synthase (eNOS) is moderated partly by blood-flow induced mechanical factors such as FSS, and has been found to be elevated in collateral arteries (Cai et al., 2004b). In animal models, inhibition of the nitric oxide synthase did not change the diameter or FSS detected by epicardial vessels, however in the microcirculation, nitric oxide inhibition abolished the effect of autoregulation, thereby resulting in an increase in FSS with increasing flow (Stepp et al., 1999). Therefore, it would appear that nitric oxide plays a strong role in regulating FSS. Conditions which may interfere in nitric oxide production such as metabolic syndrome and oxidative stress may impact on FSS through nitric oxide mediated pathways (Tousoulis et al., 2012).

7.3.3. Proteases

A number of candidate downstream proteases have also been implicated in collateral formation. Inhibition of Endothelial-specific reduction of a disintegrin-and-metalloprotease-domain-10 (*Adam10*)

increases collateral formation, consistent with its roles in VEGF-induced Notch1 activation and suppression of "pro-sprouting" signals. Endothelial-specific knockdown of *Adam17* reduces collateral formation, consistent with its roles in endothelial cell migration and embryonic vascular stabilization, but not in activation of ligand-bound Notch1 (Lucitti et al., 2012).

8. Role of inflammatory cells

A number of cells distinct from monocytes are involved in the process of arteriogenesis, with an ever broadening understanding of their roles, further implicating other simultaneous processes to elevated FSS in the recruitment and maturation of arteriogenesis. Neutrophils, a potent driver of inflammation is believed to play an important role in the early, initiation phase of arteriogenesis. Their role in augmenting arteriogenesis occurs through direct effects on endothelial cells through binding to ALK, PI3K and resulting in NOS mediated vasodilation. Furthermore, activated neutrophils through ICAM-1 amongst others, are believed to secrete midkine, an inflammatory cytokine which mediates VEGF expression and release (Lautz et al., 2018; Ohki et al., 2005). Neutrophils are also a major source of ROS in the circulation (Bagi, 2015). However, given the short half-life of neutrophils outside of the circulation (1–2 days), as well as negative results of therapeutic studies with neutrophils (Hoefer et al., 2005), the role of neutrophils appears to be limited to the early initiation phase.

In mice deficient of macrophages, whilst there is a reduced arteriogenic response, it is not absent, suggesting other cells are involved in arteriogenesis (Bergmann et al., 2006). In a study in ApoE deficient mice (Couffinhal et al., 1999), which lack functional T-cells, following femoral artery ligation, perfusion restoration was severely reduced. Whilst macrophage accumulation in the adventitia was normal, there was a reduction in VEGF, suggesting that T-Cell activation and subsequent VEGF expression is vital for arteriogenesis. In the same study, administration of VEGF achieved by adenoviral gene transfer, normalised collateral flow. Similarly, in an ischemic hindlimb model in mice lacking CD4+ T-cells (Stabile et al., 2003), there was significant reduction in the inflammatory response and collateral development, which was reversed by infusion of spleen derived CD4+ T-cells. This was also observed in mice lacking CD8+ T-cells (Stabile et al., 2006). Further studies (van Weel et al., 2007) have suggested that NK-cells play a role in initiation of arteriogenesis, whilst CD4 cells are involved throughout, whilst NK T-cells do not seem to play a role in arteriogenesis. Interestingly, administration of lymphotactin, a lymphocyte chemokine did not result in an increase in collateral development, despite increased T-cell accumulation, suggesting an excess accumulation of T-cells may result in an unfavourable environment for macrophage attraction and activity (Hoefer et al., 2005).

Mast cells have been identified in the wall of growing collateral vessels, which are believed to invade into the endothelium and release MMP-9, which may be essential for the mast cell migration (Tanaka et al., 1999; Kanbe et al., 1999). Mast cells may stimulate proliferation of SMCs by releasing FGF-2 and TGF-β and may also support arteriogenesis indirectly by inducing monocyte chemotaxis and their differentiation into macrophages through the release of inflammatory cytokines MCP-1 and TNF-α (Qu et al., 1995; Reed et al., 1995; Grutzkau et al., 1998; Norrby, 2002), and prolong monocyte survival through release of GM-CSF (Buschmann et al., 2001; Norrby, 2002). Furthermore, mast cells may stimulate monocytes/macrophages to release interleukins, particularly IL-1, which can stimulate production of MMPs in a variety of cells, or else they may directly release MMPs or serine protease capable of degrading ECM-components (Norrby, 2002).

9. Vascular remodelling

Following endothelial activation, the predominant cellular subtypes activated are inflammatory cells and progenitor cells, with subsequent release of inflammatory cytokines and enzymes resulting in degradation of the basement membrane and ECM. Endothelial cell mitosis appears to precede that of SMCs, and growth factors are released from the matrix and from monocytes at this time (Arras et al., 1998). These growth factors along with direct mitogenic activity, also influence transcription of secondary growth factors including FGF, inactivation of the MMP inhibitor TIMP, and downregulation of the ECM component elastin.

Vascular SMC (VSMC) phenotype switch from the adult, quiescent, contractile phenotype to the synthetic, proliferative migratory phenotype with resultant proliferation and migration of the VSMCs into the lumen of the pre-existing native collateral vessel (Hutcheson et al., 2013). During this phase, a neointima forms composed of SMCs in which, like in the earlier degradation of the internal elastic lamina, MMPs are involved (Scholz et al., 2001; Scholz et al., 2000). The ability of VSMCs to switch back to the contractile phenotype plays an important role in the formation of functional, mature collaterals. Experimental data has suggested that this is mediated through miR-145, which has downstream effects on a number of proteins (Hutcheson et al., 2013). One such mechanism is the effect of low endogenous miR-145 concentrations, as occurs in metabolic syndrome, which in turn results in increase of 20-hydroxyeicosatetraenoic acid (20-HETE), predominantly from neutrophils, compromising endothelial cell survival and function, leading to impaired coronary collateral formation (Joseph et al., 2017).

Following increase in diameter of the vessel, and increased thickness of endothelial wall by SMC proliferation, there is a reduction in fluid shear stress and circumferential wall sheer stress, thereby providing a negative feedback and termination of the remodelling phase. Consequently, the transformation of a small microvascular resistance vessel into a large conductance artery is now completed. After a pronounced proliferating and remodelling phase, the collateral artery is no longer distinguishable from a normal artery, except for a slightly higher collagen content between the SM layers (Wolf et al., 1998). [Fig. 3].

9.1.1. Redox stress

Through the above described in-vitro, in-vivo and animal models, it is apparent that other processes, distinct from elevated FSS are implicated in the recruitment and maturation of collaterals. One such factor is the effect of redox stress on arteriogenesis. Reactive oxygen species (ROS) including superoxide ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($^{\bullet}OH$) are derived from a number of sources, including mictochondria, xanthine oxidase, uncoupled nitric oxide synthesases and NADPH oxidase (Ho et al., 2013). There is increasing evidence that they play a pivotal role in many cardiovascular pathologies, including atherosclerosis and ischemia-reperfusion injury (Sugamura and Keaney, 2011). In the setting of coronary revascularisation (and reperfusion), an influx of ROS results in cellular destruction, leading to stunning and necrosis of tissue mediated by the effects of ROS on cell death by lipid peroxidation, interruption of survival signalling pathways by protein modification, and DNA damage (Erdogan et al., 2006).

Perhaps surprisingly, despite the apparent deleterious effects of ROS, their presence is necessary for coronary collateral growth. Ischemic pre-conditioning, whereby brief, intermittent ischemia to an organ, renders it tolerant to subsequent ischemia and reperfusion, is mediated, in part through promotion of collaterals (Chen et al., 1995). Repetitive ischemia in a rat model (and subsequent collateral recruitment) resulted in induction of $O_2^{\bullet -}$ and H_2O_2, whilst blockade of $O_2^{\bullet -}$ resulted in a reduction of coronary collaterals (Rocic et al., 2007). Similarly, Treatment with an ROS scavenger, *N*-acetyl cysteine in a canine model reduced coronary collateral development (Gu et al., 2003). Consequently, through shifting from an oxidative state to a reductive milleu there is depletion of ROS, and hence a reduction in ROS

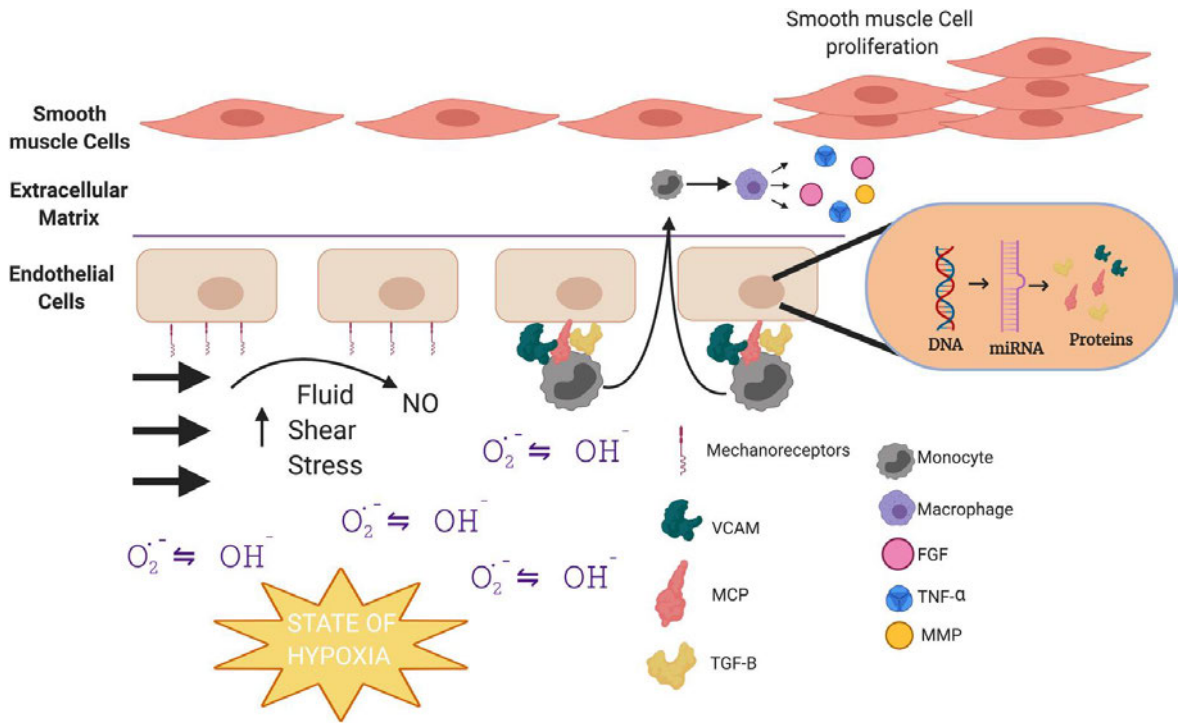

Fig. 3. Mechanisms of collateral recruitment and development.
Following occlusion of an epicardial vessel, there is an immediate increase in flow through the primitive collateral vessels, resulting in elevated fluid shear stress, which is detected by mechanoreceptors on the endothelial cell (EC) surface. In a favourable "redox window" with associated hypoxia, this EC activation results in increased expression of proteins necessary for monocyte attraction and adherence including vascular cell adhesion molecule 1 (VCAM-1), monocyte chemoattractant protein 1 (MCP-1) and transforming growth factor beta (TGF-β). The monocyte subsequently enters the extracellular matrix (ECM), differentiates into a macrophage and secretes inflammatory cytokines, including tumour necrosis factor-α (TNF-α), growth factors such as FGF-2 and matrix metalloproteinases. These create a favourable environment for SMC proliferation which in turn results in increased diameter of collateral vessels, allowing increased perfusion. There is also associated alterations in gene expression, mediated through effects of microRNA.

mediated cellular processes of survival, proliferation and migration.

However, in the setting of spontaneously elevated oxidative stress, such as with metabolic syndrome and diabetes (Tuttle et al., 2002), as well as experimentally elevated redox stress, there is inhibition of the ability to mature and develop collaterals (Rocic et al., 2007). In experimental rat models, alterations in redox state through administration or antagonism of angiotensin II resulted in either inhibition or augmentation of collateral growth depending on the baseline degree of oxidative stress (Reed et al., 2008).

Consequently, there is an emerging theory of a so called "redox window" (Yun et al., 2009) whereby some degree of ROS is required for collateral growth, although a state of oxidative stress is detrimental. In-vitro experiments have suggested this redox-window, whereby VEGF mediated endothelial cell tubular formation was inhibited by both administration of agents to block ROS formation and shift the redox state to a reductive environment and agents which shift the redox state to oxidative stress (Rocic et al., 2007). This in-vitro data was confirmed with in-vivo animal studies where administration of either agent similarly reducing collateral flow.

The mechanisms by which redox stress influences collateral growth remains an area of ongoing research. One proposed mechanism is through ROS mediation inhibition of p38 MAP kinase, which is activated when VEGF binds to FLk-1 (Rocic et al., 2007). Furthermore, signalling of VEGF is partially mediated by production of $O_2^{\cdot-}$ by NAD(*P*)H oxidase (Ushio-Fukai et al., 2002). One of the rationales cited for the disappointing results of growth factor trials to improve collateral growth has been a disregard for the redox state. Animal studies have shown that correction of the redox state results in significantly improved VEGF mediated collateral growth (Hattan et al., 2007). As such, future clinical studies aiming to stimulate the coronary collateral circulation should ensure that the effect of the redox state is taken into consideration for therapeutic trials.

The effect of angiotensin II in vascular growth is controversial, with angiotensin receptor blockers associated with inhibition of vascular growth in hindlimb ischemia (Ebrahimian et al., 2005) and poorer coronary collaterals in patients presenting with myocardial infarction (Allahwala et al., 2020b), whilst ACE inhibition increased angiogenesis in vivo (Walther et al., 2003). One explanation for these conflicting findings may be the background redox state. Angiotensin increases the production of $O_2^{\cdot-}$ by activation of NAD(*P*)H oxidase, in a variety of cell types in the vascular wall, including smooth muscle, adventitial fibroblasts, and endothelial cells (Hanna et al., 2002; Harrison et al., 2003). In a rat model of coronary collateral growth, animals analogous with metabolic syndrome in humans, had higher levels of oxidative stress than wild type animals. Administration of angiotensin receptor 1 (ATR1) blockade in high oxidative states increased coronary collateral flow, whilst in wild type rats treatment with ATR1 blockade reduced collateral flow (Reed et al., 2008). Furthermore, the effect of treatment appeared to be dose responsive with possibly varying degree of superoxide production. An appreciation of the redox state and its effect on the arteriogenic environment, simultaneous with elevated FSS and downstream cell signalling pathways is vital for future exploratory and therapeutic research.

10. The role of hypoxia in arteriogenesis

Whilst hypoxia remains the predominant promoter of angiogenesis, mediating its effects through upregulation of hypoxia-inducible factor-1α (HIF-1α) (Rey and Semenza, 2010), its role in arteriogenesis is less well established. Whilst certain territories where arteriogenesis occurs,

such as in the peripheral vasculature (Heil et al., 2006), skeletal muscle (Mac Gabhann and Peirce, 2010) and the mesentery (Walker, 2009) are not hypoxic, other territories where collaterals are found, such as coronary arteries, are hypoxic, making a clear distinction difficult. Stenoses which slowly progresses toward complete occlusion are often only minimally ischemic, and yet this is the condition where collaterals show maximal adaptation. In his seminal paper using radio-opaque bismuth-oxychloride-gelatin to create 3-dimensenionals casts of coronary arteries, Fulton (Fulton, 1965) showed that the origin of collaterals in the human coronary circulation occurs in normoxic tissue, driven instead by elevated FSS. In the peripheral artery disease setting, it is particularly apparent that collateral vessels mature at the site of occlusion, which is generally proximal to the area of hypoxia as has been demonstrated in animal studies (Scholz et al., 2002). Furthermore, as arteriogenesis predominantly occurs in territories which do not tolerate hypoxia for any extended period of time (the coronary and cerebral circulation), it appears unlikely that hypoxia would play the predominant role in its development. Indeed during acute myocardial infarction, 20% of patients display visible coronary collaterals on angiography, which whilst occurring around the site of ischemia must be formed prior to infarction to salvage the myocardium (Allahwala et al., 2020b). Whilst some animal studies have shown an upregulation of VEGF, hypoxia is not the stimulus for this, as this is expressed by the endothelium of growing collaterals, an area, by definition constantly perfused by well oxygenated blood (Schaper, 2009). In some animal models of peripheral arteriogenesis, there is upregulation of VEGF or hypoxia induced genes (Deindl et al., 2001), whilst other animal studies have similarly shown no association of hypoxia and arteriogenesis (Gray et al., 2007).

However, one of the issues with animal models of arteriogenesis is in isolating FSS from ischemia, as invariably vascular occlusion results in both elevation in shear stress and ischemia. Chillian et al. (Chilian et al., 1990) attempted to isolate the role of ischemia by administration of embolized microspheres into the microvasculature of the left circumflex in dogs, showing that whilst some collateral flow was observed, this was less than would be expected, suggesting that whilst ischemia plays a role in arteriogenesis, elevation in sheer stress greatly augments this process. Adding further evidence to the perhaps overarching role of FSS was the finding of increased collaterals between the left anterior descending artery and the right coronary artery following progressive occlusion of the left circumflex artery, clearly showing collateral growth in non-ischemic territories (Scheel et al., 1976; Scheel et al., 1977).

Consequently the presence of ischemia may augment, or be involved in the initiation of arteriogenesis and may also be dependent on the area at risk and the effect of underlying redox state. Further research is required to delineate the effects of ischemia an arteriogenesis.

10.1. Gene expression

MicroRNAs (miRNAs) are a class of non-coding RNAs which play important roles in regulation of gene expression. They are transcribed from DNA sequences and, in most cases interact with the 3′ untranslated region (3′ UTR) of target mRNAs to induce mRNA degradation and translational repression (O'Brien et al., 2018). A number of miRNAs have been implicated with coronary collateral growth and maturation, including miR-21, which has been associated with increased and inappropriate cell proliferation in the latter stages of collateral formation (Hutcheson et al., 2014). In animal models of peripheral arteriogenesis, miR-146a is associated with a reduction in collateral formation (Heuslein et al., 2018). However, the downstream effects remain uncertain, as in clinical studies, elevated miR-146a was associated with greater maturation of coronary collaterals (Wang et al., 2016), In an animal model of peripheral collaterals (Guan et al., 2017), miR-352 was downregulated with increasing FSS, and negatively correlated with collaterals.

10.2. Implications with atherogenesis

Whilst the correlation of the above discussed proteins, growth factors and cytokines with development of arteriogenesis is of ongoing research, there is a significant overlap with pathophysiology with atherosclerosis, and as such as significant implications for potential therapeutic applications. For example, both MCP-1 and ICAM-1 are markers of coronary artery disease (Hwang et al., 1997; Tang et al., 2007), whilst MCP-1 infusion results in increasing neointima formation and a change in plaque composition toward an unstable phenotype (van Royen et al., 2003) in pre-existing lesions.

This further underpins the fact that animal studies, which cannot replicate the background environment of established coronary artery disease will have this inherent limitation with respect to mechanistic research as well as future potential therapeutic agents. The significant overlap of the process of arteriogenesis and atherosclerosis underpins inherent limitations to future studies on the subject. Whether the presence of collaterals in the coronary circulation is associated with collaterals in other vascular beds is uncertain. Patients with developed coronary collaterals undergoing coronary artery bypass grafting, had less cognitive decline, suggesting possibly better developed cerebral collaterals (Dieleman et al., 2009), however the broader implications of this remain to be determined.

11. Conclusions

Arteriogenesis is the mechanism by which pre-existing, primitive arteriolar connections between arterial beds can quickly and efficiently increase in size and capacity, to allow perfusion to the myocardial bed distal to an occluded vessel.

These collaterals mature and develop as a result of increased fluid shear stress in the setting of an optimized redox state with probable hypoxia induction (Fig. 4). This series of events activate endothelial cells and creates an initial cascade of inflammatory cells to accumulate at the site of pre-existing arteriolar connections. There is subsequent activation of a multitude of downstream transcription factors, chemokines and growth factors resulting in endothelial cell and vascular smooth muscle cell proliferation and functionality. With increasing size and calibre as well as amelioration of hypoxia, there is negative feedback to this loop resulting in cessation of further molecular alterations. Whilst an understanding of the core signalling pathways by which these vessels mature is emerging, further specific cell signalling, genomic and proteomic pathways remain to be identified and specific interaction with redox signalling and hypoxia induced factors remain to be answered.

CRediT authorship contribution statement

Usaid K Allahwala: Conceptualization, Methodology, Investigation, Writing - Original Draft; **Levon M Khachigian:** Conceptualization, Resources, Writing - Review & Editing; **Daniel Nour;** Conceptualization, Methodology, Writing - Original Draft; **Anisyah Ridiandres:** Writing - Review & Editing; **Muntasir Billah:** Writing - Review & Editing; **Michael Ward:** Supervision; **James Weaver:** Supervision, Writing - Review & Editing; **Ravinay Bhindi**: Methodology, Writing - Review & Editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

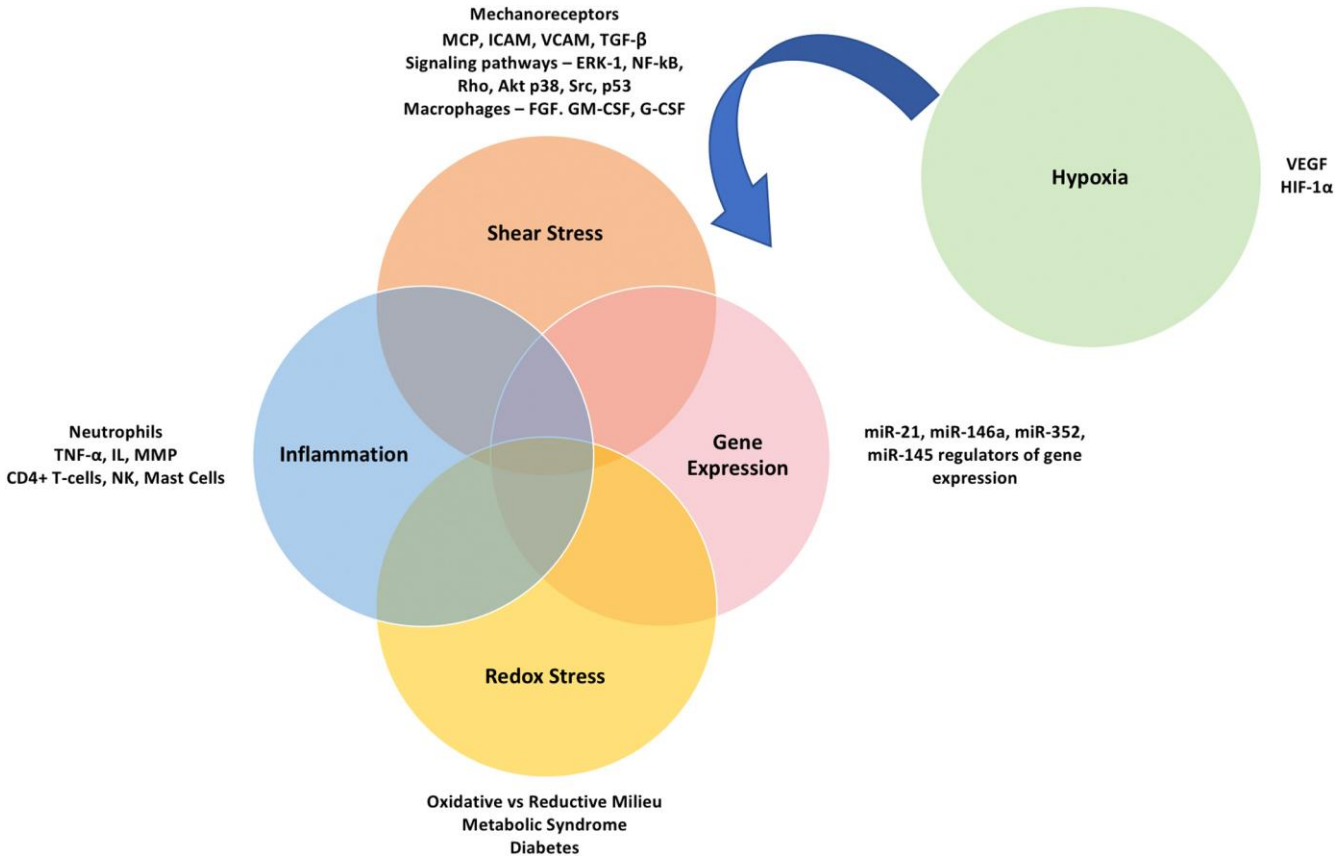

Fig. 4. Factors involved in arteriogenesis.
The process of arteriogenesis is mediated through simultaneous and integrated processes following arterial occlusion, namely elevated fluid shear stress, inflammation and redox stress, mediated through alterations in gene expression. These four factors are exquisitely linked and necessary to create a favourable arteriogenic environment. These factors mediate their effect through of activation of a multitude of downstream transcription factors, chemokines and growth factors, as shown, resulting in endothelial cell and vascular smooth muscle cell proliferation and functionality. The precise role of tissue hypoxia on arteriogenesis remains uncertain, although may augment the role of the key factor involved in arteriogenesis.

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SECTION III: ACUTE RECRUITMENT OF CORONARY COLLATERALS

Chapter 3: Recruitment of acute coronary collaterals in patients presenting with an ST elevation myocardial infarction (STEMI)

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Effect of Recruitment of Acute Coronary Collaterals on In-Hospital Mortality and on Left Ventricular Function in Patients Presenting With ST Elevation Myocardial Infarction

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Recruitment of the coronary collateral circulation is frequently observed during ST elevation myocardial infarction (STEMI) and is of uncertain significance. The aim of this study was to identify and determine the predictors and prognostic implications of the presence of robust collaterals during STEMI. All patients presenting to a large tertiary centre with a STEMI undergoing percutaneous coronary intervention from 2010 to 2018 were reviewed. Patients with poor collateral recruitment were defined as those with Rentrop grade 0 or 1 collaterals, whilst patients with robust collateral recruitment were defined as Rentrop grade 2 or 3. A total of 1,625 patients were included in the study, with 1,280 (78.8%) patients having poor collateral recruitment and 345 patients (21.2%) having robust collateral recruitment. Patients with robust collaterals were younger (63.1 vs 65.1 years, $p < 0.05$), had a longer ischemic time (628.5 minutes vs 433.1 minutes, $p < 0.0001$), and more likely to have a chronic total occlusion of a noninfarct related artery (10.4% vs 5.3%, $p < 0.001$). The presence of robust collaterals was associated with higher rates of normal or mildly impaired left ventricular function (83.5% vs 63.2%, $p < 0.0001$) and lower in-hospital mortality (2.1% vs 7.6%, $p < 0.0001$). After correcting for left ventricular function, collateral recruitment was not an independent predictor of mortality. In conclusion, in patients presenting with STEMI, the presence of robust coronary collaterals appears to be associated with improved left ventricular function. Further research is required to identify mechanisms of collateral maturation and recruitment. **(Am J Cardiol 2020;125:1455–1460)**

The coronary collateral circulation is a network of preformed anastomotic channels which connect the territory supplied by one epicardial coronary artery with that supplied by another.[1] Naturally occurring coronary collaterals appear in all species, with significant variation in their extent and development.[2] These collateral arteries therefore provide an alternative source of blood supply to myocardium that has been jeopardized by occlusive coronary artery disease, as is seen in the setting of a ST elevation myocardial infarction (STEMI). Consequently, the presence of collaterals may help to preserve myocardial function and improve clinical outcomes.[3] Coronary collaterals are rapidly recruited, often observed during coronary angiography for primary percutaneous coronary intervention (pPCI), and whilst some[4,5] observational studies have shown a prognostic benefit with collaterals, not all have agreed.[6] We sought to determine the prevalence, predictors and impact of the presence of collaterals, recruited acutely during a STEMI on clinical outcomes.

Methods

We reviewed patients presenting to our tertiary centre with a diagnosis of STEMI, undergoing pPCI or rescue PCI for unsuccessful thrombolysis, from July 2010 until December 2018. All patients had pretreatment with aspirin, unless they were unable to tolerate per oral medications. Therapeutic intra-arterial heparin was administered at the beginning of the procedure, whilst intracoronary nitrates prior to intervention was not routinely administered, due to the emergent nature of the procedure. Glycoprotein IIb/IIIa inhibitor use was left to operator discretion.

Patients who had had a prior coronary artery bypass graft, or spontaneous coronary artery dissection were excluded. Electronic medical records were reviewed to identify procedural characteristics, presence of preinfarct angina, in-hospital course, left ventricular (LV) function, and biochemical results. LV function was assessed by transthoracic echocardiogram following STEMI, or if not performed, then based on ventriculography at the time of the procedure. Invasive hemodynamics

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See page 1459 for disclosure information.

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at the beginning of the coronary angiogram, including heart rate (HR) and aortic systolic blood pressure (SBP) were recorded, and defined as "starting HR" and "starting SBP."

Analysis of coronary angiography was performed to determine the presence and maturity of collaterals, graded according to the Rentrop classification,[7] where grade 0 = no filling of collateral channels; grade 1 = filling of the side branches of the infarct related artery (IRA); grade 2 = Partial filling of the epicardial vessel of the IRA; grade 3 = complete filling of the IRA. For the analysis, patients were divided into those with poor collateral recruitment (Rentrop grade 0 or 1) and robust collateral recruitment (Rentrop grade 2 or 3) as has been done in previous studies.[8,9] The predominant donor vessel, was defined as the epicardial coronary artery which supplied the greatest number of collaterals to the IRA.

Ischemic time was defined as the time from onset of continuous chest pain, to first angiographic image obtained during PCI. This was chosen rather than balloon time, as the end point was the presence of collaterals at the beginning of the case. A chronic total occlusion (CTO) was defined as the presence of a 100% occlusion in a non-IRA, as has been described previously.[10] Prehospital arrest and ventricular arrhythmia during the case was defined as sustained ventricular arrhythmia with loss of cardiac output necessitating cardiopulmonary resuscitation and/or defibrillation. LV impairment was defined as an LV ejection fraction of ≤50%. Project approval by the local human ethics committee was obtained prior to data analysis.

Continuous variables were presented as means (± standard deviation) or as medians and interquartile ranges, if the distribution of data was not normal. Categorical variables were reported as percentages. The baseline characteristics, PCI procedural findings, and in-hospital outcomes were compared between patients with poor versus robust collateral recruitment. Comparisons between groups were performed using Pearson's chi-square test for all categorical variables and a student's *T* Test for all continuous variables normally distributed or the Kuskal-Wallis *H* test for continuous data not distributed normally.

Multivariate logistic regression analyses were performed to determine variables associated with the recruitment of robust collaterals and in-hospital mortality. The multivariate model was built by forward linear regression, with entry and exit criteria set at the $p < 0.10$. Cumulative event rates were calculated according to the Kaplan-Meier method and compared using the log-rank test. Multivariable Cox's proportional hazards regression was performed to identify predictors of outcomes. All tests were 2-sided, and a $p < 0.05$ was considered statistically significant. Analyses were performed using SPSS (v24, IBM, New York, NewYork).

Results

A total of 1,795 patients were identified during the study period, with 110 patients excluded due to previous coronary artery bypass graft, 13 due to spontaneous coronary artery dissection, 21 due to no image acquired of the non-IRA prior to pPCI, 14 due to no culprit lesion identified and 12 facilitated PCIs. Of the remaining 1,625 patients, 1,280 (78.8%) had poor collateral recruitment, whilst 345 (21.2%) had robust collateral recruitment (Figure 1).

Patients' with robust collateral recruitment were younger (63.1 vs 65.1 years, $p < 0.05$), and had a longer ischemic time (628.5 minutes vs 433.1 minutes, $p < 0.0001$). Cardiovascular risk factors were similar, although patients with robust collaterals were less likely to be treated with an angiotensin converting enzyme (ACE) inhibitor or angiotensin receptor blocker (25.9% vs 31.8%, $p < 0.05$) or an aldosterone antagonist (0% vs 1.6%, $p < 0.05$). Patients with robust collaterals were more likely to have the right coronary artery (RCA) as the IRA compared with the left anterior descending artery or left circumflex (35.3% vs 12.1% vs 14.2%, $p < 0.0001$). Other baseline differences are shown in Table 1.

The presence of robust collaterals was associated with lower in-hospital mortality (2.1% vs 7.6%, $p < 0.0001$) and lower rates of LV impairment (37.6% vs 69.2%, $p < 0.0001$) than in those with poor collaterals (Table 2). One hundred and ninety-six patients underwent rescue PCI for failed thrombolysis, and were less likely to have robust collaterals compared with those undergoing pPCI (12.8% vs 22.7%, $p < 0.0001$). In patients undergoing rescue PCI, presence of robust collaterals was associated with lower rates of LV impairment (50% vs 78.9%, $p < 0.002$) but was not

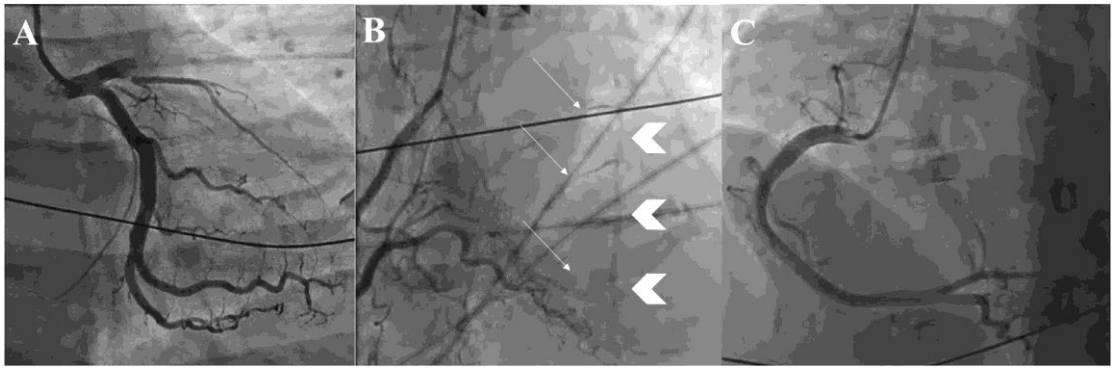

Figure 1. Angiographic appearance of poor and robust collateral recruitment. (A) Coronary angiogram illustrating an occluded left anterior descending artery in the setting of an anterior STEMI. (B) Robust collaterals (Rentrop 2-3) arising from the RCA (thin arrows), opacifying the occluded left anterior descending artery (LAD) in a retrograde manner (solid arrows). (C) No collaterals arising from the RCA in the setting of an anterior STEMI.

Table 1
Baseline characteristics and angiographic findings

Variable	Rentrop 0 or 1 (n=1280)	Rentrop 2 or 3 (n=345)	p value
Age *(years)*	65.1 (± 13.9)	63.1 (± 13.0)	<0.05
Women	23.7%	19.7%	0.13
Body Mass Index *(kg/m^2)*	27.2 (± 4.7)	27.6 (± 4.9)	0.17
Hypertension			
Hypercholesterolemia	46.1%	40.4%	0.07
Smoker	38.7%	35.6%	0.33
Never			0.90
Ex	39.2%	38.9%	
Current	27.6%	27.9%	
Diabetes Mellitus	33.1%	32.1%	
Family History of coronary	16.3%	13.5%	0.26
disease before age 50	27.3%	32.7%	0.06
Ischemic Time *(min)*	433.1 (± 596)	628.5 (± 785)	<0.0001
Medications			
ACE-I/ARB	31.8%	25.9%	<0.05
Beta blockers	13.0%	11.4%	0.45
Statins	22.0%	22.7%	0.77
Aldosterone Antagonist	1.6%	0%	<0.05
Nondyhdropyridine CCB	16.0%	12.4%	0.65
Thrombolysis	13.6%	7.3%	<0.001
Prior angina pectoris	92.9%	91.8%	0.48
Starting Heart Rate (beats/min)	80.0 (±19.8)	78.6 (±18.7)	0.23
Starting Systolic Blood Pressure (mm Hg)	122.4 (± 27.9)	126.9 (± 28.1)	<0.01
Previous stent	10%	10.4%	0.84
Culprit coronary artery			<0.0001
Left anterior descending	674 (87.9%)	93 (12.1%)	
Left circumflex	206 (85.8%)	34 (14.2%)	
Right	400 (64.7%)	218 (35.3%)	
Thrombolysis in Myocardial Infarction Flow >1	39.6%	7.2%	<0.0001
% Stenosis in predominant donor vessel	36.5 (± 23.8)	45.4 (± 25.2)	<0.0001
PCI performed	92.4%	92.5%	1.0
No of stents	1.1 (± 0.6)	1.2 (± 0.7)	<0.05
Length of stent *(mm)*	28.3 (± 14.8)	32.6 (± 17.4)	<0.0001
Presence of Chronic Total Occlusion in non-IRA	5.3%	10.4%	<0.001
Glycoprotein IIb/IIIa Inhibitor use	55.1%	62.6%	<0.05

associated with differences in in-hospital mortality. In those undergoing pPCI, the presence of robust collaterals was associated with lower in-hospital mortality (2.2% vs 7.8%, p < 0.0001) and less LV impairment (36.8% vs 67.2%, p < 0.0001).

In multivariate regression analysis, the predictors of robust collateral recruitment were younger age (OR:0.85 95%CI:0.75 to 0.97, p < 0.05, for every 10 years older), the RCA as the IRA (OR: 3.71 95%CI: 2.64 to 5.22, p < 0.0001) TIMI flow 0 or 1 (OR: 10.36 95%CI:5.72 to 18.78, p < 0.0001, the presence of a CTO in a remote vessel (OR: 2.65 95%CI: 1.61 to 4.37, p < 0.001) and ischemic time (OR: 2.51 95%CI: 1.44 to 4.40, p < 0.001 for every hour delay) (Table 3).

The independent predictors of in-hospital mortality were older age (OR: 1.88 for every 10 years; 95%CI: 1.38 to 2.52, p < 0.0001), presence of diabetes mellitus (OR:2.53; 95%CI: 1.09 to 5.86, p < 0.05), prehospital arrest (OR: 4.59; 95%CI: 2.09 to 10.08 p < 0.0001) and LV impairment (OR: 7.71; 95%CI: 2.30 to 25.86, p < 0.001), whilst collateral recruitment, the presence of a CTO, and gender were not (Supplementary table 1 & 2).

The mean follow-up for those with robust collaterals was 776.8 days and 751.7 days for those with poor collaterals (p = 0.62). The presence of poor collaterals was associated with an increased risk of mortality longer term (HR: 2.1, 95%CI: 1.16 to 3.88, p < 0.05) (Figure 2). However, after adjusting for LV impairment, collateral formation did not remain a predictor of long term mortality (Supplementary Table 3).

Discussion

This is a large scale analysis of the presence and prognostic impact of rapidly recruited collaterals in patients presenting with STEMI. 25% of patients undergoing coronary angiography have the ability to recruit sufficient collaterals during balloon occlusion to prevent ischemia.[11] The predictors of rapid recruitment of coronary collaterals have however, remained uncertain. We found that patients with robust collaterals were younger than those with poorer collaterals, similar to prior studies,[12] possibly due to impaired endothelial nitric oxide synthase pathways and increased oxidative stress with older age.[13]

Table 2
Clinical outcomes

Variable	Rentrop 0 or 1 (n=1280)	Rentrop 2 or 3 (n=345)	p value
Cardiac Arrest	11.0%	11.9%	0.63
Inotropes	13.8%	16.5%	0.23
Ventricular arrhythmia during case	6.5%	4.4%	0.16
Left Ventricular Functional impairment*			<0.0001
None/Mild	766 (62.9%)	282 (83.4%)	
Moderate	347 (28.5%)	43 (12.7%)	
Severe	105 (8.6%)	13 (3.8%)	
Left Ventricular Impairment	843 (69.2%)	127 (37.6%)	<0.0001
In-hospital Mortality	7.6%	2.1%	<0.0001
Intensive Care Unit admission	16.4%	13.2%	0.18
Peak Troponin *(ng/L)*	27,722.5 (+/-6.2)	30,333.3 (+/-5.3)	0.44

* 1,556 patients had assessment of left ventricular function performed.

Table 3
Independent predictors of robust coronary collaterals

Variable	OR	95%CI	p value
Right Coronary Artery	3.71	2.64-5.22	<0.0001
Chronic Total Occlusion in a remote vessel	2.65	1.61-4.37	<0.0001
Thrombolysis in Myocardial Infarction Flow <2	10.36	5.72-18.78	<0.001
Ischemic Time (every hour delay)	2.51	1.44-4.40	<0.001
Age (every 10 years older)	0.85	0.75-0.97	<0.05

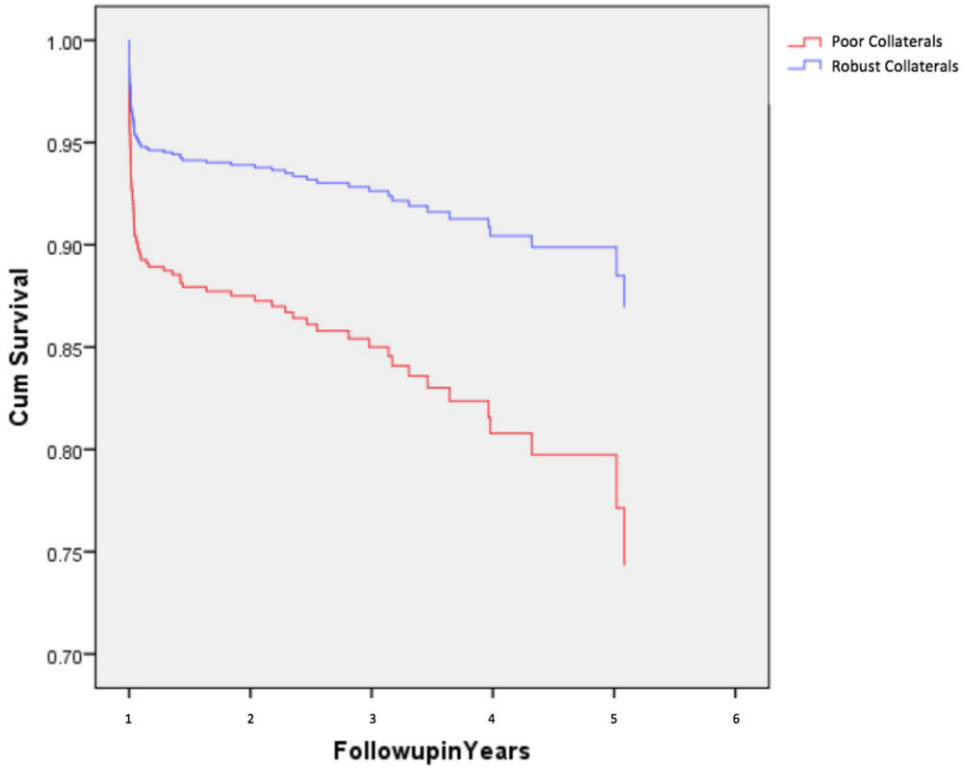

Figure 2. Kaplan Meier curve for survival in patients with robust and poor collaterals.

The recruitment and maturation of coronary collaterals is believed to occur through the process of arteriogenesis, which refers to the transformation of pre-existing arterioles into functional collateral arteries. The primary driver is believed to be an increased pressure gradient and shear stress through these pre-existing channels.[14]

As such, it is unsurprising that in those with robust collaterals, there is a greater ischemic time, which would result in greater shear stress exposure to the immature collaterals. Indeed those with TIMI 2 or 3 flow in the IRA at the onset of angiography were less likely to have robust collaterals, reflecting a reduction in collateral shear stress and flow. Similarly, those with robust collaterals, had a higher initial SBP, which may suggest exposure to higher shear stress.

Robust collaterals were more likely to be seen when the IRA was the RCA, as has been seen in other series.[15] This may reflect the lower resistance myocardium subtended by the RCA, allowing more rapid and robust collateral recruitment. Furthermore, those with a CTO in a remote vessel were more likely to develop robust collaterals. Whilst the hemodynamics and flow undoubtedly contribute to the recruitment of collaterals, certain growth factors have also been implicated in arteriogenesis.[16,17] Conceivably in patients with a CTO, there would be greater levels of these circulating growth factors, which may contribute to more robust collateral recruitment.

In patients with robust collaterals, the donor vessel had a greater degree of luminal stenosis, which is in keeping with previous reports.[18] Whilst previous studies have suggested that a history of angina is a strong predictor of collaterals,[19] we did not see this in our study. This may reflect patient histories of chest pain being unreliable,[20] or else reflect the relatively high incidence of preinfarction angina in our cohort. Furthermore, ischemic preconditioning; the induction of cyclical ischemia to vascular beds around the time of an ischemic insult, has been a proposed mechanism of reducing the degree of infarction.[21] Whilst there have been conflicting reports[22,23] as to whether ischemic preconditioning improves collateral blood flow, it is plausible that the greater degree of donor vessel obstruction, as well as higher rates of CTO may provide preconditioning resulting in improved collateral recruitment.

Both ACE-inhibitors and/or angiotensin receptor blockers and aldosterone antagonist use was associated with poorer collateral recruitment. Previous studies have suggested a negative effect on coronary collateralisation in patients taking ACE-inhibitors,[24,25] possibly due to lower aldosterone levels resulting in lower vascular endothelial growth factor concentrations. Similarly, we found that patients who had robust collaterals were less likely to have received GPIIb/IIIa inhibitors, which although may reflect differing angiographic findings between both groups, may be related to direct platelet effects, whereby activation of platelets has been shown to be associated with poorer collateral flow.[26]

In the present study, robust coronary collaterals were associated with a significantly lower risk of in-hospital mortality and improved myocardial function compared with those with poorer collateral recruitment. This is similar to the findings in other reports,[5,8,9] although Hara et al[6] showed that patients with very robust coronary collaterals (Rentrop grade 3), had a similar mortality rate to those patients without coronary collaterals. The authors argued that this may reflect worse a clinical profile, although there was no benefit observed in their study even after correcting for these confounders. The authors of that study included patients with previous bypass grafts, which may undoubtedly affect shear stress and collateral recruitment and hence interpretation of results. Whilst these results may also reflect ethnic differences between populations, it is possible that the putative advantage of robust collaterals is lost with the greater ischemic time in these patients. Interestingly in our cohort, despite the longer ischemic time seen in patients with robust collaterals, they had improved LV function and lower mortality, suggesting an apparent paradoxical situation in some patients, where greater time of coronary occlusion actually resulted in improved outcomes mediated through robust coronary collateral recruitment. Robust collaterals are associated with less microvascular and endothelial dysfunction,[27] which may provide a mechanism for the improved recovery in ventricular function in this cohort of patients.

The protective effect of acute collateral recruitment seemed to persist following the index event with a maintenance of survival advantage in follow up. We have shown that the ability to recruit collaterals during subsequent infarcts is maintained[28] and hence, it is possible that these patients may maintain a protective effect in the setting of stent thrombosis or repeat infarct. Alternatively, it is possible that the ability to recruit coronary collaterals in the acute setting may extend to recruitment of collaterals in other vascular territories,[29] resulting in a protective mechanism from thrombotic disease elsewhere, which may also improve longer term prognosis. The reasoning for the longer term prognostic impact of robust coronary collaterals requires further investigation.

This study was conducted in a retrospective manner in a single centre, which has inherent limitations. It is possible that some apparent collaterals may not have been visualized due to inadequate time of image acquisition whilst performing angiography of the nonculprit artery during the STEMI. However, robust collaterals are generally seen relatively quickly with a previous study suggesting that collaterals opacify the epicardial vessel in 20 to 30 frames,[30] which in the setting of a cine acquisition of 15 frames per second, does not require prolonged injections and acquisition than standard care.

In this large, detailed analysis of patients presenting with STEMI, the presence of robust collaterals, seen in 20% of patients, was associated with improved LV function. Further research should be undertaken to ascertain the mechanisms by which robust collateral recruitment occurs.

Disclosures

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Authors' Contribution

Usaid K Allahwala: Conceptualization, Methodology, Investigation, Writing - Original Draft; James C Weaver: Methodology, Writing - Review & Editing; Gregory I Nelson: Methodology, Writing - Review & Editing. Daniel Nour: Investigation Max Ray: Investigation Jonathan

L Ciofani: Investigation Michael Ward: Conceptualization; Gemma Figtree; Methodology, Writing - Review & Editing; Peter Hansen; Methodology, Writing - Review & Editing Ravinay Bhindi Methodology, Writing - Review & Editing.

Supplementary materials

Supplementary material associated with this article can be found in the online version at https://doi.org/10.1016/j.amjcard.2020.02.023.

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Supplementary Table 1: Predictors of Mortality

Variable	Died	Alive	p-value
Age (years)	**74.1 (+/- 13.9)**	**64.1 (+/- 13.5)**	**<0.0001**
Women	**36.2%**	**22.0%**	**<0.001**
Body Mass Index (kg/m^2)	**25.8 (+/-4.7)**	**27.3 (+/-4.8)**	**<0.01**
Hypertension	**55.3%**	**44.1%**	**<0.05**
Hypercholesterolemia	42.8%	37.7%	0.32
Smoker			**<0.05**
Current	**18.9%**	**33.8%**	
Former	**28.4%**	**27.9%**	
Never	**52.7%**	**38.4%**	
Diabetes	**26.1%**	**15.1%**	**<0.01**
Family History of CAD before age 50	**6.9%**	**29.8%**	**<0.0001**
Culprit Vessel			**<0.05**
LAD	55.2%	46.6%	
LCx	19.0%	14.5%	
Right	25.7%	38.9%	
Thrombolysis in Myocardial Infarction Flow >1	24.8	33.3%	0.07
Rentrop grade 2 or 3	**6.7%**	**22.3%**	**<0.0001**
Chronic Total Occlusion in remote vessel	**11.4%**	**6.0%**	**<0.05**
Pre-Hospital Arrest	**36.2%**	**9.5%**	**<0.0001**
Inotropes	**39.0%**	**12.7%**	**<0.0001**
Heart Rate (bpm)	**88.3 (+/- 23.5)**	**79.1 (+/-19.2)**	**<0.0001**
Systolic Blood Pressure (mmHg)	**104.3 (+/-31)**	**124.7 (+/-27.3)**	**<0.0001**
Arrhythmia during case	**21.9%**	**4.9%**	**<0.0001**
LV impairment	**94.0%**	**60.5%**	**<0.0001**
Ischemic Time (mins)	476.9 (+/- 667.2)	475.1 (+/-645.1)	0.98
Thrombolysis	8.9%	12.5%	0.28

Supplementary Table 2: Multivariable predictors of In-hospital Mortality

Variable	OR	95% CI	p-value
Age (every 10 years older)	1.88	1.38-2.52	<0.0001
Diabetes	2.53	1.09 - 5.86	<0.05
Pre-Hospital Arrest	4.30	2.09-10.08	<0.0001
LV Impairment	7.71	2.30-25.86	<0.001

* Variables included in the initial model, which were removed (Collateral formation; p=0.22, Female sex; p=0.13, CTO in remote vessel p=0.21)

Supplementary Table 3: Multivariable Predictors of long term mortality

Variable	OR	95% CI	p-value
Age (every 10 years older)	1.71	1.36 – 2.14	<0.0001
Diabetes	3.10	1.68 – 5.69	<0.0001
Pre-hospital arrest	2.17	1.16 – 4.06	<0.05
LV Function			<0.0001
Normal/Mild impairment	1		
Moderate impairment	1.88	0.98 – 3.62	
Severe impairment	7.72	3.95 – 15.1	

Chapter 4: The effect of recurrent ST Elevation myocardial infarction on the ability to recruit acute coronary collaterals

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ORIGINAL ARTICLE

Spontaneous coronary collateral recruitment in patients with recurrent ST elevation myocardial infarction (STEMI)

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Abstract
The spontaneous recruitment of acute coronary collaterals in the setting of an ST elevation myocardial infarction (STEMI) is seen frequently in those patients undergoing primary percutaneous coronary intervention (pPCI) and is associated with improved clinical outcomes. However, it is unknown whether in patients who present with a recurrent STEMI, the degree of collateral recruitment remains the same as in the index procedure. We reviewed all patients presenting to our tertiary centre with a STEMI undergoing primary or rescue percutaneous coronary intervention (PCI) from July 2010 until December 2018. We identified patients who presented with a recurrent STEMI following their index procedure. We defined patients with poor collateral recruitment as Rentrop grade 0 or 1, whilst patients with robust collateral recruitment as Rentrop grade 2 or 3. Of the 1795 patients who were identified, there were 27 cases in 25 patients who presented with a repeat STEMI following their index procedure. The median time between cases was 12.8 days (IQR 2.3–589.5 days). Compared to the index case, there was no statistically significant difference in the degree of collateral recruitment in recurrent presentations ($Z = -0.378$, $p = 0.70$). In those patients presenting more than 6 months following the index procedure, the median time between cases was 654.5 days (IQR 479.5–1151.9). There was no difference in the degree of collateral recruitment in recurrent presentations ($Z = 0.000$, $p = 1.0$). Cases which had poorer collateral recruitment in recurrent presentations were less likely to be current smokers (0% vs 50%, $p < 0.001$) and less likely to have diabetes (0% vs 27.3%, $p < 0.05$) The recruitment of spontaneous coronary collaterals remains constant in recurrent STEMI presentations suggesting an innate biological process rather than merely a manifestation of alteration of haemodynamic blood flow. Further investigations to identify these processes is required.

Keywords Collaterals · Coronary collaterals · STEMI · Acute coronary syndrome · Rentrop

Introduction

Coronary collaterals are preformed anastomotic channels which connect the territory supplied by one epicardial coronary artery with that supplied by another, which have the ability to rapidly develop and increase in size and function [1]. These collaterals provide an alternate blood supply source to the myocardium subtended by the occluded vessel.

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Previous studies have suggested that the rates of spontaneously recruited collaterals during an ST elevation myocardial infarction (STEMI) to be in the region of 20–30%, and have been associated with improved outcomes in some [2–6] observational studies but not all have agreed [7–11]. Similarly, in the experimental setting, coronary collaterals may be rapidly recruited in up to 25% of patients undergoing coronary angiography [12].

There have been no studies to date assessing recruitment of spontaneous collaterals in patients presenting with recurrent STEMI, in particular whether the degree of collateral formation during the first presentation is similar (or different) to the degree of collaterals during a recurrent STEMI. We sought to determine whether the ability to recruit collaterals in the acute setting during a STEMI was constant in patients who presented with multiple episodes of coronary artery occlusion.

Methods

We reviewed all patients presenting to our tertiary centre with a diagnosis of STEMI, undergoing primary percutaneous coronary intervention (pPCI) or rescue PCI following unsuccessful thrombolysis, from July 2010 until December 2018. Within this cohort, we identified patients who had presented on more than one occasion for repeat pPCI for a recurrent STEMI. We included patients with stent thrombosis, using previously accepted criteria [13] as well as patients with de novo lesions in the same, or remote artery. Patients with prior coronary artery bypass graft (CABG) or a subsequent CABG following their first presentation or a final diagnosis of spontaneous coronary artery dissection (SCAD) were excluded. Approval by the local ethics committee was obtained prior to investigation.

Analysis of coronary angiography was performed to determine the presence and maturity of collaterals, graded according to the Rentrop classification [14], where grade 0 = no filling of any collateral channel; grade 1 = filling of the side branches of the infarct-related artery; grade 2 = partial filling of the epicardial vessel of the infarct-related artery and grade 3 = complete filling of the epicardial vessel. Poor collateral recruitment was defined as either Rentrop grade 0 or 1 whilst robust collateral recruitment was defined as Rentrop grade 2 or 3, as has been done in previous studies [4, 8, 10, 11, 15–18]. We also classified the collaterals by the collateral connection (CC) grade [19], whereby GG grade 0 = no continuous connection, CC grade 1: threadlike continuous connection and CC grade 2 = side branch like connection. The CC grade has been found to closely correlate with invasively determined collateral haemodynamics. Patients with CC grade 2 were considered large collateral connections, whilst CC grade 0 or 1 were considered small collateral connections, as has been previously described [17].

Statistical analysis

Continuous data were presented as mean (± standard deviation), whilst categorical data were presented as number (%) in the setting of normal distribution. Comparison between the index presentation and recurrent presentations with respect to the degree of acute collateral recruitment was performed by the non-parametric Wilcoxon signed-rank test. Comparison of characteristics between patients with the same degree of collateral formation during recurrent presentations and those with poorer collateral recruitment were performed using Pearson's Chi square test for all categorical variables and the student's *T* test for all continuous variables.

All tests were two-sided, and a $p < 0.05$ was considered statistically significant. All analyses were performed using SPSS (version 24, IBM, New York, NY).

Results

A total of 1795 patients presenting with a STEMI who underwent pPCI were identified during the study period. Of these, there were 27 repeat presentations in 25 patients. The mean age was 65.4 (± 12.8) with 24% females.

Of the repeat presentations, 22 cases (81.5%) had the same infarct related artery, whilst 5 (18.5%) cases were in a remote vessel. Of the 22 cases involving the same IRA as the index case, 13 (59.1%) were definite stent thrombosis (Fig. 1). Thirteen (59.1%) cases were repeat occlusion of the right coronary artery (RCA), whilst 9 (37.5%) were repeat occlusion of the left anterior descending artery (LAD). The median time between repeat presentations was 12.8 days (IQR 2.3–589.5 days) (Figs. 2, 3 illustrate 2 patient examples).

In the index cases, 9 (33.3%) had robust recruitment of collaterals, whilst 18 (66.7%) had poor collateral recruitment. Compared to the index case, 20 cases had the same degree of collateral recruitment, 4 cases had improved collateral recruitment, whilst 3 had poorer collateral recruitment. A non-parametric Wilcoxon signed-rank test showed that there was no statistically significant difference in the degree of collateral recruitment in recurrent presentations ($Z = -0.378$, $p = 0.70$). Eleven cases (40.7%) occurred 6 months following the index presentation, with a median time between cases of 654.5 days (IQR 479.5–1151.9). There was no difference in the degree of collateral recruitment in recurrent presentations ($Z = 0.000$, $p = 1.0$).

When assessing degree of collateralisation based on the 4 different Rentrop grades, at the index procedure, 14 (51.8%) had Rentrop grade 0, 4 (14.8%) had Rentrop grade 1 whilst 9 (33.3%) had Rentrop grade 2. No patients had grade 3 collaterals. Similarly, there was no difference in the degree

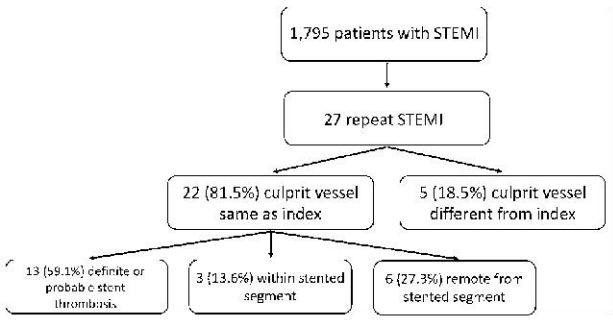

Fig. 1 Flow chart of included patient encounters

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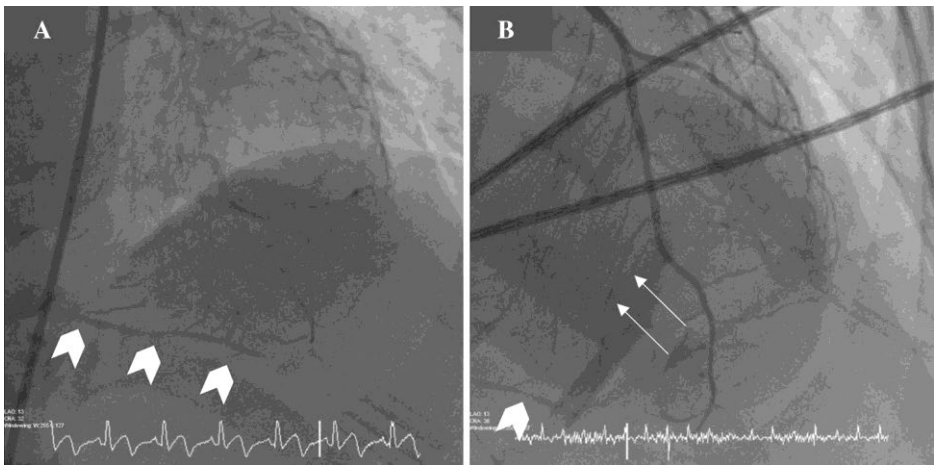

Fig. 2 Inferior STEMI with collaterals from the LAD

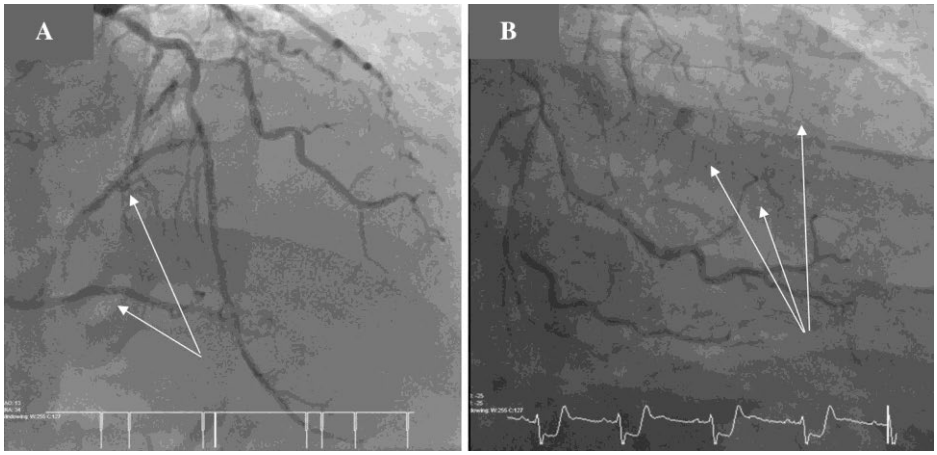

Fig. 3 Inferior STEMI and subsequent anterior STEMI in the same patient

of collateral formation in patents in recurrent presentations ($Z=0.000$, $p=1.0$) with 16 patients having the same degree of collateralisation, 6 with poorer collateralisation and 5 with improved collateralisation.

In patients presenting with the same culprit vessel during the secondary presentation with STEMI, 3 patients (13.6%) had poorer collateral recruitment compared to their initial presentation, with no improvement in the degree of collateral recruitment. In patients presenting with a different culprit vessel in their recurrent STEMI, 4 patients (80%) had a different degree of collateral recruitment, 3 better and one worse than the index procedure, which was statistically significant ($p<0.05$).

In cases where the recurrent presentation resulted in poorer collateral recruitment compared with the index procedure, there was a trend toward an older age (80.0 vs 64.3, $p=0.34$), more likely to be females (33.3% vs 8.3%, $p=0.54$) and higher rates of TIMI 2 or 3 flow in the infarct related artery (IRA) prior to intervention (66.7% vs 20.8%, $p=0.31$). Patients with poorer collateral recruitment in recurrent presentations were less likely to be current smokers (0% vs 50%, $p<0.001$) and less likely to have diabetes (0% vs 27.3%, $p<0.05$), although there were no differences between other cardiovascular risk factors. The RCA was less likely to be the IRA in patients with poorer collateral recruitment in recurrent presentations (56.5% vs 0%, $p<0.0001$).

With respect to CC grade, of the 27 cases, at index, 4 cases (14.0.8%) has large calibre collateral connections, whilst 23 cases (85.2%) has small calibre collateral connections (17 with CC 0 and 6 with CC 1). Compared to the index procedure, 23 cases had the same calibre of collateral connections, 1 patient has a less calibre of collateral connections, whilst 3 had a larger calibre of collateral connections. A non-parametric Wilcoxon signed-rank test showed that there was no statistically significant difference in the calibre of collateral connections in recurrent presentations ($Z=-1.0$, $p=0.32$).

Discussion

This is the first study to date, to assess the recruitment of collaterals in patients presenting with recurrent STEMI. In a study by Lee et al. [20] 97 patients with either an acute coronary syndrome (ACS) and near or total coronary occlusion, or a chronic total occlusion (CTO), had comprehensive coronary physiology performed prior to, and following revascularisation to determine the extent of coronary collaterals. They found that 43.5% of patients with an ACS had a pressure derived collateral pressure index (CPI) of ≥ 0.25, the threshold for ischaemia, whilst 74.3% of patients with a CTO had a CPI of ≥ 0.25. The rate of recruitable collaterals sufficient to prevent ischaemia reduced to 65% following PCI, and fell to 8.8% at 1 year follow up. Whilst there was no comment on the degree of visualised and recruitable collaterals, it is in keeping with the findings of our present study, whereby repeated occlusions of a vessel results in recruitment of collaterals. The relatively low rate seen in this study may reflect the short duration of coronary occlusion at 2 min, as well as only including patients with a CPI high enough to prevent ischaemia, which may not correlate with the Rentrop classification [19]. In a study by Werner et al. [21], in the setting of CTO recanalisation, they found that in the case of spontaneous reoclusion, the degree of invasively determined collateral flow was of a similar in degree to that prior to revascularisation. However, in those patients who did not have spontaneous re-occlusion, experimental balloon occlusion did not yield a significant recruitment of collaterals in the majority of patients, with only 18% being able to recruit sufficient collaterals to prevent ischaemia. Those with larger calibre collaterals (CC grade 2) were more likely to retain collateral function. Whilst comparing collateral recruitment in the acute setting following CTO revascularisation represents a different clinical scenario to that which we studied in this study, it does suggest the innate ability of collaterals to be rapidly recruited.

The acute recruitment of coronary collaterals is commonly seen in patients undergoing pPCI in the setting of a STEMI. In this cohort, we found that 1/3rd of STEMI cases had recruited robust coronary collaterals, which was slightly higher than previously published reports of 20–23% [4, 22]. Of these patients, we have shown for the first time, that in those with a repeat STEMI presentation, the majority of cases are able to recruit the same degree of collaterals, suggesting this process is an innate and specifically related biological processes rather than merely a manifestation of alteration of haemodynamic blood flow. Whilst the Rentrop classification is based upon the ability of collaterals to perfuse the occluded vessel, the collateral connection grade is specifically adjudicated on the size of the collateral vessel itself and may be a more specific score about the collateral physiology and maturation. We found that the CC grade was similar in repeat STEMI presentations, suggesting that the collateral vessel architecture and size is stable in the acute setting. Previous studies [23] have suggested that poorer CC grade is associated with worse endothelial and smooth muscle cell dysfunction suggesting the ability to recruit differing calibre collaterals is a manifestation of microvascular function and likely to be reproducible in the acute setting. We found that regardless of the initial size of the collaterals, the ability to recruit a similar size of collaterals was preserved, that is irrespective of the CC grade, repeat collateral recruitment was similar.

The recruitment and maturation of coronary collaterals occurs through the progression of arteriogenesis, whereby pre-existing arterioles are transformed into functional collateral arteries with acquisition of viscoelastic and vasomotor properties [24]. The primary driver is believed to be an increased pressure gradient and shear stress through these pre-existing channels [25], as occurs following coronary artery occlusion. However, as in our case, the majority of patients do not recruit these collaterals, despite an occluded artery, and presumably increased flow through these immature arterioles.

In those patients in whom repeat presentations with STEMI were associated with poorer collateral recruitment, we found they were less likely to be smokers, i.e. smoking appeared to provide a protective effect by improving the degree of coronary collateral recruitment. Previous studies have suggested that smoking may be associated with improved coronary collateral formation [26], believed to be a manifestation of the effects of chronic hypoxia and endothelial dysfunction [27]; however, we have previously shown that in clinical practice, smoking did not improve collateral recruitment [28]. However, whilst hypothesis generating, a definitive determination of the effect of smoking requires assessment in larger numbers.

We also found that patients with poorer subsequent collateral formation were less likely to have diabetes mellitus, suggesting a beneficial effect on coronary collateral recruitment in those with diabetes. Previous studies [29] have similarly suggested that those with diabetes are more likely to develop coronary collaterals that those without, possibly due to the chronic degree of subclinical hypoxia and ischaemia which may promote their formation.

Furthermore, robust collaterals were more likely to be seen when the infarct-related artery was the right coronary artery, as has been seen in other series [15]. This may reflect the lower resistance myocardium subtended by the RCA, allowing more rapid and robust collateral recruitment.

The use of drug-eluting stents has been implicated in the impairment of collateral recruitment and function [30],

although this was not seen in our study. The majority of patients in our study were treated with a drug-eluting stent, rather than a bare metal stent, and hence further assessment as to whether the local release of the antiproliferative drug may impact on vasomotion and recruitment is necessary.

Limitations

Whilst this retrospective, single-centre review, with relatively small numbers, has with it inherent limitations, this is the first study to systematically review patients to determine the degree of acute collateral recruitment in repeated STEMI presentations. The findings have implications with respect to the underlying driver of collateral recruitment which may have therapeutic implications in the future. Furthermore, the relatively small samples size was insufficient to detect effects on clinical outcomes.

Conclusion

The early and acute recruitment of coronary collaterals is seen commonly in patients presenting with STEMI. In those with a repeat STEMI presentation, the degree of collateral recruitment is similar to that of their index presentation, suggesting inherent biological processes are the drivers of their recruitment rather than merely a manifestation of ischaemia, hypoxia and increased shear stress. However this appears limited to repeat STEMI with the same culprit vessel, suggesting local resistance and capacitance factors also likely play a large role in collateral recruitment. Further research to delineate these mechanisms is required.

Compliance with ethical standards

Conflicts of interest All authors declare that they have no conflict of interest.

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Chapter 5: Prognostic implications of the rapid recruitment of coronary collaterals during ST Elevation Myocardial Infarction (STEMI): A meta-analysis of over 14,000 patients

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Prognostic implications of the rapid recruitment of coronary collaterals during ST elevation myocardial infarction (STEMI): a meta-analysis of over 14,000 patients

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Abstract
Acute coronary collateralisation of an infarct-related arterial (IRA) territory may be identified during angiography for ST elevation myocardial infarction (STEMI). Whether the presence or absence of these collaterals affects outcomes remains uncertain. A search of EMBASE, MEDLINE and Cochrane Library, using the Preferred Reporting Items for Systematic reviews and Meta-Analysis (PRISMA) guidelines was conducted to identify studies which reported on the association between coronary collaterals and in-hospital and longer term mortality, left ventricular ejection fraction (LVEF), risk of repeat acute myocardial infarction (AMI) and repeat revascularisation. Patients with Rentrop grade 0 or 1 were defined as poor collaterals whilst those with Rentrop grade two or three were defined as those with robust collaterals. Studies were eligible if they included patients ≥ 18 years of age who had immediate coronary angiography for STEMI. Included studies were observational which recorded the degree of collateral blood flow to the IRA. Two investigators reviewed all citations using a predefined protocol with final consensus for all studies, the data from which was then independently entered to ensure fidelity of results. Inverse variance random effects model for the meta-analysis along with risk of bias assessment was performed. 20 studies with a total of 14,608 patients were identified and included in the analysis. Patients with robust collaterals had lower mortality (OR 0.55, 95% CI 0.48–0.64), both in-hospital (OR 0.47, 95% CI 0.35–0.63) and longer term (OR 0.58, 95% CI 0.46–0.75). Patients with robust collaterals also had a higher mean LVEF (SMD 0.23, 95% CI 0.10–0.37). There was no difference in the rates of AMI or repeat revascularisation between patients with robust or poor collaterals. The presence of robust collaterals during STEMI is associated with reduced in-hospital and longer term mortality and improved left ventricular function. These findings have implications for prognostication and identifying patients who require close monitoring following STEMI.

Keywords Coronary collaterals · Rentrop · STEMI · Collateral

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Highlights

- The presence of robust collaterals, visualised during ST elevation myocardial infarction (STEMI), perfusing the territory supplied by the occluded vessel, reduce risk of all-cause mortality.
- The presence of robust collaterals visualised during a STEMI reduce both short and long term mortality.
- The presence of robust collaterals visualised during a STEMI is associated with higher LVEF.
- The degree of collaterals does not predict risk of AMI or revascularisation.
- The molecular and cell signalling pathways by which collaterals are recruited should be further investigated.

Introduction

During angiography for percutaneous coronary intervention (PCI) in the setting of ST elevation myocardial infarction (STEMI), the finding of coronary collaterals, perfusing the territory distal to the occluded, infarct related artery (IRA), is frequently identified. Whilst the presence of a bystander chronic total occlusion (CTO), with collaterals perfusing the pre-existing CTO is a well described predictor of poorer outcomes [1], the rapid recruitment of collaterals to perfuse the acutely occluded IRA is a separate and distinct entity. Whether the presence of these collaterals is associated with improved prognosis remains uncertain. We performed a systematic review and meta-analysis to examine the impact of rapidly recruited coronary collaterals on risk of mortality, left ventricular function, recurrent acute myocardial infarction (AMI) and repeat revascularisation, in patients presenting with STEMI.

Methods

Search strategy

We followed the Preferred Reporting Items for Systematic reviews and Meta-Analysis (PRISMA) guidelines to formulate and conduct the search strategy [2]. We performed a computerised systematic search through MEDLINE, EMBASE, and the Cochrane Library databases. The search was conducted in MEDLINE from 1946–January 10th 2020, EMBASE from 1974–January 10th 2020 and Cochrane Library from 2003–January 10th 2020 and then repeated until March 15th 2020 to identify newer published data. All relevant subject headings as well as free text terms relating to coronary collaterals and STEMI were used. The keywords were searched as text words as well as exploded medical subject headings when feasible. The search strategy for MEDLINE was as follows; (1) Collateral Circulation/(12,137), (2) collateral*.mp. (45,969), (3) Rentrop.mp. (303), (4) CCS.mp (5746), (5) Collateral Connection Score.mp. (3099), (6) 1 or 2 or 3 or 4 or 5 (51,682), (7) ST Elevation Myocardial Infarction/ (3099), (8) STEMI.mp. (10,410), (9) 7 or 8, (10) 6 and 9 (120). The search strategy for EMBASE was (1) Collateral circulation/ (11,049), (2) collateral artery/(409), (3) coronary artery collateral circulation/(1756), (4) collateral*.mp. (56,811), (5) Rentrop.mp. (569), (6) CCS.mp. (9630), (7) Collateral Connection Score.mp. (5), (8) 1 or 2 or 3 or 4 or 5 or 6 or 7 (66,357), (9) ST segment elevation myocardial infarction/ (36,557), (10) STEMI.mp. (27,144), (11) 9 or 10 (41,450), (12) 8 and 11 (434). The search strategy is shown in Appendix 1. We further screened references from the retrieved and included studies as well as prior meta-analyses for any relevant studies which were not retrieved through the initial search.

Data source and searching

Articles were eligible for inclusion if they included patients ≥ 18 years of age who had immediate coronary angiography for STEMI, within 12 h of onset of chest pain or if evidence of ongoing ischaemia. Eligible study designs were ones which recorded the degree of collateral blood flow to the IRA prior to intervention. To standardise the degree of collaterals, we included studies, which quantified the collaterals based upon the Rentrop classification [3], where grade 0 = no filling of any collateral channel; Grade 1 = filling of the side branches of the infarct related artery; Grade 2 = Partial filling of the epicardial vessel of the infarct related artery' Grade 3 = complete filling of the epicardial vessel. For the analysis, we grouped patients with Rentrop grade zero or one as poor collateral recruiters, whilst those with Rentrop grade two or three were classified as robust collateral recruiters, a dichotomy which was done in the majority of previously published studies, as this corresponds with the degree of perfusion of the occluded vessel.

Two investigators (U.A., D.N.) reviewed all citations identified through the literature search using a predefined protocol. Articles that clearly did not meet inclusion criteria were excluded at the title and/or abstract level. The remaining articles were selected for full text review. When limited information was available from the abstract, full text was always obtained. If further details were sought, the corresponding authors of the study were contacted to obtain further information. Disagreements regarding the selection and quality assessment of articles were resolved through discussion, and full consensus was achieved at each stage of review. Both investigators (U.A., D.N.) also independently

entered results and outcomes into separate predetermined tables to ensure fidelity of results. Ethics approval was not deemed necessary given this was a meta-analysis of previously published data.

The outcomes of interest were all-cause mortality, left ventricular function, risk of recurrent AMI and need for repeat revascularisation. We further classified time course of mortality as in-hospital and short term, defined as ≤ 6 months follow up, or longer term mortality, defined as ≥ 12 months follow up. The Newcastle-Ottawa Scale (NOS) [4] was used to assess the quality of observational studies and risk of bias. The overall quality of the study was determined semi-quantitatively using the Agency for Healthcare Research and Quality (AHRQ) standards [5], whereby studies were graded of good quality, fair quality or poor quality, based upon scoring of the NOS.

Data analysis

For dichotomous outcomes, odds ratio was chosen to present data, whilst for continuous variables, the standardised mean difference (SMD) was used to present data. For continuous variables, the mean and standard deviation was recorded. If studies only presented medians and interquartile ranges, the data was transformed into mean with standard deviations using previously validated methods [6]. The program Review Manager (version 5.3) was used to conduct an inverse variance random effects model for the meta-analysis. The Cochrane Q-statistic (I^2) was used to assess the consistency among studies, with $I^2 < 25\%$ considered low, $I^2 > 50\%$ moderate, and $I^2 > 75\%$ high heterogeneity [7]. Publication bias was estimated visually by funnel plots, where publication bias was considered unlikely if the plot resembles an inverted funnel, and was further tested by using the weighted regression test of Egger [7].

Results

After removing duplicate studies, a total of 554 studies were screened of which, a final 20 studies with 14,608 patients were included in the analysis (Fig. 1). Twelve of the studies [8–19] were prospective observational studies, 6 [20–25] were retrospective observational, and 2 [26, 27] were retrospective analysis of randomised control trials of patients presenting with STEMI. One study included only patients ≥ 65 years [21], two included only patients with an anterior STEMI, whereby the left anterior descending artery was the IRA [14, 24], one included only patients with inferior STEMI [19] and one included only patients with cardiogenic shock complicating STEMI [18]. Included studies reported on rapidly recruited collaterals, perfusing the territory supplied by the IRA, distinct to preformed collaterals perfusing an existing CTO. All but two studies reported the degree of collateralisation using the Rentrop or equivalent scoring tool. In the study by Alsanjari et al. [8], collaterals were graded as "collaterals" or "no collaterals". Given the fact that patients with visible collaterals would almost certainly be Rentrop grade two or three, we included this group as robust collateral recruiters whilst those without collaterals were defined as poor collateral recruiters. In the study by Wang et al. [24], patients were grouped as those with no collaterals, defined as Rentrop grade zero, and those with collaterals, which were defined as Rentrop grade 1–3, of which 77% were grade one. For the purposes of this analysis, we characterised patients with visible collaterals as having robust collaterals. Two studies [8, 11] reported outcomes at 6 months, and longer term, with time specific data included in the analysis models.

Included studies were published between 1998 to 2020 with follow up periods between in-hospital alone to 5 years. The number of patients included in each study was between 96 to 3,340 with a mean age of 62.3. The percentage of patients with robust collaterals ranged between 10% and 41.3% with an average of 25.0% across all the studies. The thrombolysis in myocardial infarction (TIMI) flow of 0 in the infarct related artery at the time of angiography, whereby there is complete, persisting occlusion of the IRA, and is a strong predictor of collateral recruitment [20], ranged between 67.3% and 100%. The results of each study included in the analysis are summarised in Table 1. The NOS score for each study is presented in Supplementary Table 1, with 12 studies of a good quality, two fair quality and 6 of a poor quality.

Mortality

Patients with robust collateral recruitment had a lower risk of mortality compared to those with poor collateral recruitment (OR 0.55, 95% CI 0.48–0.64) throughout all included studies, with a very low degree of heterogeneity between studies ($I^2 = 17\%$, $p = 0.24$) (Fig. 2). Asymmetrical appearance of the funnel plot suggested that smaller studies, which do not show a significant mortality difference, were unpublished. Publication bias was supported by Eggers regression analysis ($p < 0.01$).

In the 11 studies assessing in-hospital and short term mortality, there was a lower rate of mortality in those patients with robust collaterals compared to those with poor collaterals, (OR 0.47, 95%CI 0.35–0.63) (Fig. 3). There was a low degree of heterogeneity between these studies ($I^2 = 23\%$, $p = 0.22$). Publication bias was suggested by both an asymmetrical appearance of the funnel plot, whereby smaller studies which do not show a significant mortality difference were unpublished, and Eggers regression analysis ($p < 0.01$).

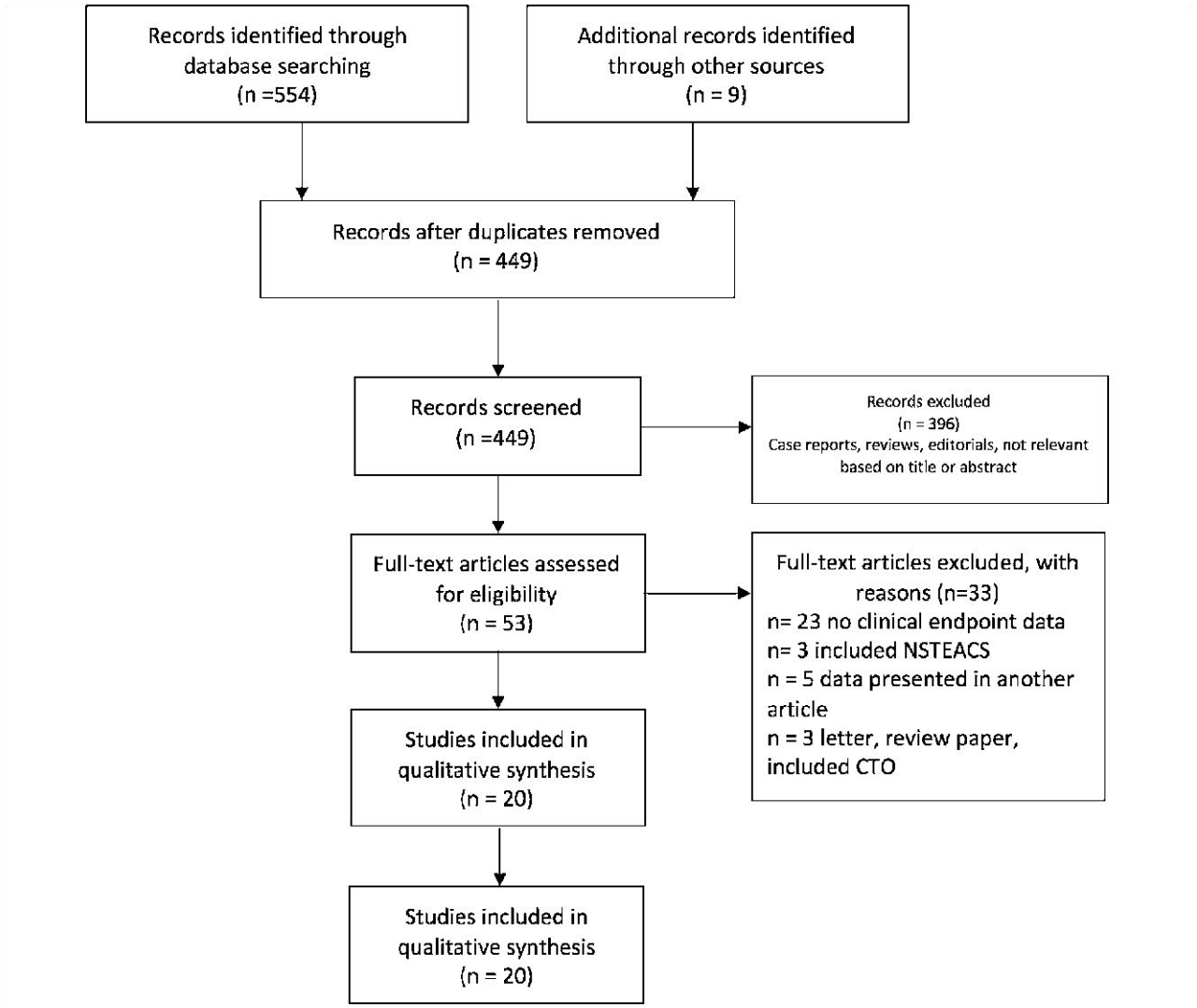

Fig. 1 Flow diagram of search strategy

In the 11 studies assessing longer term mortality, patients with robust collateral recruitment had a lower risk of mortality compared to patients with poor collateral recruitment (OR 0.58, 95% CI 0.46–0.75) (Fig. 4). In these studies, there was a very low degree of heterogeneity (I^2 = 22%, p = 0.23), whilst there was no evidence of publication bias, with a symmetrical appearance of the funnel plot and Eggers regression analysis (p = 0.13).

As the study of Alsanjari et al. [8] and Hara et al. [11] accounted for 65.3% of the weighted analysis for mortality, sensitivity analysis was performed excluding these 2 heavily weighted studies. After their exclusion, patients with robust collateral recruitment had a lower risk of mortality compared to those with poor collateral recruitment, (OR 0.40, 95% CI 0.31–0.52, p < 0.0001) with no evidence of heterogeneity (I^2 = 0%, p = 0.58) and no evidence of publication bias, with a symmetrical appearance of the funnel plot and by Eggers regression analysis (p = 0.35). Similarly, patients with robust collaterals had a lower risk of in-hospital and short term mortality compared to those with poor collaterals (OR 0.36, 95% CI 0.26–051,p < 0.0001) with no evidence of heterogeneity (I^2 = 0%, p = 0.94) or evidence of publication bias with a symmetrical appearance of the funnel plot and by Eggers regression analysis (p = 0.47). Patients with robust collaterals had a lower risk of longer term mortality compared to those with poor collaterals (OR 0.47, 95% CI 0.28–0.78, p < 0.01) with low degree of heterogeneity (I^2 = 27%, p = 0.20) and no evidence of publication bias, with a symmetrical appearance of the funnel plot and by Eggers regression (p = 0.34) (Supplementary Table 2).

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Table 1 Included studies in meta-analysis

Paper	Year	Patients	Robust collaterals	Rentrop grade	Study type	Follow up	% Females	Age	LAD IRA	TIMI 0 flow in IRA	NOS quality
Allahwala	2020	1625	345 (21.2%)	Yes	Retrospective observational	In-Hospital	371 (22.8%)	64.7	767 (47.2%)	1093 (67.3%)*	8 Good
Alsanjari	2019	1944	322 (17%)	No	Prospective observational	In-hospital & 5yrs	469 (24.1%)	65.1	748 (38.5%)	1783 (91.7%)	8 Good
Antoniucci	2002	1164	264 (23%)	Yes	Prospective observational	6 months	158 (22.1%)	63.8	588 (50.5%)	950 (81.6%)*	8 Good
Chu	2019	346	108 (31.2%	Yes	Retrospective observational	12 months	72 (20.8%)	67.7	156 (45.1%)	N/A	7 Good
Desch	2010	235	69 (29.4%)	Yes	Prospective observational	2.2 years	58 (24.7%)	65	115 (48.9%)	208 (88.5%)	7 Good
Elsman	2004	1059	106 (10%)	Yes	Retrospective observational	12 months	205 (19.4%)	59.4	509 (48.1%)	1059 (100%)*	7 Poor
Freund	2020	95	33 (34.7%)	Yes	Retrospective analysis of randomised control trial	4 years	26 (27.4%)	66	38 (40%)	90 (94.7%)	5 Fair
Hara	2016	3340	770 (23%)	Yes	Prospective observational	In-hospital & 5yrs	772 (23.1%)	65	1470 (44%)	3340 (100%)	8 Good
Hernandez	2017	947	212 (22%)	Yes	Retrospective observational	2.4 years	186 (19.6%)	60.8	363 (38.4%)	880 (92.9%)	8 Good
Kajiya	2014	96	33 (34.4%)	Yes	Prospective observational	4 years	31 (32.3%)	68.5	19 (29.7%)	N/A	5 Poor
Kim	2006	247	54 (21.9%)	Yes	Prospective observational	12 months	50 (20.2%)	59	118 (47.8%)	228 (92.3%)	5 Fair
Perez-castellano	1998	180	32 (17.8%)	Equivalent	Prospective observational	In-Hospital	33 (18.3%)	64	180 (100%)	205 (86%)	6 Good
Rechciński	2013	330	78 (23.6%)	Yes	Prospective observational	2 years	97 (29.4%)	56.2	122(37.0%)	217 (67.8%)	7 Poor
Sen	2017	1375	278 (20.2%)	Yes	Prospective observational	30 days	327 (23.8%)	57.4	573 (41.7%)	1375 (100%)*	8 Good
Shen	2014	389	60 (15.4%)	Yes	Prospective observational	6 months	74 (19%)	63.6	197 (50.6%)	389 (100%)*	8 Good
Sorajja	2007	318	119 (37.4%)	Yes	Retrospective analysis of randomised control trial	30 day & 6 months	65 (20.4%)	60	120 (37.7%)	272 (85.6%)	6 Poor
Valim	2011	105	22 (20.9%)	Yes	Prospective observational	In-hospital	36 (34.3%)	64.2	N/A	N/A	2 Poor
Wang	2011	189	78 (41.3%)	No	Retrospective observational	12 months	32 (16.9%)	56.2	189 (100%)	N/A	6 Poor
Yaylak	2015	235	88 (37.4%)	Yes	Prospective observational	In-hospital	45 (19.1%)	55	0 (0%)	210 (89.4%)	6 Good

Table 1 (continued)

Paper	Year	Patients	Robust collaterals	Rentrop grade	Study type	Follow up	% Females	Age	LAD IRA	TIMI 0 flow in IRA	NOS quality
Ying	2014	389	60 (15.4%)	Yes	Retrospective observational	6 months	74 (19.0%)	63.6	197 (50.6%)	N/A	8 Good

*TIMI 0 or 1 flow

IRA infarct related artery, *LAD* left anterior descending artery, *N/A* not applicable, *NOS* newcastle ottawa scale, *TIMI* thrombolysis in myocardial infarction. See supplementary Table 1 for complete newcastle ottawa scale for each study

Left ventricular function

Four studies reported on rates of left ventricular impairment, which was defined as < 50% in three studies [8, 13, 20] and not defined in another [18].There was no difference in rates of left ventricular impairment between those with robust collateral recruitment compared to those with poor collateral recruitment (OR 0.69, 95% CI 0.29–1.66) There was, however, a high degree of heterogeneity between studies ($I^2 = 93\%$, $p < 0.0001$) with no evidence of publication bias, with a symmetrical appearance of the funnel plot and by Eggers regression ($p = 0.12$) (Fig. 5). A total of 13 studies reported mean left ventricular ejection fractions (LVEF), which was performed in-hospital in 11 studies [10, 15, 16, 19, 20, 22, 23, 25–28], and 6 months in two studies [9, 17]. Patients with robust collaterals had a significantly higher standard mean LVEF compared to those with poor collaterals (standard mean difference (SMD) 0.23, 95% CI 0.10–0.37). There was however a high degree of heterogeneity between studies ($I^2 = 81\%$, $p < 0.0001$) with no evidence of publication bias, with a symmetrical appearance of the funnel plot and by Eggers regression ($p = 0.41$) (Fig. 6).

AMI and repeat revascularisation

Ten studies analysed the effect of collateral recruitment on risk of recurrent AMI. There was no difference between patients with robust collaterals or poor collaterals on risk of AMI (OR 0.82, 95% CI 0.50–1.34). There was very low heterogeneity between studies ($I^2 = 0\%$, $p = 0.94$) with no evidence of publication bias, with a symmetrical appearance of the funnel plot and by Eggers regression ($p = 0.06$) (Fig. 7).

Seven studies analysed the effect of collateral recruitment on risk of repeat revascularisation. There was no difference between patients with robust collaterals or poor collaterals on risk of repeat revascularisation (OR 0.99, 95% CI 0.73–1.34). There was very low heterogeneity between studies ($I^2 = 0\%$, $p = 0.57$) with no evidence of publication bias, with a symmetrical appearance of the funnel plot and by Eggers regression ($p = 0.15$) (Fig. 8).

Discussion

In this meta-analysis of 20 studies with more than 14,500 patients, we found that in those presenting with STEMI, the angiographic appearance of robust collaterals is associated with lower mortality, both in the short term and longer term, with an associated higher LVEF. More robust coronary collaterals, with partial, or complete retrograde perfusion of the occluded epicardial artery, allow oxygenated blood to perfuse the myocardial territory subtended by the IRA.

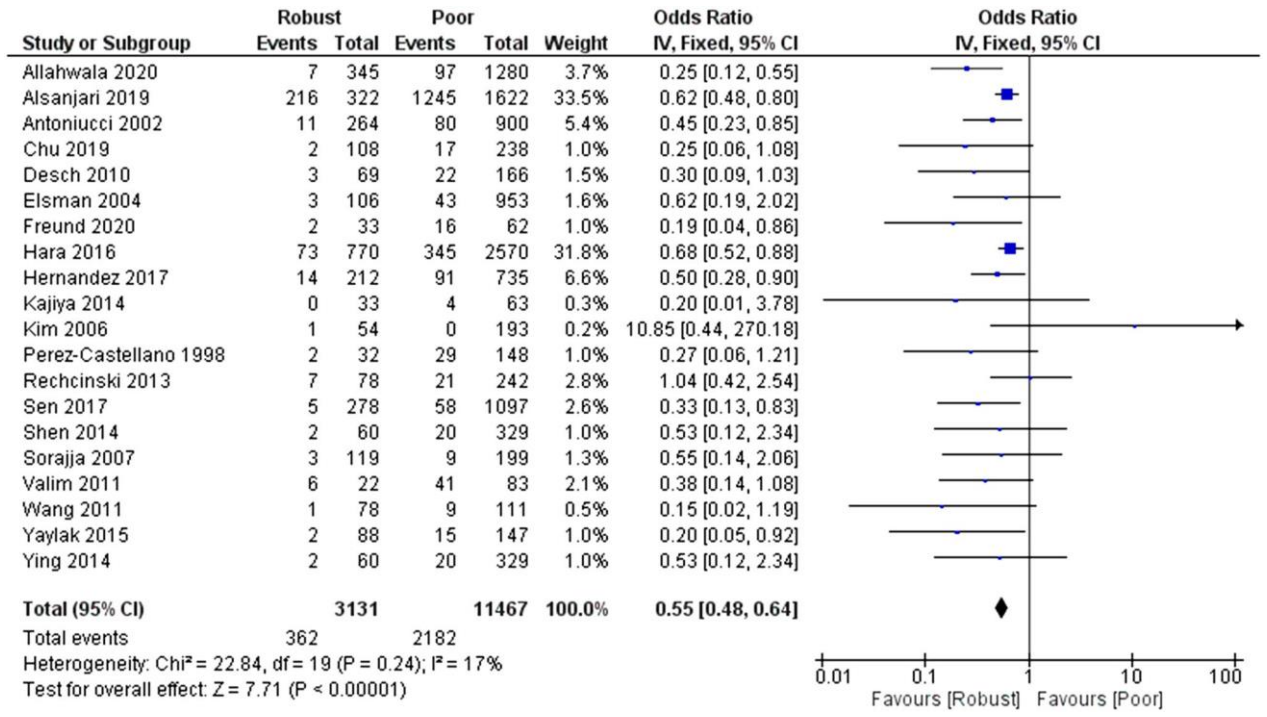

Fig. 2 Risk of mortality in patients with robust vs poor collaterals

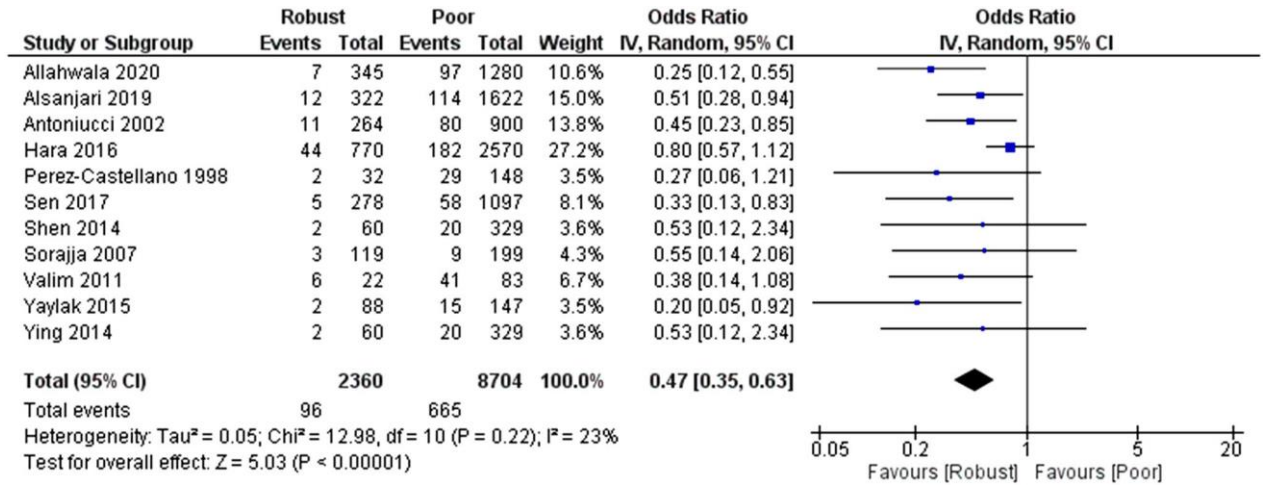

Fig. 3 Risk of in-hospital and short term mortality in patients with robust vs poor collaterals

It is perhaps unsurprising that increased perfusion would result in improved LVEF. Whilst the rates of left ventricular impairment did not meet statistical significance, three of the four studies [8, 13, 18] did not report LVEF. In the studies which did report mean ejection fractions, this was significantly higher in patients with robust collaterals compared to those with poor collaterals. It is possible that a greater proportion of those patients with poor collaterals had more severe left ventricular impairment, which may explain these apparent conflicting findings. Whilst the absolute difference in LVEF was small, there was a sustained benefit derived from all studies suggesting the protective effects of collaterals is maintained.

Perhaps driven by the protective effect on left ventricular function, mortality, both in-hospital and short term, as well as longer term was lower in those with robust

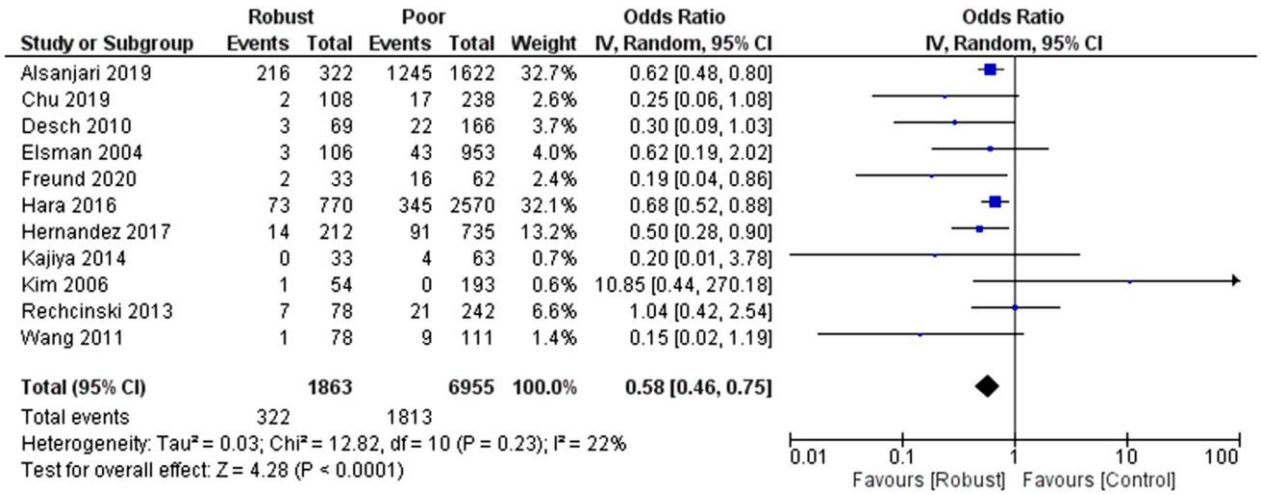

Fig. 4 Risk of long-term mortality in patients with robust vs poor collaterals

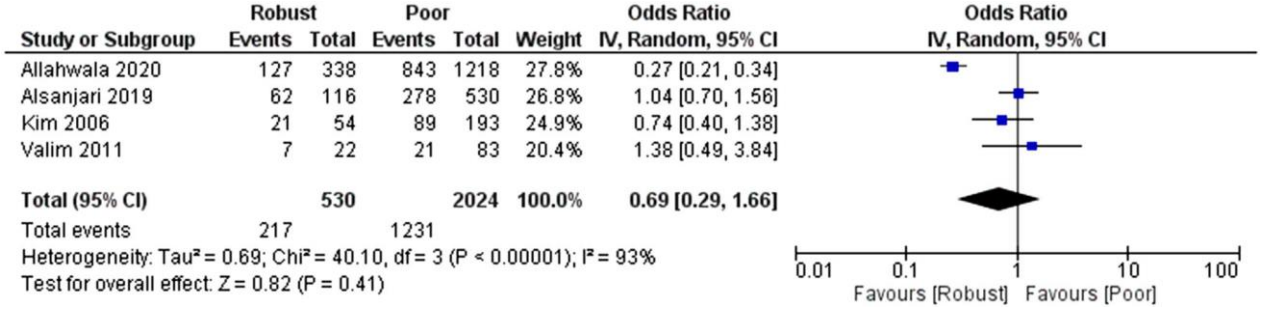

Fig. 5 Risk of left ventricular impairment in patients with robust vs poor collaterals

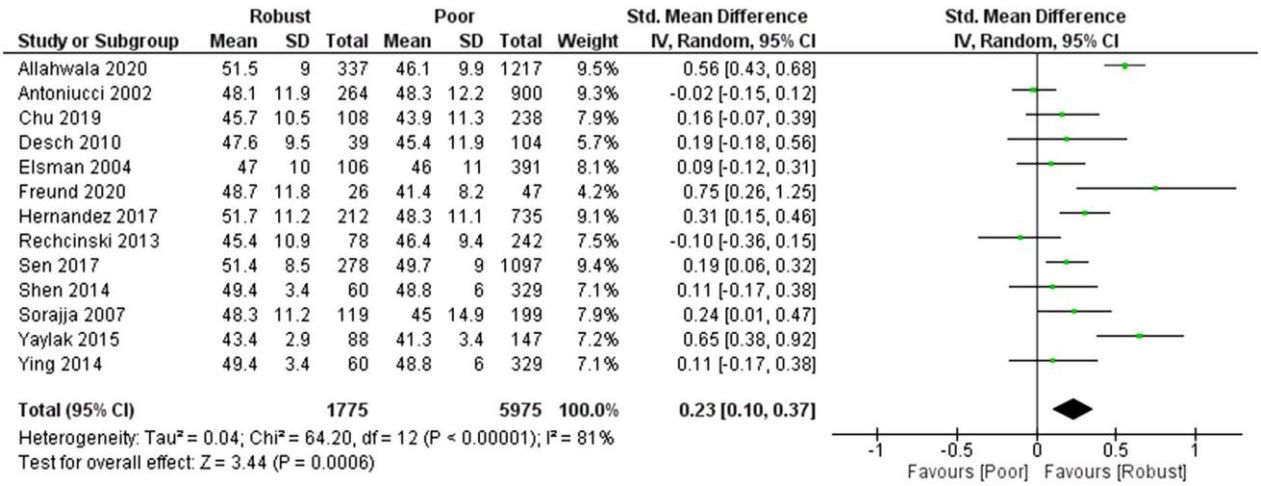

Fig. 6 Left Ventricular Ejection Fraction in patients with robust and poor collaterals

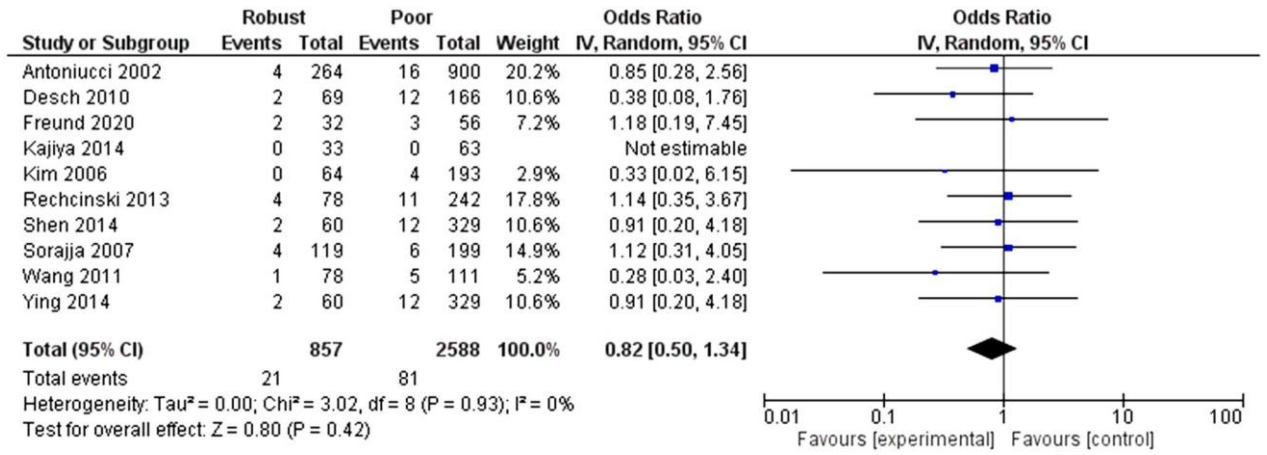

Fig. 7 Risk of recurrent acute myocardial infarction in patients with robust vs poor collaterals

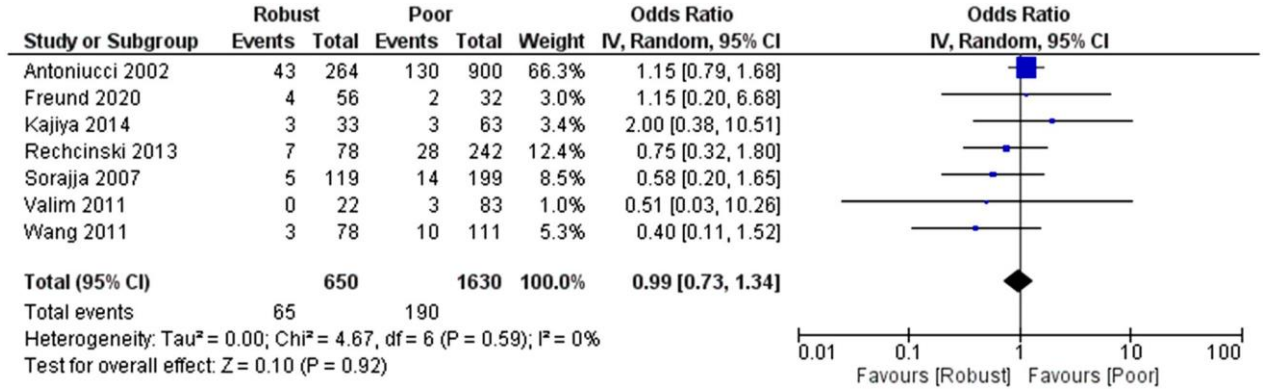

Fig. 8 Risk of repeat revascularisation in patients with robust vs poor collaterals

collaterals. Despite contemporary advancements in management of STEMI, as well as improved post infarct management, mortality rates at 1 year remain at 4.3–4.5% [29]. It is possible that the degree of intrinsic collateral recruitment may explain this remaining mortality penalty, despite optimal management and timely revascularisation. With respect to short-term mortality outcomes, there was high agreement amongst the studies. Furthermore, two studies [8, 11] reported both short and longer term outcomes. The study by Hara et al. [11] showed a trend toward reduced in-hospital mortality in those with robust collaterals (OR 0.80 95% CI 0.57–1.12), whilst at long term follow up of 5 years, there was a clear survival advantage (OR 0.68 95% CI 0.52–0.88). This delay may be explained by the protective effects of collaterals on left ventricular function, with subsequent longer term survival advantage [30]. Alsanjari et al. [8] showed a significant in-hospital mortality benefit which was sustained out to 5 years, suggesting the acute benefit of myocardial salvage persists over time. Even after excluding the studies by Alsanjari et al. [8] and Hara et al. [11] which accounted for a large percentage of weight for the analysis, the presence of robust collaterals remained predictive of total, short-term and long term mortality without evidence of heterogeneity or publication bias, suggesting a sustained and ubiquitous protective effect of collaterals on mortality.

The study by Kim et al. [13], was the only study which suggested a trend toward higher mortality with those with robust collaterals, although this did not reach significance. This may be an issue with respect to appropriate power, as the study was designed as an MRI based study, and found beneficial effects of robust collaterals with respect to smaller infarct size and smaller area at risk. Alternatively, the phenomenon of "collateral decay [31]" which has been described in the setting of a CTO, whereby robust collaterals seen at the time of an acute infarct are no longer evident on repeat angiography may conceivably apply in the STEMI setting to explain these apparent incongruous findings.

There were no differences in risk of recurrent acute myocardial infarction or repeat revascularisation in patients with robust or poor coronary collaterals. Whilst the ability to recruit collaterals is stable in subsequent STEMI presentations [32], it appears that spontaneous collateral recruitment may be protective following onset of acute myocardial infarction rather than influencing risk of subsequent vascular events. Therefore, the innate ability to recruit collaterals appears to be protective once an infarct has commenced rather than preventative, as it does not preclude acute plaque rupture and thromboembolic occlusion.

The incidence of angiographically evident robust coronary collaterals was on average 25% of patients presenting with STEMI. Whilst the mechanisms by which some patients are able to recruit collaterals is uncertain, a combination of patient specific factors (younger age), anatomical considerations (right coronary artery as the culprit vessel) as well as history of angina and presence of more severe bystander coronary disease, mediated through multiple growth factors and cytokines have been previously postulated [20]. Given the protective effects of collateral recruitment, further research is required to ascertain whether adjuvant pharmacological or mechanical treatment may facilitate in their recruitment, to improve outcomes.

The findings of the study are similar to a previous meta-analysis on the impact of collaterals in STEMI [28], although our meta-analysis includes six more studies with 4000 more patients Furthermore, the previous analysis included one study which appraised the effect of collaterals to a concomitant CTO during STEMI, a quite distinct situation to rapidly recruited collaterals to the IRA during STEMI, which may introduce some degree of bias in the results. This confirmation of results, further emphasizes the need to identify biological and mechanistic basis for collateral recruitment.

Limitations

Despite the relative consistent findings between studies of our analysis, there are important limitations which need to be considered. Firstly, all included studies were observational studies, including two which were based on the initial angiographic findings in randomised control trials, prior to intervention, and presented unadjusted outcomes, which may introduce bias into the selection of data although the very nature of spontaneously recruited collaterals suggests that only observational studies are possible. The baseline demographics were relatively similar; however the IRA was variable and in some studies, excluded specific IRA (51,923). Furthermore patient's background, in particular prior history of angina and or coronary artery disease, particularly disease in the contralateral donor vessel and prior history of angina were not considered and as such may have influenced the degree of collateral recruitment and hence outcomes. Although these collaterals were supplying the territory subtended by an acutely occluded vessel in the setting of a STEMI, it is possible that they were preformed rather than acutely recruited. However, given that the majority of the culprit lesions in patients with STEMI have a mild to moderate degree of stenosis prior to occlusion [33, 34], this is unlikely to be the case. Given included studies ranged from 1998 to 2020, it is conceivable that patient characteristics and management strategy changes may have influenced outcomes, which is an inherent limitation of pooled results. However, whilst studies have shown that patients with STEMI are younger and more likely to have traditional cardiovascular risk factors as compared to 20 years ago, there has been no change in mortality in patients who underwent reperfusion therapy [35]. Given the protective effects of collaterals is likely in the short term prior to revascularisation, it is unlikely however that there is significant bias from including these studies. Another anatomical consideration was that included studies looked at the presence of retrograde filling via contralateral collaterals, and the impact of the presence of anterograde, bridging collaterals was not taken into consideration. Whilst the majority of observed collaterals are from the contralateral vessel, nevertheless this may impact on coronary perfusion and may independently affect prognosis. Finally, it is possible, that there may have been insufficient statistical power to detect associations for some outcomes.

Conclusions

The ability to recruit robust coronary collaterals during a STEMI is associated with a lower in-hospital, short term and longer term mortality as compared to those patients who cannot recruit sufficient collaterals. Similarly, robust collateral recruitment is associated with improved left ventricular function following STEMI, which may be the mechanism by which this survival advantage is achieved. These findings have implications in identifying patients who may benefit from closer monitoring and prognostication in the post infarct setting.

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Compliance with ethical standards

Conflicts of interest None of the authors have relevant conflicts of interest.

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Appendix 1: Search Strategy

Database: MEDLINE(R) All including Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Daily and Versions(R) <1946-current>

Search Strategy: January 10th 2020

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1 Collateral Circulation/ (12137)
2 collateral*.mp. (45969)
3 Rentrop.mp. (303)
4 CCS.mp. (5746)
5 Collateral Connection Score.mp. (2)
6 1 or 2 or 3 or 4 or 5 (51682)
7 ST Elevation Myocardial Infarction/ (3099)
8 STEMI.mp. (10410)
9 7 or 8 (11544)
10 6 and 9 (120)

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Database: Embase <1974 to 2020 January 10>

Search Strategy: January 10th 2020

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1 collateral circulation/ (11049)
2 collateral artery/ (409)
3 coronary artery collateral circulation/ (1756)
4 collateral*.mp. (56811)
5 Rentrop.mp. (569)
6 CCS.mp. (9630)
7 Collateral Connection Score.mp. (5)
8 1 or 2 or 3 or 4 or 5 or 6 or 7 (66357)
9 ST segment elevation myocardial infarction/ (36557)
10 STEMI.mp. (27144)
11 9 or 10 (41450)
12 8 and 11 (434)

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Supplementary Table 1: Newcastle Ottawa Scale for Included Studies

Study Ref	**Selection**				**Comparability**	**Outcome**			**NOS Quality Score**
	Case Definition	*Representativeness of the cases*	*Selection of cases*	*Definition of Cases*	*Comparability of cohorts*	*Ascertainment of Exposure*	*Same method of ascertainment for cases and controls*	*Adequacy of follow-up of cohorts*	
Allahwala 2020	*	* controls are poor collaterals	*	*	* multivariate analysis to correct for confounders	* Collateral grading blinded to outcome	*	*	8 Good
Alsanjari 2019	*	* controls are poor collaterals	*	*	* multivariate analysis to correct for confounders	* Collateral grading blinded to outcome	*	*	8 Good
Antoniucci 2002	*	*	*	*	* multivariate analysis to correct for confounders	*	*	*	8 Good
Chu 2019	*	only >65 included	*	*	* multivariate analysis to correct for confounders	*	*	*	7 Good
Desch 2010	*	*	*	*	* multivariate analysis to correct for confounders	*	*	*	7 Good
Elsman 1004	*	*	*	*	No correction for confounders	*	*	*	7 Poor
Freund 2020	0 – included STEMI patients up to 48 hrs post symptoms	Subgroup analysis of trial with small numbers of total with significant exclusion criteria	*	*	* multivariate analysis to correct for confounders	Collateral grading by 1 physician alone	*	*	5 Fair

Hara 2016	*	*	*	*	* multivariate analysis to correct for confounders	*	*	*	8 Good
Hernandez 2017	*	*	*	*	* Propensity matching analysis	*	*	*	8 Good
Kajiya 2014	Patients underwent CT first, no inclusion or exclusions described	Patients underwent CT first, no inclusion or exclusions described	*	*	No correction for confounders	*	*	*	5 Poor
Kim 2006	Only patients with successful PCI and CMR included	Select population alone	*	*	* multivariate analysis to correct for confounders	*	*	Very low mortality rate at 1 year suggest incomplete follow up	5 Fair
Perez-Castellano 1998	*	Only anterior STEMI included	*	*	* multivariate analysis to correct for confounders	*	*	Only in-hospital outcomes	6 Good
Rechciński 2013	*	*	*	*	No correction for confounders	*	*	*	7 Poor
Sen 2017	*	*	*	*	*	*	*	*	8 Good
Shen 2014	*	*	*	*	*	*	*	*	8 Good
Sorajja 2007	*	Subgroup analysis of trial	*	*	No correction for confounders	*	*	*	6 Poor
Valim 2011	Only patients in cardiogenic shock	Small number of patients over long inclusion time	*	No definition on method of grading collaterals	No correction for confounders	*	Uncertain	Only in-hospital outcomes	2 Poor

Wang 2011	*	Only anterior STEMI included	*	*	No correction for confounders	*	*	*	6 Poor
Yaylak 2015	*	Only inferior STEMI included	*	*	*	*	*	Only in-hospital outcomes	6 Good
Ying 2014	*	*	*	*	*	*	*	*	8 Good

Supplementary Table 2: Odds ratio of outcomes after exclusion of 2 studies.

Outcome	No. of Studies	OR	Heterogeneity (I^2)	Publication Bias [Eggers Regression]
Mortality	18	0.40 [0.31-0.52]	0%	0.35
In-Hospital Mortality	9	0.36 [0.26-0.51]	0%	0.47
Long term Mortality	9	0.47 [0.28-0.78]	27%	0.34

SECTION IV: CHRONIC RECRUITMENT OF CORONARY COLLATERALS

Chapter 6: Predictors and Prognostic Implications of Well-Matured Coronary Collaterals in patients with a chronic total occlusion (CTO)

This chapter is presented as the published work: Allahwala UK, Kott K, Bland A, Ward M, Bhindi R. (2020). Predictors and Prognostic Implications of Well-Matured Coronary Collateral Circulation in Patients with a Chronic Total Occlusion (CTO). *International Heart Journal*. 61(2): 223-230. doi: 10.1536/ihj.19-456

CLINICAL STUDY

Predictors and Prognostic Implications of Well-Matured Coronary Collateral Circulation in Patients with a Chronic Total Occlusion (CTO)

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Summary

The predictors and prognostic implications of well-matured collaterals in those with a chronic total occlusion (CTO) are unknown. We sought to identify the determinants of collateral maturation and to determine its effects on procedural outcomes and prognosis.

Patients presenting for CTO percutaneous coronary intervention (PCI) between April 2010 and July 2019 were included. Patients with a previous coronary artery bypass (CABG) to the CTO and those with only bridging collaterals were excluded. The degree of collateral maturation was determined by the Rentrop grading classification. Demographic, biochemical, and anatomical factors and procedural and longer-term outcomes were identified.

A total of 212 patients were included in the study. Patients with well-matured collaterals were more likely to be females (29.7% versus 15.2% versus 0%, $P < 0.005$ for Rentrop grade 3, 2, and 0 or 1, respectively), less likely to have chronic kidney disease (CKD) (8.8% versus 4.5% versus 19.2%, $P < 0.05$) and less likely to have had a prior CABG (15.6% versus 18.7% versus 19.2%). Patients with well-matured collaterals had lower neutrophil-to-leukocyte ratio (NLR) (2.8 versus 4.0 versus 5.7, $P < 0.0001$). Patients with well-matured collaterals were more likely to have procedural success (90.5% versus 62.5% versus 34.6%, $P < 0.0001$). The degree of collateral maturation was not associated with longer-term mortality.

Improved collateral maturation was associated with female sex and lower rates of CKD and CABG and a lower NLR. Those with well-matured collaterals had a significantly higher rate of procedural success but not improved prognosis.

(Int Heart J 2020; 61: 223-230)

Key words: Coronary artery disease (CAD), Rentrop, Percutaneous coronary intervention (PCI)

A chronic total occlusion (CTO) of the coronary circulation is defined as complete or near-complete occlusion of an epicardial vessel, present for at least 3 months,[1] angiographically appreciated via opacification of the distal vessel via the collateral circulation. The prevalence of a CTO identified during coronary angiography is estimated to be between 30 and 50%,[2,3] with 6.6% of patients presenting with an ST elevation myocardial infarction also having a CTO in a non-infarct-related artery.[4]

The presence of well-developed coronary collaterals has been associated with improved prognosis in most studies in the setting of stable disease;[5,6] however, studies have used conflicting definitions of a "sufficient" collateral circulation.[5] While the presence of well-developed collaterals is often described as "benign" and an indication to avoid either percutaneous or surgical revascularization,[7] conflicting studies have suggested that well-developed collaterals in patients with a CTO may be associated with increased ventricular arrhythmia and greater appropriate defibrillator therapy.[8]

Similarly, in the setting of an acute coronary syndrome, the ability to acutely recruit collaterals to supply the myocardial territory subtended by the culprit vessel has been associated with improved outcomes,[9] although one study suggested better developed collaterals associated with poorer outcomes.[10]

Furthermore, while there have been correlations between patient demographics, culprit vessel characteristics, and circulating angiogenic mediators,[11] the predictors of the coronary collateral recruitment and maturation remain uncertain.

Given the uncertainty of the factors associated with collateral recruitment, as well as prognostic implications of the degree of collateral maturation, we sought to determine the demographic, anatomic, and biochemical determinants of collateral maturation in patients presenting for planned CTO percutaneous coronary intervention (PCI).

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We also sought to determine the impact of the coronary collateral circulation on procedural outcomes as well as prognosis.

Methods

All patients presenting for CTO PCI at our dedicated tertiary center between April 2010 and July 2019 were included in the review. Patients with a CTO of a vessel supplied by a coronary artery bypass graft (CABG) or a CTO of a bypass graft were excluded from the study as this may impact on the ability to recruit collaterals of the native circulation. To standardize definition of collateral maturation, all angiograms were reviewed to determine the degree of collateral formation, scored based on the Rentrop classification,[12] whereby grade 0 is defined as no filling of collaterals or recipient vessel, grade 1 as filling of side branches of the recipient artery via collaterals without visualization of the epicardial segment, grade 2 as filling of the epicardial segment via collateral channels, and grade 3 as complete filling of the epicardial segment of the recipient artery via collateral channels. Reviewers were blinded to the outcome of the CTO PCI procedure.

For the analysis, patients were grouped as Rentrop grade 3 collaterals, Rentrop grade 2 collaterals, or Rentrop grade 0 or 1 collaterals. We also conducted analysis of those with Rentrop grade 3, which we defined as *complete collaterals,* and those with Rentrop grade 0, 1, or 2 collaterals which we defined as *incomplete collaterals*.

Along with Rentrop classification, all angiograms had calculation of the Japan-CTO (J-CTO) score[13] performed. Patients with only bridging collaterals, that is, no collaterals providing retrograde filling via a different epicardial vessel, were excluded from the analysis. PCI success was based on operator self-reporting. Electronic medical records were reviewed to identify biochemical and hematological blood tests, taken within 4 weeks prior to CTO PCI procedure, procedural characteristics, and in-hospital course along with longer-term outcomes and biochemical results. Chronic kidney disease (CKD) was defined by an eGFR < 45 mL/minute/m^2.

Statistical analysis: Continuous variables were presented as means (± standard deviation) or as medians and interquartile ranges, if the distribution of data was not normal. Categorical variables were reported as percentages. The baseline characteristics, PCI procedural findings, and in-hospital outcomes were compared between differing degrees of collateral formation. Comparisons between groups were performed using Pearson's chi-square test for categorical variables for normally distributed and the Kruskal-Wallis H test for categorical variables which were not normally distributed. The analysis of variance (ANOVA) test was used for comparing continuous variables or the independent student's *t*-test when comparing two groups. Cumulative event rates were calculated according to the Kaplan-Meier method and compared using the Breslow generalized Wilcoxon score. All tests were two-sided, and a $P < 0.05$ was considered statistically significant. All analyses were performed using SPSS (version 24, IBM, New York, New York).

Results

A total of 212 patients were included over the study period, of which 39 (18.4%) were females with a mean age of 69.2 (± 10.2). Within the study population, 26 patients (12.3%) had Rentrop 0 or 1 collaterals, 112 (52.8%) had Rentrop grade 2 collaterals, and 74 (34.9%) had Rentrop grade 3 collaterals. With respect to the culprit vessel, the RCA was the commonest 119 (56.1%) followed by the LCx, 48 (22.6%), and the LAD 45 (21.1%).

Patients with well-matured collaterals were more likely to be female (29.7% versus 15.2% versus 0%, $P < 0.005$ for Rentrop grade 3, 2, and 0 or 1, respectively), although there was no difference in age or BMI. Cardiovascular risk factor profiles were similar between the three groups, although those with well-matured collaterals had lower rates of CKD (8.8% versus 4.5% versus 19.2%, $P < 0.05$). Patients with well-developed collaterals were also less likely to have a history of a previous CABG (15.6% versus 18.7% versus 19.2%, $P < 0.05$). Median ejection fraction, previous acute myocardial infarction, and medication usage at baseline were similar between all groups. Similarly, disease complexity, as assessed by the syntax score, was similar between all groups (Table I).

With respect to hematological blood results, patients with well-matured collaterals had a higher leukocyte concentration (1.9×10^9/L versus 1.6×10^9/L versus 1.9×10^9/L, $P < 0.01$) and a lower NLR (2.8 versus 4.0 versus 5.7, $P < 0.0001$) (Table I). Other full blood count parameters were similar between the three groups. Similarly, there was no difference with respect to baseline biochemistry results between either group (Table II).

Patients with well-matured collaterals had a higher PCI success rate as compared to those with poorer developed collaterals (90.5% versus 62.5% versus 34.6%, $P < 0.0001$) and were more likely to have successful CTO PCI performed via the retrograde approach (32.8% versus 18.6% versus 0%, $P < 0.05$). Patients with well-developed collaterals were less likely to use adjunctive intravascular imaging as compared to those with poorer developed collaterals (2.7% versus 5.4% versus 19.2%, $P < 0.05$). Other procedural characteristics were similar between both groups. The second-generation drug-eluting stents and total contrast volume used during the procedure were similar in both groups (Table III).

We subsequently reviewed electronic medical records to determine the impact on prognosis with respect to the degree of collateral maturation. The mean follow-up for patients were similar between the three groups: 686.8 days for Rentrop grade 3, 680.1 days for Rentrop grade 2, and 692.2 for Rentrop grade 1 or 0 ($P = 0.99$).

There was no difference in longer-term survival when analyzing patients by degree of collateral maturation with respect to mean survival in the overall population (2574.6 days versus 2387.5 days versus 1955.2 days, $P = 0.50$) (Figure 1). When grouping patients as those with complete collaterals (Rentrop grade 3) compared to incomplete collaterals (Rentrop grade 0, 1 or 2), there was a trend toward a reduction in longer-term mortality (2574.6 days versus 2372.5 days, $P = 0.24$) (Figure 2). After excluding patients who underwent successful CTO PCI pro-

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Table I. Baseline Characteristics

	Rentrop 1 (*n* = 26)	Rentrop 2 (*n* = 112)	Rentrop 3 (*n* = 74)	*P*-value
Age (years)	70.9 (± 10.6)	69 (± 10.0)	68.9 (± 10.4)	0.65
BMI (kg/m^2)	29.8 (± 5.2)	28.5 (± 5.9)	28.4 (± 5.5)	0.54
Female sex (*n*) (%)	0 (0%)	17 (15.2%)	22 (29.7%)	< 0.005
CTO vessel (*n*) (%)				0.83
LAD	6 (23.1%)	24 (21.4%)	15 (20.3%)	
LCx	8 (30.8%)	24 (21.4%)	16 (21.6%)	
RCA	12 (46.1%)	64 (57.1%)	43 (58.1%)	
Smoking history (%)				0.91
Current	7.7	10.8	10.9	
Ex-smoker	34.6	36.0	41.1	
Non-smoker	57.7	53.1	47.9	
Hypertension (%)	80.8	76.8	79.2	0.85
Hypercholesterolemia (%)	88.5	79.5	77.0	0.46
Diabetes mellitus (%)	42.3	31.2	27	0.35
Family Hx of CAD (%)	19.2	31.2	36.5	0.26
Renal failure (%)	19.2	4.5	8.8	< 0.05
Prior CABG (%)	42.3%	18.7%	15.6%	< 0.05
LVEF (%) (median, IQR)	55 (50-60)	55 (50-60)	55 (40-60)	0.18
LV impairment, *n* (%)	5 (22.7%)	21 (21.9%)	19 (32.8%)	0.31
Previous AMI, *n* (%)	17 (65.4%)	58 (51.8%)	38 (51.3%)	0.42
Medications, *n* (%)				
Aspirin	22 (91.7%)	91 (87.5%)	65 (87.8%)	0.85
P2Y12 inhibitor	21 (87.5%)	90 (86.5%)	67 (93.0%)	0.38
Beta blocker	15 (65.2%)	68 (70.1%)	48 (73.8%)	0.72
ACE-I/ARB	15 (65.2%)	69 (71.9%)	39 (60.9%)	0.34
Statin	22 (95.6%)	81 (83.5%)	61 (93.8%)	0.07
CTO of stented vessel (%)	7.7%	4.5%	6.8%	0.72
JCTO score (mean ± SD)	1.7 (1.2)	1.9 (1.2)	1.7 (1.1)	0.41
Syntax score (median, IQR)	16 (10.5-20)	12.5 (9-20.5)	13.5 (8.25-21)	0.94

BMI indicates body mass index; LAD, left anterior descending artery; LCx, left circumflex artery; RCA, right coronary artery; CABG, coronary artery bypass graft; CTO, chronic total occlusion; LVEF, left ventricular ejection fraction; LV, left ventricular; AMI, acute myocardial infarction; P2Y12, clopidogrel, ticagrelor, or prasugrel; ACE-I, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; and JCTO, Japan CTO.

cedure, we again analyzed the longer-term impact of the degree of collateralization on mortality. While there was a trend toward worse outcomes in those with complete collaterals compared to incomplete collaterals, there was no difference with respect to mean survival (1659.1 days versus 2124.1 days, $P = 0.06$) (Figure 3).

Discussion

In our single-center experience, the presence of well-developed collaterals was more common in females, those without a history of renal failure, and those without a prior coronary bypass graft.

Previous clinical studies have suggested that women have less angiographically apparent collaterals than men[14-16] although these studies were done in the setting of differing degrees of coronary stenosis, not specifically CTOs, and did not quantify the degree of collateral maturation. While a specific pathophysiology of this is unknown, prior animal studies have suggested that estrogen withdrawal, such as in the case of menopause, is associated with lower rates of arteriogenesis and hence collateral recruitment.[17] It is possible that while overall rates of collaterals may be lower in women, in those that do demonstrate collaterals, they are more likely to be well matured. While other studies have suggested that older age reduces the likelihood of collateral recruitment, possibly due to reduced telomerase activity, we found no effect of age on the degree of collateral maturity.[18]

The presence of renal impairment has previously been associated with poorer angiographically determined collateral recruitment.[19,20] It has been suggested that the greater prevalence of endothelial dysfunction in patients with renal impairment may attenuate endothelial-derived nitric oxide pathways,[21,22] with resultant reduction in collateral recruitment and maturation.

We found that patients who had a history of previous CABG were less likely to have well-developed collaterals. The presence of a bypass graft is associated with accelerated atherosclerotic disease process and greater risk of development of a CTO of a bypassed vessel.[23] Early studies indicated that patients with occluded bypass grafts were able to recruit the same degree of collaterals as prior to the surgery,[24,25] although other series have suggested that the ability to recruit collaterals to the bypassed vessel is reduced.[26] As we excluded patients who had a previous bypass to the occluded vessel, the presence of a CABG itself appears to negatively impact on the collateral circulations' ability to spontaneously mature.

The neutrophil-to-lymphocyte ratio (NLR) has been

Table II. Hematological and Biochemical Results

	Rentrop 1 (*n* = 26)	Rentrop 2 (*n* = 112)	Rentrop 3 (*n* = 74)	*P*-value
WCC ($\times 10^9$) mean (± SD)	8.8 (± 3.0)	7.9 (± 2.7)	8.1 (± 2.8)	0.43
Neutrophils ($\times 10^9$) mean (± SD)	6.2 (± 2.7)	5.5 (± 2.3)	5.2 (± 2.2)	0.25
Leukocytes ($\times 10^9$) mean (± SD)	1.6 (± 0.8)	1.6 (± 0.6)	1.9 (± 1.0)	< 0.01
NLR mean (± SD)	5.7 (± 6.0)	4.0 (± 2.5)	2.8 (± 1.1)	< 0.0001
Monocytes ($\times 10^9$) mean (± SD)	0.8 (± 0.6)	0.7 (± 0.2)	0.7 (± 0.3)	0.49
Eosinophils ($\times 10^9$) mean (± SD)	0.2 (± 0.2)	0.2 (± 0.2)	0.2 (± 0.3)	0.69
Hb (g/L) mean (± SD)	130.0 (± 17.7)	131.0 (± 25.4)	128.7 (± 33.6)	0.89
Platelets ($\times 10^9$) mean (± SD)	223.3 (± 91.9)	210.1 (± 66.2)	229.0 (± 12.6)	0.37
CRP (mg/L) mean (± SD)	14.0 (± 9.1)	16.5 (± 4.3)	8.9 (± 12.7)	0.38
Albumin (g/L) mean (± SD)	35.2 (± 4.5)	37.6 (± 5.2)	35.6 (± 9.4)	0.22
Creatinine (µmol/L) mean (± SD)	90 (67-88.5)	88 (73.5-101.5)	80 (69.7-101.2)	0.35
NT pro-BNP (ng/L) mean (± SD)	41 (± 26.8)	134.5 (± 179.3)	119.6 (± 139.9)	0.75
Troponin (ng/L) median (IQR)	223 (42-440)	27 (8-140)	12 (6-38)	0.19
HbA1C (%) mean (± SD)	5.8 (± 0.35)	6.5 (± 1.4)	6.2 (1.1)	0.24
Cholesterol (mmol/L) mean (± SD)	4.5 (± 1.2)	4.1 (± 0.9)	4.1 (± 1.3)	0.74
LDL (mmol/L) mean (± SD)	2.1 (± 0.3)	2.2 (± 0.9)	2.4 (± 1.1)	0.90
HDL (mmol/L) mean (± SD)	0.95 (± 0.2)	1.1 (± 0.8)	1.1 (± 0.5)	0.93

WCC indicates white cell count; NLR, neutrophil to lymphocyte ratio; Hb, hemoglobin; CRP, C-reactive protein; NT pro-BNP, N-terminal pro-brain natriuretic peptide; HbA1C, hemoglobin A1C; LDL, low-density lipoprotein; and HDL, high-density lipoprotein.

Table III. Angiographic Findings and Procedural Outcomes

	Rentrop 1 (*n* = 26)	Rentrop 2 (*n* = 112)	Rentrop 3 (*n* = 74)	*P*-value
PCI success	9 (34.6%)	70 (62.5%)	67 (90.5%)	< 0.0001
Approach attempted				0.50
Anterograde	21 (80.8%)	79 (70.5%)	54 (73.0%)	
Retrograde	2 (7.7%)	13 (11.6%)	12 (16.2%)	
Both	3 (11.5%)	20 (17.9%)	8 (10.8%)	
Successful approach*				< 0.05
Anterograde	26 (100%)	91 (81.4%)	50 (67.2%)	
Retrograde	0%	21 (18.6%)	24 (32.8%)	
Number of stents	2.0 (0.9)	2.4 (1.1)	2.5 (1.0)	0.38
Length of stents	61.9 (33.2)	73.1 (42.3)	75.0 (30.8)	0.50
Drug-eluting stent (*n*) (%)	13 (100%)	73 (97.3%)	67 (100%)	0.51
Contrast volume (mL) (mean ± SD)	271.1 (± 122.6)	272.2 (± 124.1)	282.3 (± 135.5)	0.86
Intravascular imaging	19.2	5.4	2.7	< 0.05
Rotational atherectomy	1 (3.8%)	3 (2.7%)	2 (2.7%)	0.95
No. of wires	3 (2-3)	4 (3-5)	3 (2-5)	0.30
No. of devices	1 (1.2)	1.2 (1.0)	1.2 (1.0)	0.38
Mortality	0	0	1.3	0.39
Radiation (Gy)	2.9	2.8	2.7	0.94

* Total number of patients with successful CTO PCI = 146, Rentrop 1 = 9, Rentrop 2 = 70 and Rentrop 3 = 67. PCI indicates percutaneous coronary intervention; and Gy, Grays.

associated with survival in multiple subtypes of malignancies,[27,28] and a higher ratio is associated with poorer cardiovascular outcomes.[29] An elevated neutrophil count is associated with elevated levels of vascular endothelial growth factor (VEGF),[30] which is a primary promotor of angiogenesis,[31] but it is not believed to play a role in arteriogenesis, the proposed mechanism by which collateral arteries mature. Patients with well-developed collaterals had significantly lower NLR and a higher leukocyte count than those with poorer developed collaterals. The process of arteriogenesis, whereby preexisting arteriolar connections between epicardial vessels mature, occurs as a result of elevated shear stress detected by mechanoreceptors within the endothelial cells. This results in downstream upregulation of proteins involved in monocyte activation and macrophage differentiation with subsequent adventitial proteolysis and vascular structural development. However, numerous animal studies have also suggested that T cells play an integral role in neovascularization from collaterals,[32-34] particularly NK cells and CD-4$^+$ cells.[35] However, in a peripheral animal model, infusion of lymphotactin and subsequent T-cell accumulation did not increase arteriogenesis.[36] The association between collateral maturation and NLR suggests that the relative increase in lymphocytes, rather than absolute increase, may be more relevant to increasing collateral maturation.

The presence of well-developed collaterals was associated with a significantly higher likelihood of procedural success, which has previously been associated with significantly improved prognosis.[37] The most commonly used

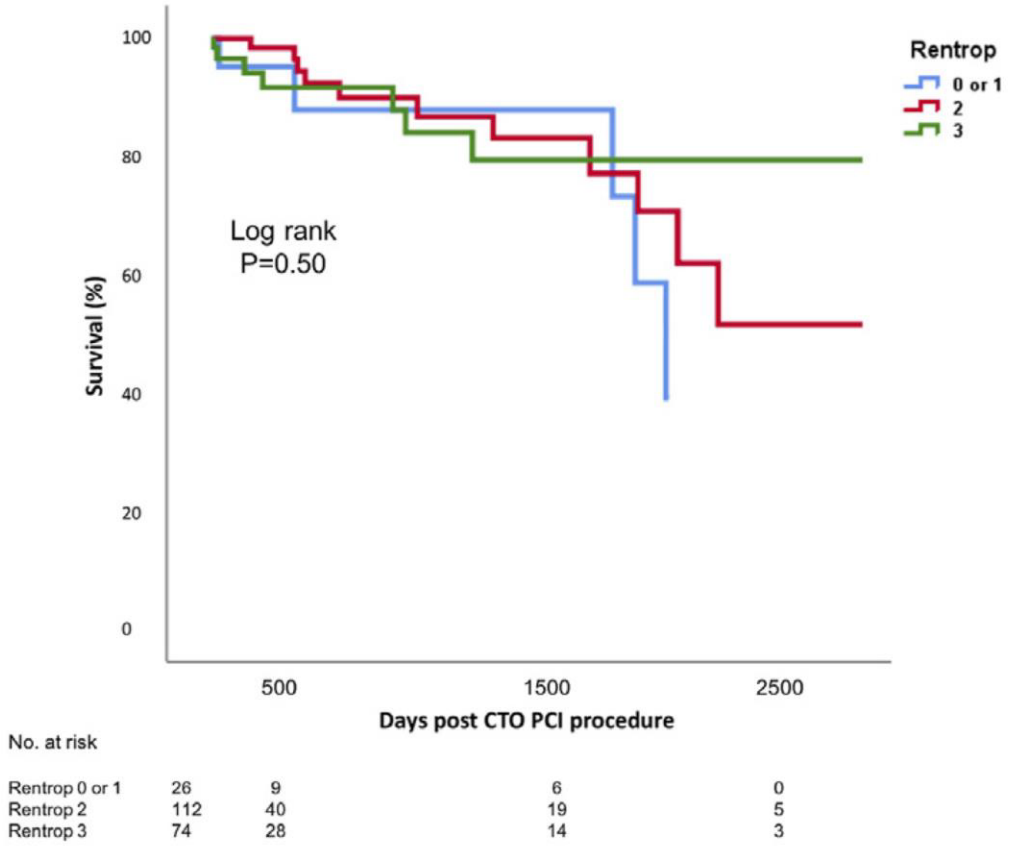

Figure 1. Kaplan-Meier cumulative survival in all patients

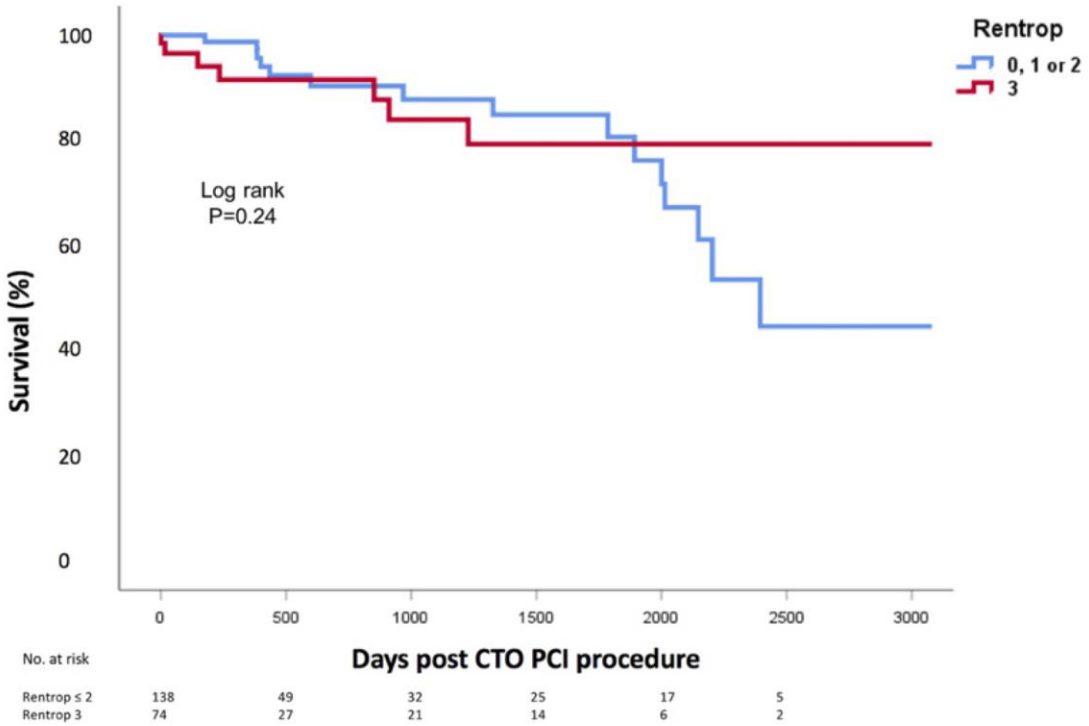

Figure 2. Kaplan-Meier cumulative survival in all patients with complete (Rentrop grade 3) or incomplete (Rentrop grade 0, 1 or 2) collaterals

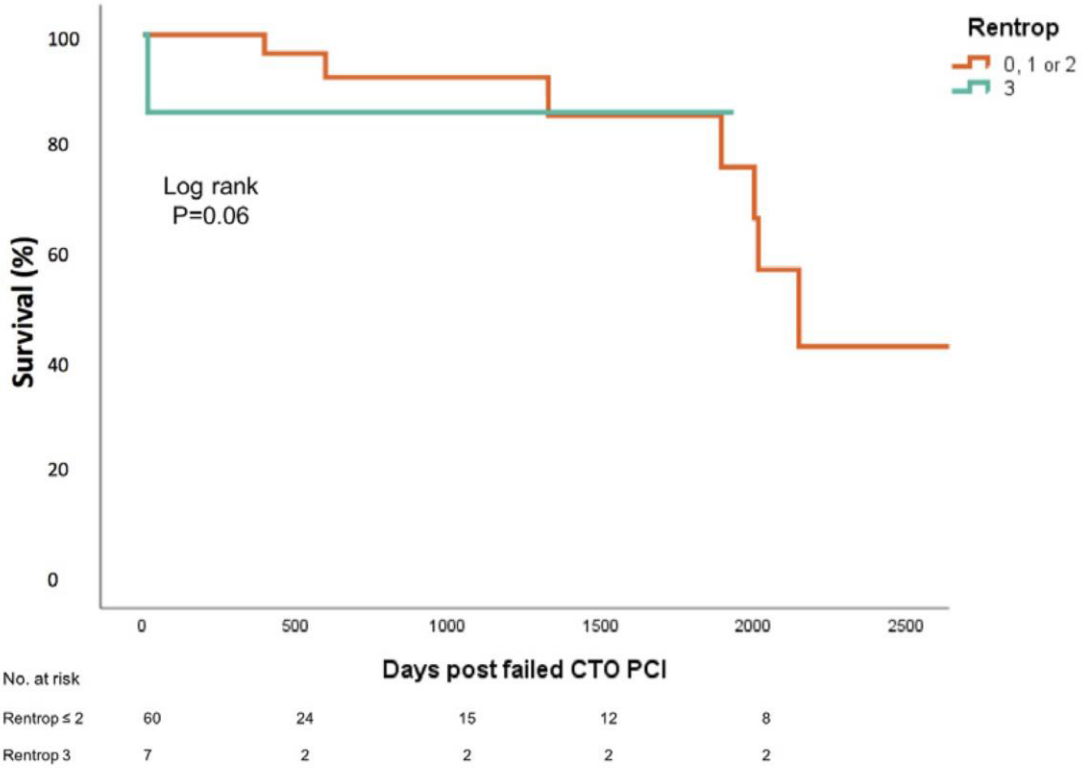

Figure 3. Kaplan-Meier cumulative survival in patients with unsuccessful CTO PCI

scoring tool for predicting successful CTO PCI, the J-CTO score,[13] does not include the presence, maturity, or size of the vessel. The more recently described PROGRESS CTO score,[38] while it includes the presence or absence of "interventional collaterals" defined as collaterals which are amenable to crossing with a guidewire and microcatheter. However, this does not consider filling of the distal vessel, which is exclusively assessed using the Rentrop grading system. We found that the degree of collateral filling of the distal vessel predicted successful anterograde and retrograde approach, suggesting that their predictive effect is not as an interventional tributary, but may reflect better distal opacification and hence visualization. The overall success rate of CTO PCI in the entire cohort was 68.9%, which is similar to the core lab adjudicated procedural success of 73% in the EXPLORE trial[39] and higher than the 59% success rate from the National Cardiovascular Data Registry.[40] While other clinical trials[41,42] have shown higher success rates, these outcomes represent "real-world" data in patients presenting for CTO PCI using both anterograde and retrograde approaches.

In longer-term follow-up in patients presenting for CTO PCI, we found no difference in all-cause mortality rates in patients with well-developed collaterals as compared to those with poorer developed collaterals. Similarly, in those patients who had failed CTO PCI, we did not find any difference in rates of longer-term mortality between groups. Patients with well-developed collaterals are often not referred for invasive revascularization as it is felt that they have sufficient perfusion to prevent ischemia. Contrary to this often held belief, invasive flow studies have shown that the territory of myocardium subtended by a CTO is in a constant state of ischemia, irrespective of the degree of collateral formation.[43] The absence of any prognostic benefit of the presence of well-matured collaterals in all patients, including those with failed CTO revascularization, suggests that angiographically well-matured collaterals should not be an indication to pursue medical management, but rather revascularization decision should be driven by the presence of anginal symptoms.

Limitations: This study in a single center, retrospective analysis of data, with which comes inherent limitations. While the overall numbers are small, we identified a number of factors associated with well-matured collaterals, including hematological factors. Given the proposed mechanism of collateral maturation and recruitment occurs based on monocyte and lymphocyte shift into the adventitial space, this provides clinical support for fundamental scientific theory. However, larger studies are required to confirm these findings and in particular to identify the molecular and cell signaling pathways by which this collateral recruitment occurs. Furthermore, we only included patients undergoing CTO PCI, and as such, patients treated with medical management or surgery were excluded, which may confound the results. However, this study also aims to assess whether collateral maturity predicts PCI success. Furthermore, we showed no benefit in prognosis in those with well-developed collaterals, which is often cited as a reason patients are not revascularized. These results will also require further larger dedicated studies to confirm.

Conclusion

Patients with well-matured collaterals are more likely to be females, with lower rates of renal dysfunction and history of prior CABG. A lower NLR and higher lymphocyte count are also associated with well-matured collaterals, underpinning the role of leukocytes in collateral development and maturation. While well-matured collaterals are a strong predictor of CTO PCI success, they do not seem to provide a survival advantage over those patients with less well-developed collaterals, and hence the decision to undertake CTO PCI should remain on the presence of symptoms. Newer scoring tools to predict the likelihood of successful CTO PCI may benefit from including these factors.

Disclosure

Conflicts of interest: None.

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Chapter 7: Prognostic impact of collaterals in patients with a coronary chronic total occlusion (CTO): A meta-analysis of over 3,000 patients

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ORIGINAL STUDIES

Prognostic impact of collaterals in patients with a coronary chronic total occlusion: A meta-analysis of over 3,000 patients

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Abstract

Objectives: To assess the prognostic implications of the degree of coronary collaterals on outcomes in patients with a CTO.

Background: Coronary chronic total occlusions (CTO) are identified frequently in patients undergoing coronary angiography and have been associated with poorer prognosis. Whether the degree of coronary collaterals, the hallmark of CTOs impacts prognosis, is unknown.

Methods: A search of EMBASE, MEDLINE, and Cochrane Library was conducted to identify studies reporting on coronary collaterals and risk of all-cause mortality, acute myocardial infarction (AMI) and successful percutaneous coronary intervention (PCI). Patients with Rentrop grade 0 or 1 collaterals were defined as poor collaterals, while Rentrop grade 2 or 3 were defined as robust collaterals.

Results: Twelve studies with a total of 3,369 were included. Patients with robust collaterals did not have lower rates of AMI (OR: 0.89, 95%CI: 0.39–2.04) or lower rates of all-cause mortality (OR: 0.81, 95% CI: 0.42–1.58), however were more likely to have successful PCI (OR: 4.04, 95%CI: 1.10–14.85).

Conclusion: The presence of robust collaterals is not associated with lower rates of AMI or mortality, but does increase the likelihood of successful CTO PCI. These results have importance implications with respect to the indications for CTO PCI as well as selecting appropriate patients to undergo the procedure.

KEYWORDS

chronic total occlusion, collaterals, coronary collateral, CTO, Rentrop

1 | INTRODUCTION

A coronary chronic total occlusion (CTO) occurs when there is complete or near complete occlusion of an epicardial vessel, present for 3 months.[1] Radiographically this is appreciated by filling of the occluded vessel by collaterals, generally in a retrograde manner, within the aforementioned historical timing. The incidence of CTOs ranges between 18 and 52% depending on the cohort presenting for coronary angiography,[2-5] with 6.6% of patients with an ST-elevation myocardial infarction (STEMI) having a concurrent CTO.[6]

The presence of a CTO in stable disease is also associated with higher mortality,[3] greater recurrence of ventricular arrhythmia[7] and ongoing anginal symptoms. Despite collaterals perfusing the territory subtended by an occluded vessel, these are insufficient to prevent ischemia during increased demand.[8] Three recently published randomized trials, EXPLORE,[9] Euro-CTO,[10] and DECISION-CTO,[11] however,

failed to show any significant prognostic improvement with CTO percutaneous coronary intervention (PCI). However, the role of CTO PCI remains to improve symptoms and quality of life.[12,13]

Despite the demonstrated symptomatic benefit of CTO PCI, the overall rates of revascularization remain low, with registry data indicating only 10% of patients with a CTO undergoing PCI, and 25% being referred for surgery.[3] While an array of differing reasons for this lower rate of revascularization are possible, the perception that the degree of collaterals which supply the occluded vessel, via a different epicardial vessel, means these patients are more stable and may not require revascularization.

However, whether the degree of collateralization to the CTO affects prognosis is uncertain. In the setting of an acute coronary syndrome, those patients able to recruit better developed collaterals have an improved prognosis[14] although whether this remains true in the setting of a CTO is uncertain.

We performed a systematic review and meta-analysis to examine the impact of the degree of collaterals on outcome in patients with a CTO, namely mortality, acute myocardial infarction (AMI) and successful CTO PCI.

2 | METHODS

2.1 | Search strategy

The preferred reporting items for systematic reviews and meta-analyses (PRISMA) guidelines were followed for the search strategy.[15] We performed a computerized systematic search through MEDLINE, EMBASE, and the Cochrane Library databases from 1946 to September 2019. All relevant subject heading and free text terms relating to CTO and coronary collaterals were included. We used the following search terms "coronary occlusion" OR "chronic total occlusion" OR "CTO" or "total chronic occlusion" and "collateral circulation" or "collateral" or "Rentrop" or "CCS" or "collateral connection score". These keywords were searched as text words as well as exploded medical subject headings when feasible. The search strategy example for MEDLINE is as follows: 1 Coronary Occlusion/(3083), 2 chronic total occlusion.mp. (2211), 3 CTO.mp. (3230), 4 total chronic occlusion.mp.,[14] 5 Collateral Circulation/(12029), 6 collateral*.mp. (45320), 7 Rentrop.mp. (296), 8 CCS.mp. (5536), 9 collateral connection score.mp. (2), 10 1 or 2 or 3 or 4 (6144), 11 5 or 6 or 7 or 8 or 9 (50823), 12 10, and 11 (604). The search strategy is shown in Appendix 1. We further screened the references of the retrieved studies and prior meta-analyses for any relevant studies not retrieved through the initial search.

2.2 | Data source and searching

Articles were eligible for inclusion if they were published in English and included patients ≥18 years of age. Eligible study designs included observational cohorts of patients with an identified CTO on invasive angiography. Only patients in whom the coronary collaterals were quantified using the Rentrop grading classification,[16] were included, where grade 0 = no filling of any collateral channel; Grade 1 = filling of the side branches of the infarct related artery; Grade 2 = Partial filling of the epicardial vessel of the infarct related artery' Grade 3 = complete filling of the epicardial vessel. To standardize degree of collateral recruitment, patients with Rentrop 0 or 1 were classified as poor collaterals, while those with Rentrop 2 or 3 were classified as robust collaterals, as was done in the majority of studies. We excluded studies, which quantified collaterals during an acute coronary syndrome. The outcomes of interest were all-cause mortality, AMI, and likelihood of PCI success.

Two investigators (U.A. and D.N.) independently reviewed all citations identified through the literature search using a predefined protocol. Articles that clearly did not meet inclusion criteria were excluded at the title and/or abstract level. The remaining articles were selected for full text review. When limited information was available from the abstract, full text was always obtained. If further details were sought, the corresponding authors of the study were contacted to obtain further information. Disagreements regarding the selection and quality assessment of articles were resolved through discussion, and full consensus was achieved at each stage of review.

The Newcastle-Ottawa Scale (NOS)[17] was used as an assessment tool for selection, comparability, and outcome assessment. Study quality was rated on a scale from 1 (very poor) to 9 (high).

2.3 | Data analysis

Two presented abstracts[18,19] included overlapping data, and hence only the larger study[18] was included in the analysis for the outcomes of all-cause mortality and AMI, while the smaller study[19] was included for the analysis of PCI success, as this was not reported in the subsequent study. The included studies were grouped for pooling according to those with poor collaterals (Rentrop grade 0 or 1) and robust collaterals (Rentrop grade 2 or 3). The odds ratio was chosen as the primary outcome. The program review manager (version 5.3) was used to conduct an inverse variance random effects model for the meta-analysis. The Cochrane Q-statistic (I^2) was used to assess the consistency among studies, with $I^2 < 25\%$ considered low, $I^2 > 50\%$ moderate, and $I^2 > 75\%$ high heterogeneity.[20]

3 | RESULTS

A total of 1,489 studies were screened, with a final 12 relevant studies with a total of 3,369 patients who met the selection criteria (Figure 1). All included studies were observational and included patients undergoing PCI[18,19,21-28] or medical management only.[29-31] Complete articles[21,24,26-31] and abstracts[18,19,22,23,25] presented at meetings were both included if they met the relevant criteria.

Publication dates for the included studies were between 1971 and 2019, with the follow-up period for patients ranging from

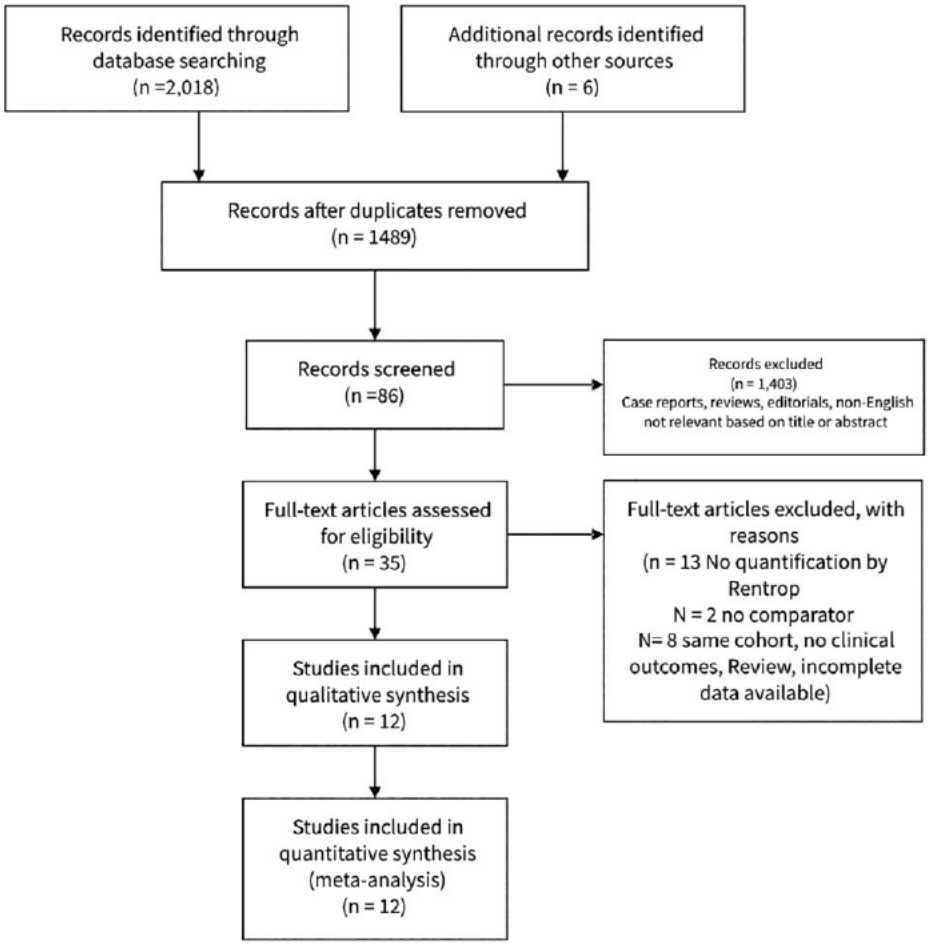

FIGURE 1 Flow diagram of included studies

TABLE 1 Publications included in the meta-analysis

Name	Year	Study types	Management of CTO	Patients	Robust collaterals	Follow-up	% Females	Mean age	NOS
Helfant	1971	Article	Medical (revascularisation excluded)	89	45 (50.6%)	1.81 years	Unknown	Unknown	6
Nestico	1985	Article	Medical (revascularisation excluded)	359	183 (51.0%)	6.8 years	29.0%	67	8
Hansen	1989	Article	Medical therapy	96	29 (30.2%)	10 years	9.3%	48.8	8
Rha	2010	Abstract	PCI	177	99 (55.9%)	6 months	Unknown	Unknown	3
Kazik	2010	Abstract	PCI	55	48 (87.3%)	7–10 years	14.5%	54	7
Moynagh	2010	Abstract	PCI	960	633 (65.9%)	Nil	Unknown	63.1	8
Jaguszewski	2010	Abstract	PCI	238	119 (50%)	Nil	28%	61	5
Rha (2)	2010	Abstract	PCI	225	146 (64.9%)	6 months	Unknown	Unknown	3
Luo	2015	Article	PCI	108	102 (94.4%)	Nil	42.6%	60.8	8
Di Serafino	2016	Article	PCI	442	219 (49.5%)	24 months	19%	65.5	6
Yang	2017	Article	PCI	216	132 (61.1%)	18 months	15%	61.4	5
Allahwala	2019	Article	PCI	275	242 (88%)	1.9 years	18.4%	69.2	6
Tian	2019	Article	PCI	285	195 (68.4%)	Nil	23.9%	63.9	5

Abbreviations: CTO, coronary chronic total occlusions; PCI, percutaneous coronary intervention.

6 months to 19 years. The numbers of patients in each study were variable and ranged between 55 and 960 patients. The percentage of patients with robust collaterals ranged between 30.2% and 94.4%. Most studies used the Rentrop classification for determination of the degree of collateral recruitment, although three studies used other grading systems, which were analogous[29-31] to the Rentrop system. The results of the studies that evaluated effect of the collateral circulation on outcomes are shown in Table 1.

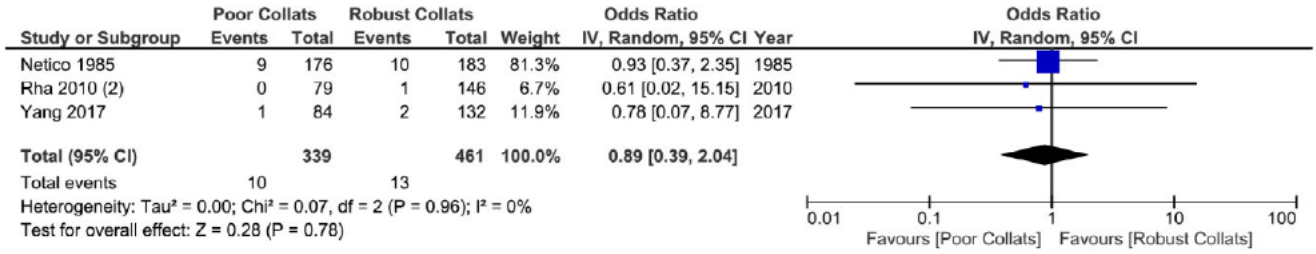

FIGURE 2 Risk of acute myocardial infarction in patients with robust versus poor collaterals [Color figure can be viewed at wileyonlinelibrary.com]

FIGURE 3 Risk of mortality in patients with robust versus poor collaterals [Color figure can be viewed at wileyonlinelibrary.com]

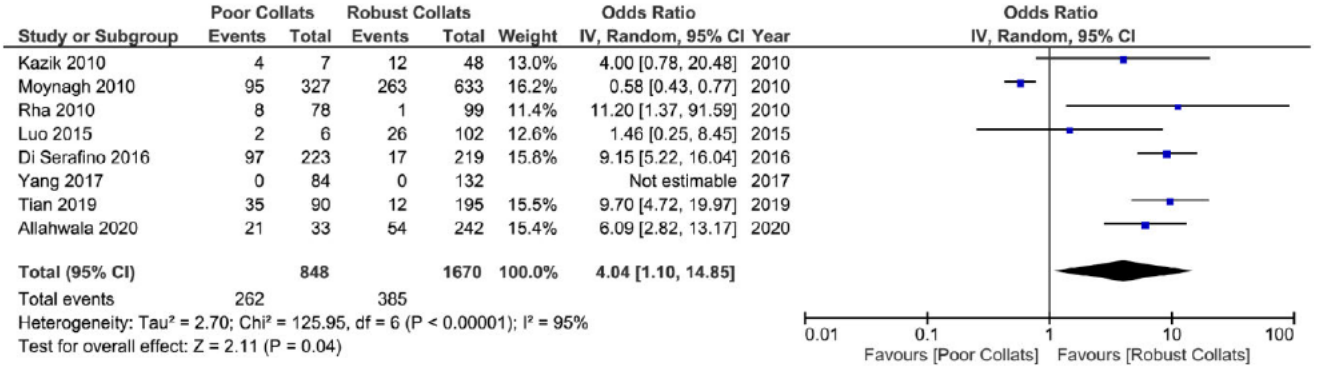

FIGURE 4 Likelihood of successful percutaneous coronary intervention in patients with robust versus poor collaterals [Color figure can be viewed at wileyonlinelibrary.com]

There was no difference in risk of AMI in patients with robust collaterals compared to those with poor collaterals (OR: 0.89, 95%CI: 0.39–2.04) among the three studies which reported this outcome. There was a very low degree of heterogeneity between studies (I^2 = 0%) (Figure 2).

Similarly, there was no difference in the risk of all-cause mortality in patients with robust collaterals compared to those with poor collaterals (OR: 0.81, 95% CI: 0.42–1.58) among the six studies which reported on this outcome. There was a very low degree of heterogeneity between studies (I^2 = 22%) (Figure 3).

There was a significantly higher likelihood of successful revascularization in patients with robust collaterals compared to those with poor collaterals (OR: 4.04, 95%CI: 1.10–14.85) among the eight studies which reported this outcome. There was a high degree of heterogeneity between studies (I^2 = 95%) (Figure 4).

4 | DISCUSSION

In our meta-analysis of 12 studies including 3,369 patients with a chronic total occlusion, the presence of robust collaterals did not result in lower rates of all-cause mortality or AMI, but was associated with a greater likelihood of successful revascularization.

In patients with stable coronary disease, the degree of ischemic burden has consistently been associated with poorer prognosis as assessed by stress echocardiography,[32,33] nuclear imaging,[34] and

cardiac myocardial resonance imaging (CMR).[35,36] Given patients with a CTO are in a chronic state of ischemia,[8] it is unsurprising that this cohort of patients is associated with poorer outcomes. While it may be expected that more robust angiographically assessed coronary collaterals would be associated with a lower degree of ischemic myocardium, previous studies have shown no correlation between the Rentrop classification of collaterals and invasively determined myocardial perfusion.[37] This is likely a reflection of the complex interplay of effects of the microcirculation, collateral steal, and endothelial function as occurs in the setting of a CTO.[38] It is thus unsurprising that given there appears to be little correlation between angiographic collateral assessment and myocardial perfusion, no difference in mortality rates were seen in our study. While a number of reasons are used to justify the lower rates of invasive management strategy in patients with a CTO, the degree of collateral formation should not justify this approach.

This finding is different to a previous meta-analysis,[39] which found a 36% reduction in mortality in those patients with robust collaterals compared to those with poorer collaterals. However, while all 12 of the studies included in our analysis were chronic occlusions, the previous meta-analysis, which also had 12 studies, included six in the setting of an AMI, one within a month of an AMI, one without angiographic grading of collaterals, and one with a mean diameter stenosis of 80%. Clearly, such a heterogenous group may have implications on prognosis, particularly as in the setting of an ACS, the degree of collateral recruitment is associated with improved outcomes.[14] The same may not however be true in the setting of stable coronary disease. Furthermore, well-developed coronary collaterals do not correlate with PET derived myocardial blood flow,[40] suggesting robust collaterals do not prevent ischemia, and hence may not sufficiently obviate the risk of subsequent mortality.

Another mechanism for an absence of mortality benefit in those patients with robust collaterals may be an increase in the risk of ventricular arrhythmias in those patients as compared to those with poorer developed collaterals, as has been previously reported.[7,41] This apparent paradoxical finding has been attributed to greater hibernating myocardium in the peri-infarct zone with electrical instability especially in the border zone of partially necrotic areas, and may explain no benefit seen in the setting of robust collaterals.

In a substudy of the EXPLORE trial,[42] in which patients presenting with a STEMI and a concurrent CTO, were randomized to either medical management or PCI, 23.1% of patients had what the authors described as "collateral decay" whereby the degree of angiographically appreciated collaterals were less robust at the time of CTO PCI, as compared to diagnosis during their index STEMI procedure, at a mean of 5 days later.[9] Furthermore, those with collateral decay had a significant improvement in left ventricular function, which was not observed in those patients without decay. This suggests that the degree of collaterals may be dynamic, reflecting hemodynamic, inflammatory and biochemical variations, which may explain why no prognostic benefit with robust collaterals was observed.

We found no correlation between the degree of collateral recruitment and future risk of AMI. The ability to recruit collaterals upon repeated acute coronary occlusion appears to be stable, regardless of which epicardial vessel is occluded.[43] Similarly, in the setting of a CTO, coronary collateral flow does not completely regress following PCI,[44] suggesting the ability to recruit collaterals is maintained. Despite this, we found no difference in risk of AMI in those with robust collaterals. This may be a manifestation of the relatively few number of events included in the studies, or alternatively may reflect the fact that even with collateral recruitment, ischemia in the CTO territory persists. Furthermore, some studies have suggested that drug eluting stent implantation[45] may impair collateral recruitment and function, which may also influence the outcomes.

Patients with a robust collaterals were more likely to have successful PCI than in those with poorer collaterals. Only one study[25] suggested that those with poorer collaterals had a higher success likelihood of CTO PCI, although they initially classified patients on the degree of ipsilateral collaterals, which may have introduced bias into the study. The greater likelihood of successful CTO PCI is likely multifactorial, including a degree of selection bias, with those with poorer collaterals having true or perceived nonviable myocardium, better visualization of the true distal vessel, as well as a retrograde option for revascularization. Despite the fact that a better degree of collateral recruitment improves CTO PCI success likelihood, the presence of robust collaterals is often a reason why physicians may defer to medical treatment, believing less need to revascularize given the angiographically apparent collaterals.

5 | LIMITATIONS

There are important limitations with our analysis, which need to be considered. First, the degree of collaterals is a visual estimate, and not all studies reported how a consensus was reached. More objective measures of collateral function while less affected by interobserver variability are cumbersome and without risk, and hence cannot be routinely justified to be carried out. There was a degree of heterogeneity between the included studies, particularly as three studies[29-31] were in the era preceding modern PCI strategies. However, they do provide insight into the natural history of patients with a CTO who do not undergo revascularization and hence are of benefit to consider and likely still relevant in the contemporary era. While we have shown no difference in rates of AMI or mortality in patients with robust collaterals compared to poorer collaterals, we cannot conclude whether the degree of collaterals should influence patient selection for PCI. Indeed, while PCI is more likely to be successful in patients with mature collaterals, this does not seem to impact on prognosis. This finding is in keeping with randomized data of the effect of revascularization in stable coronary disease with respect to prognostic outcomes.[46,47]

6 | CONCLUSION

In patients with a CTO, the degree of collateralisation does not affect risk of mortality or AMI. However, patients with robust collaterals are

more likely to have successful CTO PCI. The degree of collateralisation should not be used to defer or justify revascularization.

CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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SECTION V: CORONARY PHYSIOLOGY AND BIOMARKERS IN THE SETTING OF CORONARY COLLATERALS

Chapter 8: Applicability and Interpretation of Coronary Physiology in the Setting of a Chronic Total Occlusion

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Circulation: Cardiovascular Interventions

CONTEMPORARY REVIEW IN INTERVENTIONAL CARDIOLOGY

Applicability and Interpretation of Coronary Physiology in the Setting of a Chronic Total Occlusion

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ABSTRACT: Concurrent coronary artery disease in a vessel remote from a chronic total occlusion (CTO) is common and presents a management dilemma. While the use of adjunctive coronary physiology to guide revascularization is now commonplace in the catheterization laboratory, the presence of a CTO provides a unique and specific situation whereby the physiological assessment is more complex and relies on theoretical assumptions. Broadly, the physiological assessment of a CTO relies on assessing the function and regression of collaterals, the assessment of the microcirculation, the impact of collateral steal as well as assessing the severity of a lesion in the donor vessel (the vessel supplying the majority of collaterals to the CTO). Recent studies have shown that physiological assessment of the donor vessel in the setting of a CTO may overestimate the severity of stenosis, and that after revascularization of a CTO, the index of ischemia may increase, potentially altering the need for revascularization. In this review article, we present the current literature on physiological assessment of patients with a CTO, management recommendations and identify areas for ongoing research.

A coronary chronic total occlusion (CTO) is defined as the complete occlusion of a coronary artery, defined as thrombosis in myocardial infarction grade 0 flow, present for at least 3 months.[1] Radiographically, this is appreciated by the filling of the distal occluded vessel by collaterals. The incidence of a CTO ranges between 11% and 52%,[2–6] with 6.6% of patients with an ST elevation myocardial infarction having a concurrent CTO.[7] A CTO is associated with higher mortality,[3] greater occurrence of ventricular arrhythmias,[8] and symptoms of angina.[9]

The presence of concurrent coronary artery disease in vessels remote from a CTO is common, with ≈50% of patients with a CTO needing revascularization of another vessel.[10] Deciding whether a vessel needs revascularization or not should not only be based on angiographic characteristics but can be improved with adjunctive use of coronary physiology.[11–14]

Broadly, the physiological assessment and considerations for CTOs can be described as:

1. Physiological assessment of collateral function
2. Physiological assessment of lesions in the donor vessel
3. Changes in the myocardial territory supplied by the donor vessel after CTO revascularization
4. Effect of collateral steal

Key Words: catheterization ■ incidence ■ microcirculation ■ myocardial infarction ■ thrombosis

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In this article, we summarize the current understanding of coronary hemodynamics of collaterals and donor vessels in the setting of a CTO and the current evidence base for its implementation in clinical practice.

ANATOMIC AND PHYSIOLOGICAL ASSESSMENT OF COLLATERAL FUNCTION

Assessment of both the presence and functionality of collaterals is vital to determine the likelihood of symptoms, prognosis, and strategies for percutaneous revascularization. Traditionally, angiographic assessment has been the preferred method of quantifying coronary collaterals because of ease and familiarity. The Rentrop Classification and the collateral connection grade are 2 anatomic methods of describing collaterals. The Rentrop classification[15] is based on the degree of filling of the occluded epicardial vessel, whereas the collateral connection grade[16] is based on the size of the collaterals, rather than their ability to opacify the epicardial vessel. Given the size and caliber of a collateral is often more relevant when attempting CTO percutaneous coronary intervention (PCI), this is often preferentially used in predicting the chance of collateral crossing(Table 1; Figure 1).

While these anatomic methods of grading collaterals are practical and easy to calculate, they do not necessarily correlate with perfusion. Initial studies using coronary wedge pressure through a fluid-filled balloon catheter[17] and blood flow velocity[18] were found to be better predictors of myocardial ischemia than angiographic grading of collateral vessels alone.[19] In fact, Werner et al[16] showed that there was no relationship between Rentrop classification and functional assessments of collateral function in patients with a CTO.

The quantification of the collateral contribution of total coronary flow can be determined by flow and pressure indices, which have been derived from experimental models of collateral recruitment.

The velocity-derived collateral flow index[20] can be calculated by dividing blood flow velocity in the occluded vessel (either during balloon coronary occlusion or spontaneously in the setting of a CTO) by blood flow velocity in the absence of balloon occlusion. Given in a true CTO, theoretically, all flow distal to the occlusion is from collaterals and the velocity-derived collateral flow index can be directly measured after successful antegrade crossing. However, given the technically challenging and operator-dependent issues with flow Doppler wire measurements, pressure-wire assessment modalities have been developed to characterize collateral flow in a quicker and more reproducible manner.

The pressure-derived collateral flow-index (CFI_P) is calculated as $(P_{distal}–CVP)/(P_{proximal}–CVP)$, where P_{distal} refers to pressure distal to the CTO, and $P_{proximal}$ refers to aortic pressure. The more developed the collateral circulation, the closer the CFI_P to 1. The CFI_P correlates well with velocity-derived collateral flow index[21] and is easier to obtain with better reproducibility as its signal is not critically dependent upon the position of the guidewire tip in the epicardial segment.[20] A CFI_P of <0.23 has been used as the cutoff for the presence of inducible ischemia and has been associated with more ischemic events at 1 year as compared with patients with a higher CFI_P.[22] Other studies have suggested that in patients with single vessel disease or no coronary disease, a CFI_P<0.25 is an independent predictor of long-term cardiovascular mortality at 10 and 15 years.[23,24]

These methods provide good insight into the acute recruitment of collaterals in the setting of balloon occlusion of an epicardial vessel. However, these cutoffs have not been tested in the setting of a CTO. Furthermore, the method of determining collateral flow and pressure distal to a CTO relies on passing a coronary wire distal to the occlusion in an antegrade manner and then using a microcatheter to pass a Doppler or flow wire to this location.

As a result of these limitations, and the inefficient method of acquiring these recordings, which would increase procedural time for an already lengthy procedure, as well as an absence of evidence for its clinical applicability, collateral physiological assessment has, to date, remained only a research tool.

CORONARY PHYSIOLOGY OF THE DONOR VESSEL

Hemodynamic assessment of the coronary circulation, using pressure as a surrogate for flow can be used to identify the ischemic potential of a lesion, and, in clinical practice most commonly involves measurement of either the fractional flow reserve (FFR) or instantaneous wave-free ratio (iFR). FFR is a lesion-specific index, defined as the ratio of maximal hyperemic myocardial blood flow, or maximum vasodilatation, across a stenotic artery to the maximal myocardial blood flow across the same artery in the theoretical absence of the stenosis. The FFR can simply be derived as a ratio of the mean distal coronary pressure (P_d) at a point past the stenosis to the aortic pressure (P_a) during maximal hyperemia where $FFR=P_d/P_a$. As with FFR, iFR also relies on the proportional relationship of pressure and flow that occurs with constant intracoronary resistance. However, instead of requiring pharmacological vasodilatation to achieve this, it is measured over a diastolic wave-free period in the cardiac cycle where intracoronary resistance is naturally constant.[25] iFR is calculated as the ratio of distal to proximal pressures over this wave-free period.

Table 1. Anatomic and Functional Classification of Collaterals

Anatomic	
Rentrop classification	
0	No filling of collaterals or recipient vessel
I	Filling of side branches of the recipient artery via collateral channels without visualization of the epicardial segment
II	Partial filling of the epicardial segment via collateral channels
III	Complete filling of the epicardial segment of the recipient artery via collateral channels
CC grade	
0	No continuous connection between donor and recipient artery
1	Continuous, thread-like connection (diameter ≤0.3 mm)
2	Continuous, small, side-branch-like size of the collaterals throughout its course (diameter ≥0.4 mm)
Functional	
Collateral flow index	
CFI≥0.23	High collateralization
CFI<0.23	Low collateralization (associated with inducible ischemia)

CC indicates collateral connection; and CFI, collateral flow index.

Although FFR is reported to be independent of changing hemodynamics,[26] it is closely related to coronary flow.[27,28] Hence, in the setting of a donor vessel supplying collaterals to perfuse the territory of myocardium subtended by the CTO, the total amount of blood flow through the donor vessel is higher. As a result, this would influence any pressure gradient across a lesion, with an FFR that would be lower than if the vessel was not supplying the collaterals (as there would be a smaller myocardial territory supplied by the donor vessel). Similarly, after revascularization of the CTO, there would be a reduction in donor flow through collaterals as they regress, resulting in a higher FFR across the same lesion in the donor vessel. Indeed, relatively small invasive hemodynamic studies have shown, that after CTO PCI, the absolute coronary flow in the donor vessel supplying the CTO territory is significantly reduced, as a result of a reduction in myocardial territory perfused and a reduction in collateral donation[29] (Figures 2 and 3).

In vivo animal models pioneered by Gould[30] and Young et al[31]have demonstrated a close relationship between the severity of a stenosis and change in flow with resultant pressure gradient alterations. That is, a less severe lesion can accommodate a larger increase in flow, before there is a change in pressure gradient (ie, FFR) across that stenosis, as compared with a more severe stenosis.

FFR IN THE DONOR VESSEL

Numerous case reports have reported marked increases in the FFR of a donor vessel after successful CTO PCI (Table 2).[32–37] Of the 6 cases, 5 showed an increase in donor vessel FFR of 0.12 with the other showing an increase of 0.09. Importantly, in 4 of these cases, the FFR crossed the threshold of ischemia (0.80) and altered treatment to defer.

Systematic studies have also investigated the effect on FFR of the donor vessel pre- and post-CTO PCI. Sachdeva et al[38] in a study of 50 patients with an intermediate lesion (30%–70%) in the donor vessel performed coronary hemodynamic assessment. Fourteen patients had a successful CTO PCI, of which 9 (64.3%) patients had an ischemic donor vessel FFR<0.80. Of these 9, after CTO PCI, 6 reverted to nonischemic FFR (mean change of 0.10±0.04), whereas 3 remained ischemic (mean change of −0.01±0.04). Four patients remained nonischemic, with a mean change in FFR of 0.002±0.04. The interrogated artery in most cases (92.9%) was the left anterior descending. Ladwiniec et al[29] showed that in 34 consecutive patients with successful CTO PCI, there was a mean increase in the donor vessel FFR of 0.02 with an associated reduction in absolute flow and hyperemic flow in the donor vessel. They also showed that a greater increase in FFR was seen with greater coronary stenosis as assessed by angiography and coronary flow velocity-pressure gradient, with a reduction in flow across the stenosis associated with an increase in FFR.

In a series of 8 consecutive patients undergoing CTO PCI,[39] 4 had Rentrop Grade ≥2 collaterals, whereas the remainder had Rentrop Grade 1 or 0 collaterals. In the patients with the anatomically well-developed collaterals, there was a significant increase in the FFR of the donor vessel after CTO PCI, whereas in the group with anatomically poorly developed collaterals, there was no difference in the FFR, suggesting the importance of collateral flow in interpretation of the FFR.

Disease in the donor vessel itself is also vital for prediction of the change in FFR after PCI of the CTO vessel. In the setting of a single focal lesion within the donor artery, the change in FFR would be expected to be significantly greater than if the donor vessel had diffuse disease with a superimposed focal stenosis, as the presence of diffuse disease results in a lower coronary flow reserve (CFR) but limited reduction in FFR.[40] Consequently, a reduction in total coronary flow would not be expected to result in a significant change in FFR of the donor vessel after CTO PCI.

The assumption, and to some extent validation from clinical studies, is that with successful CTO PCI, the territory of myocardium supplied by the donor vessel is significantly reduced and hence flow reduces in the donor vessel, and the FFR would increase. However, with more contemporary approaches to CTO PCI, including dissection re-entry approaches, which tend to involve longer stented segments, greater disruption of the vascular architecture and a greater tendency

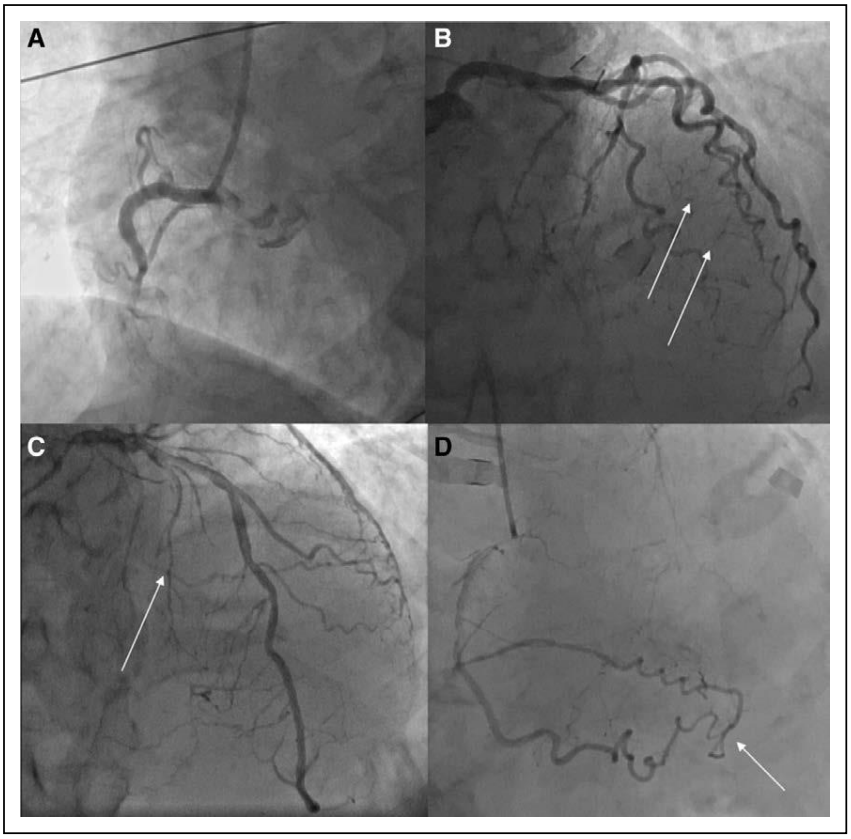

Figure 1. Anatomic classification of coronary collaterals.
Angiographic appearances of collaterals. **A**, Rentrop 0: occluded proximal right coronary artery without any collateral filling. **B**, Rentrop 1: partial filling of side branches of left anterior descending chronic total occlusion (CTO) from left circumflex artery. **C**, Rentrop 2: partial filling of the posterior descending artery by left anterior descending septal and epicardial collaterals. **D**, Rentrop 3: complete filling of right coronary artery CTO by right ventricular branch epicardial collateral. Collateral filling indicated by white arrows.

for side branch occlusion, the proportion of myocardium perfused by the donor vessel may remain large even after CTO PCI. Hence, whether these findings of a small, but perhaps not clinically relevant change in FFR, may be applied generally to patients with a CTO, or may need to be determined on an individualized basis, is uncertain. Furthermore, the initial case reports of large changes in FFR after CTO PCI may be an indication of the inherent variability with pressure wire assessment or else bias in reporting. Even in the systematic studies, there is a variation in FFR changes after CTO PCI, which may reflect the inherent variability with wire assessment but also the heterogenous nature of the donor vessel, with respect to degree of stenosis, location within the vessel (proximal or distal), and presence or absence of diffuse disease. The large discrepancy in changes in FFR underscore the importance of needing systematic, large scale studies.

IFR IN THE DONOR VESSEL

Similar to FFR, iFR is a pressure index that relies on flow, during the phase of the cardiac cycle where resistance is constant. As such, the iFR would also be expected to increase with CTO PCI and reduction of the territory supplied by the donor vessel.

In a study of 34 patients[41] who underwent CTO PCI to the right coronary artery, FFR, iFR, and FFR_{coll} was performed immediately before, after, and 4 months after CTO PCI of the donor vessel (mean stenosis of 41% by QCA). The FFR_{coll} is an invasive measure to determine the collateral flow contribution to total myocardial flow, determined by $(P_w–P_v)/(P_a–P_v)$, where the P_w is the coronary wedge pressure (which in the setting of a CTO is analogous to the pressure distal to the CTO), and P_v is the central venous pressure. This measure allowed interpretation of the effect of both angiographic and physiological coronary collateral regression on FFR and iFR.

Immediately after CTO PCI, there was a significant, but small absolute increase in the iFR (0.86–0.88; $P<0.05$), whereas there was no difference in FFR (0.76–0.75; $P=0.27$) in the major donor vessel. In the minor donor vessel, the vessel contributing the lesser collateral contribution to the CTO by angiography, there was no change in FFR or iFR. At 4 months, there was a significant increase in the iFR (0.86–0.90; $P=0.003$) and FFR (0.76–0.79; $P<0.05$). At 4 months, in the donor vessel, 18% of the FFR and 25% of the iFR were reclassified from ischemic to nonischemic. There was no change in the FFR_{coll} immediately after CTO PCI, but a significant reduction at 4 months (0.31–0.18; $P<0.0001$). Although there is less variation in the iFR value change after CTO PCI compared with FFR, this may reflect the small numbers of patients who have been studied, and further studies are needed.

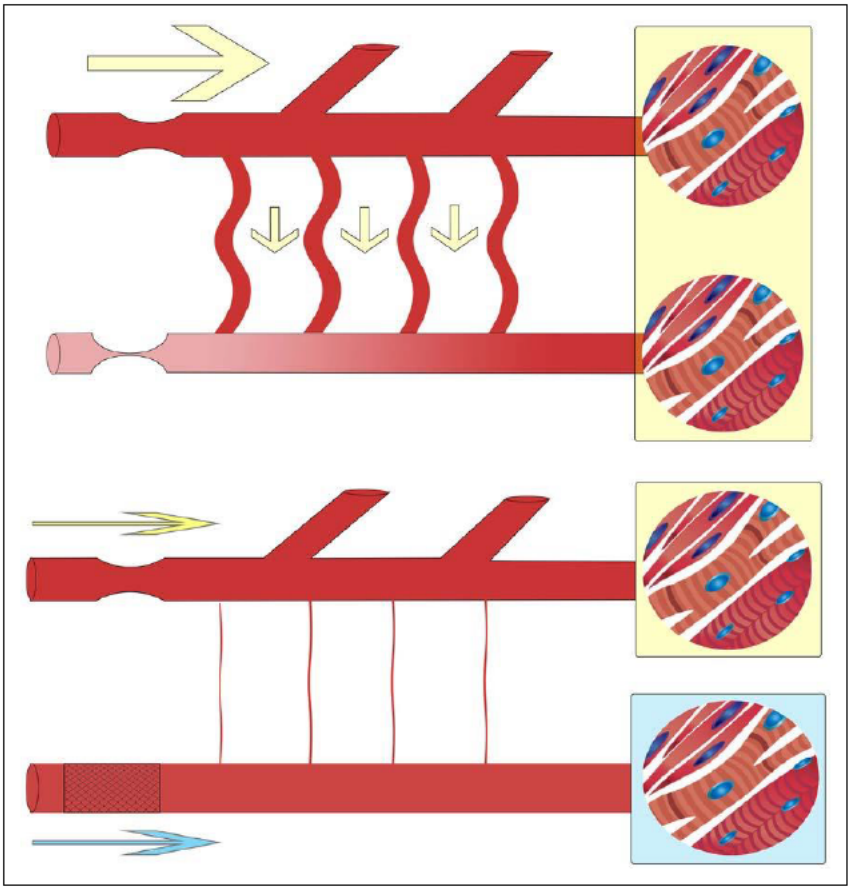

Figure 2. Changes in flow after chronic total occlusion (CTO) revascularization and summary figure.
In the top, the donor vessel is supplying the CTO vessel distal to the occlusion via collaterals. As a result, the territory of myocardium supplied by the donor vessel is larger (in yellow box) and overall flow in the donor vessel and across the proximal stenosis is greater. In the bottom, there is restoration of anterograde flow in the CTO with the myocardium subtending that vessel, now supplied by the previously occluded vessel (blue box). Consequently, there is regression of the collaterals with a smaller territory of myocardium supplied by donor vessel (yellow box), resulting in less flow in the donor vessel (narrower yellow arrow).

COLLATERAL REGRESSION

It is evident that both hyperemic and resting indices of lesion severity require an appreciation of the territory of myocardium supplied. As such, it is imperative to determine when there is regression of the collaterals (immediate or late), in the setting of a CTO, and whether this is a dynamic or fixed process. It is readily apparent that after CTO PCI, with restoration of anterograde blood flow, angiographically appreciable collaterals regress and are not visible. However, there remains some degree of blood flow through these collaterals. There has been significant variation in studies relating to the speed of regression of collaterals, and an appreciation of this controversy is paramount to subsequent interpretation of hemodynamic indices of the donor vessel.

Werner et al[42] found that immediately after CTO PCI in 103 patients (median 31 minutes after baseline recording), the velocity-derived collateral flow index decreased from a median of 0.44± 0.33 to 0.22± 0.15, with no further change at 5 months. However, this may have been a result of the difficulty of obtaining reliable and reproducible flow signals, as the CFI_p decreased by 23% immediately after recanalization of the CTO and a further 23% at follow-up at 5 months. The mechanism of this regression was felt to be related to changes in flow and consequently tone in the coronary circulation. In the setting of a CTO, both the collateral vessel and the microvascular arteriole are in a state of maximal hyperemia [see Section on collateral steal] and hence resistance in both are relatively low. After CTO PCI, the arteriolar tone increases, as a result of the autoregulatory potential of the microvasculature to respond to improved perfusion, and hence resistance increases. In the study by Werner et al,[42] the resistance index in the collateral circulation was much greater than arteriolar resistance index, both immediately after CTO PCI, and at 4 months, suggesting this preferential increase in tone contributes to the early collapse and closure of the collateral circulation.

Similarly, invasive measures of flow in the donor vessel pre- and post-CTO PCI show a reduction in absolute blood flow, with the change in flow associated with markers of endothelial activation and arteriogenesis.[43] In a small study of 18 patients undergoing CTO PCI, Petronio et al[44] found that the coronary collateral circulation, as assessed by contrast echocardiography, regressed significantly by 15 minutes after PCI. Werner et al[45] showed that in 21 patients with CTO, after PCI to the occluded segment, the CFI reduced by >50% immediately, with no further reduction after 24 hours. Zimarino et al,[46] in 42 patients with a CTO, noted that after PCI of the occluded segment, there was a rapid reduction in blood flow attributable to the collateral circulation. With subsequent balloon inflations of the vessel, there was

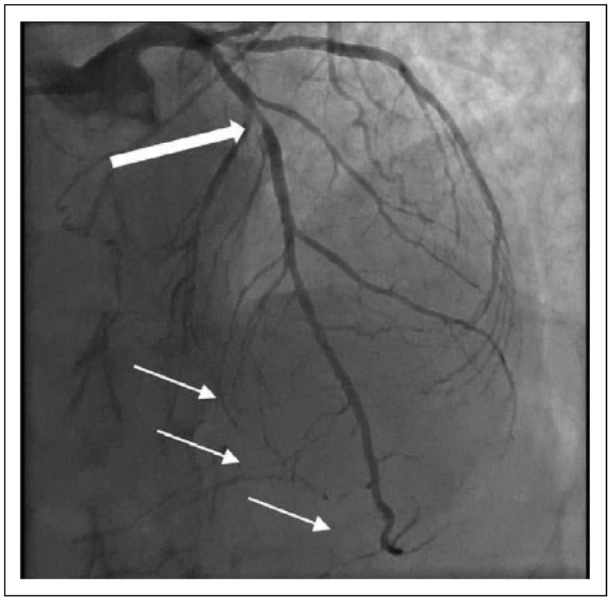

Figure 3. Clinical example of moderate lesion in a donor vessel.
A moderate lesion in proximal left anterior descending (LAD; thick white arrow) supplying collaterals to perfuse the chronic total occlusion (CTO) of the right coronary artery (RCA; thin white arrows). Fractional flow reserve (FFR) performed of LAD lesion 0.77. After RCA CTO percutaneous coronary intervention, repeat FFR of LAD was 0.80. The increase in FFR reflects a smaller territory perfused by the LAD after RCA CTO revascularization.

evidence of ST elevation and symptomatic chest pain, suggesting early (and irreversible) regression of the collaterals. Indeed, other studies have also suggested that after CTO PCI, pressure derived recruitable collateral function diminishes rapidly within an hour to its baseline and is not immediately recruitable with balloon occlusions of the newly revascularized vessel,[45,46] suggesting early, and possibly irreversible collateral regression.

However, not all studies agree with this rapid regression of anatomically and functionally active collaterals. Lee et al[47] measured the CFI_p in 74 patients with a CTO, as well as 23 patients with an acute coronary syndrome with collaterals. They found that immediately after PCI and restoration of blood flow, there was no change in the CFI_p in either CTO patients or acute coronary syndrome patients. At 1 year, however, there was a significant reduction in CFI_p from baseline. They argued that immediate anatomic remodeling of preexisting collaterals is implausible and requires time to develop. Other studies have similarly suggested that the collateral supply remains relatively unchanged for at least 24 hours after PCI of the occluded segment. Perera et al[48] measured CFI_p at 5 minutes, 24 hours, and 6 months after CTO or high-grade lesion, revascularization. They, similar to Lee et al,[47] found that there was no significant change in CFI_p from baseline at 5 minutes or 24 hours post; however, there was a significant reduction in in CFI_p at 6 months.

It thus seems apparent that while there is an immediate significant reduction in blood flow in the collateral vessel after CTO PCI, there remains some degree of flow. The natural history of these vessels remains uncertain and require further studies to elucidate.

COLLATERAL STEAL

Collateral (or coronary) steal is defined as an absolute or relative fall in coronary blood flow to a vascular region in favor of another supply area under conditions of hyperemia, that is, a CFR of <1.[49,50] This variation in regional blood flow under hyperemic conditions is estimated to occur in 10% to 30% of patients with coronary artery disease and in 25% of patients with a CTO.[29] The phenomenon of coronary steal has also been proposed as the reason for worse prognosis in patients with acute coronary syndromes receiving dihydropyridine calcium channel blockers,[51] and the mechanism for more ischemic events in patients with well-developed collaterals receiving nifedipine.[52]

The effect of vasodilators, such as adenosine, on flow in the collateral-dependent myocardium is often paradoxical. Multiple surgical and nuclear perfusion studies have shown that vessels with severe stenoses are in a constant state of maximal vasodilation in an effort to maintain resting perfusion.[53,54] Therefore, there is limited, if any, vasodilatory reserve, and no response to pharmacological vasodilators such as adenosine. The effect is similar in the setting of CTOs.[53,55,56] However, not only is there no increase in flow of the collateral-dependent myocardium under pharmacological stress,

Table 2. Summary of Published Case Reports of Change in Donor Vessel FFR After CTO PCI

	CTO Vessel	Donor Vessel	Pre CTO-PCI FFR	Post CTO-PCI FFR	Change	Change in Strategy
Iqbal et al[32]	RCA	LAD	0.72	0.84	0.12	Deferred
Sachdeva and Uretsky[33]	RCA and LCx	LAD	0.71	0.83	0.12	Deferred
Melikian et al[34]	RCA	LAD	0.75	0.84	0.09	Deferred
Matsuo and Kawase[35]	RCA	LAD	0.81	0.93	0.12	Remained negative
Sachdeva[36]	RCA	LMCA	0.73	0.85	0.12	Stented because of tandem LAD lesions
Kurisu et al[37]	LAD	RCA	0.84	0.96	0.12	Remained negative

CTO indicates chronic total occlusion; FFR, fractional flow reserve; LAD, left anterior descending; LCx, left circumflex artery; LMCA, left main coronary artery; PCI, percutaneous coronary intervention; and RCA, right coronary artery.

there is often a reduction in perfusion because of the phenomenon of coronary or collateral steal.

The mechanism of collateral steal is based on redistribution of blood flow from the collateral circulation to the microcirculation of the donor vessel. This occurs because of a fall in the perfusion pressure at the origin of the collateral vessels because of increased resistance to flow during hyperemia, comparative to the microcirculation of the donor vessel, which is able to vasodilate.[57] The ability to vasodilate results in an increase in conductance (and reduced resistance) above that of the low and fixed conductance of the collateral-dependent myocardium.

As illustrated in Figure 4, in the theoretical situation of a CTO of a vessel with collateral flow, we assume total perfusion of 50 mL/minute in the collateral circulation and 100 mL/minute in the donor vessel microcirculation. Under vasodilatory stress, the microcirculation of the donor vessel will vasodilate to a greater degree than the exhausted collateral dependent myocardium, resulting in increased flow in the donor vessel myocardium, 125 mL/minute, and thus a reduction in collateral flow (25 mL/minute). This results in net myocardial perfusion during hyperemia to be less than at resting conditions of the myocardium subtended by the occluded vessel. Depending on whether a lesion in the donor vessel is proximal or distal to the majority of collaterals, this will have impacts on the pressure gradient across this lesion.

In a study of 100 patients coronary artery disease undergoing angiography, Seiler et al[58] found that 10% of patients displayed coronary steal, with a 40% reduction in collateral flow during intravenous adenosine in these patients. In patients with well-developed collaterals, the effect of intravenous adenosine on reduction of coronary flow was greater, suggesting more coronary flow in patients with better collateral circulation.

Werner et al[56,59] found that in patients with a CTO, 1 of 3 have of patients exhibit collateral steal, whereas 1 of 3 have no change in flow and 1 of 3 have an increase in blood flow in the collateral dependent myocardium. The authors suggest that either a significant reduction in pressure in the donor artery during adenosine infusion (FFR<0.80) or an absence of vasodilatory reserve in the collateral-dependent myocardium along with well-developed collaterals were necessary for collateral steal to occur, while collateral steal is unlikely to occur in the setting of well-developed collaterals.

MICROVASCULAR DYSFUNCTION

The microcirculation may be assessed through the direct measurement of flow within the epicardial vessels to make a determination of the microcirculation. The CFR is defined as the ratio of hyperemic blood flow (Q_{max}) to resting myocardial blood flow (Q_{rest}), where

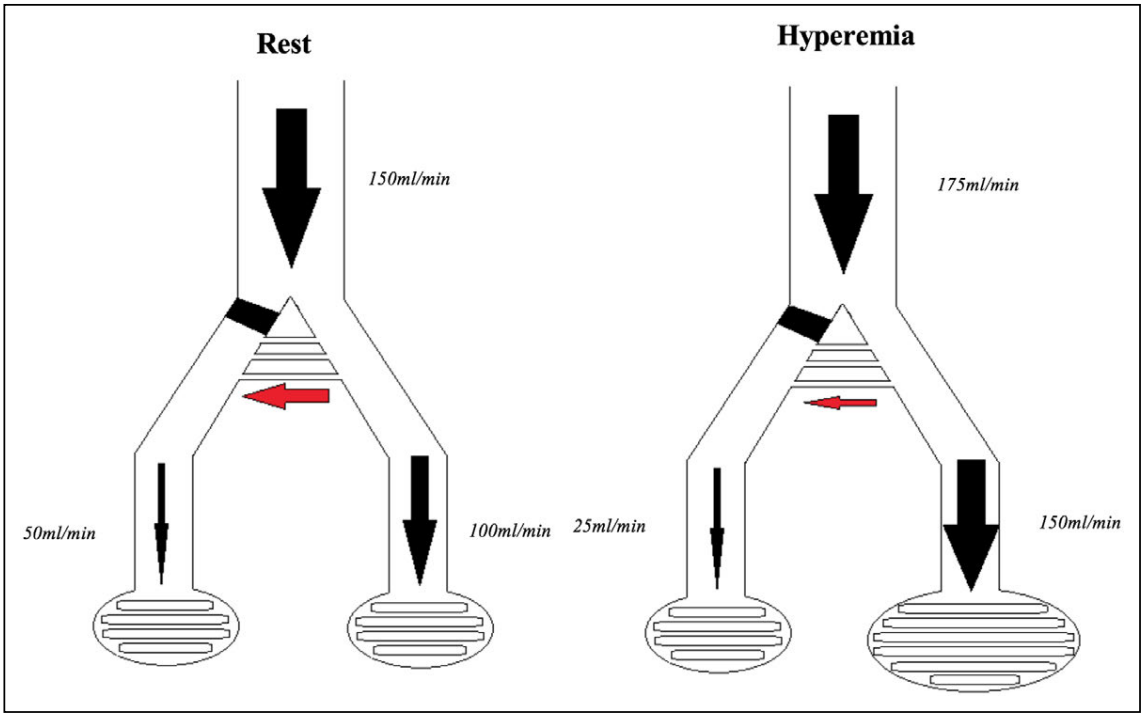

Figure 4. Mechanism of collateral steal.
During resting conditions, perfusion of the myocardium subtended to the chronic total occlusion is supplied by the donor vessel through collaterals. The microcirculation is in a chronic state of vasodilation to increase perfusion pressure. As a result, 100 mL/min flow reaches the donor vessel myocardium and 50 mL/min in the collateral dependent myocardium. Under hyperemia, the donor vessel microcirculation is able to vasodilate far greater than the maximally exhausted collateral dependent myocardium, resulting in preferentially more flow to the donor vessel myocardium (150 mL/min) with a reduction in the collateral-dependent myocardium (25 mL/min). The coronary flow reserve=flow during hyperemia/flow at rest=25 mL/50 mL=0.5 (<1 denotes collateral steal).

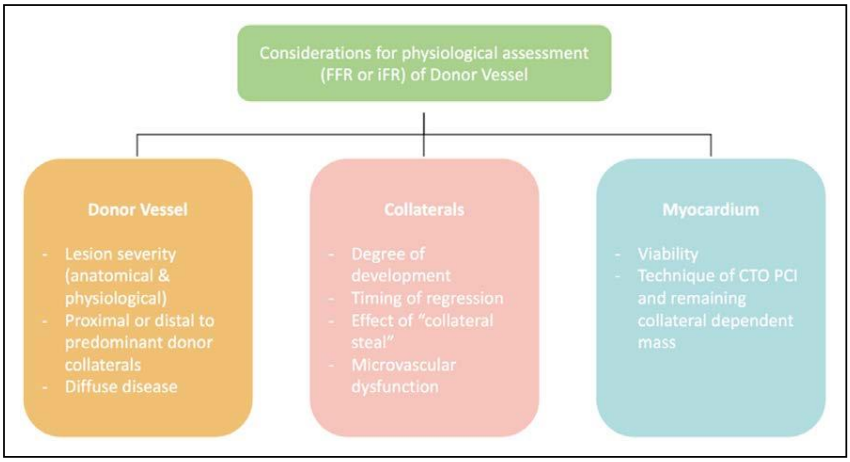

Figure 5. Factors affecting physiological assessment of the donor vessel in the setting of chronic total occlusion (CTO).
FFR indicates fractional flow reserve; iFR, instantaneous wave-free ratio; and PCI, percutaneous coronary intervention.

$CFR=Q_{max}/Q_{rest}$. The CFR is a combined measure of the capacity of the major resistance components, which are the epicardial coronary arteries and microvascular bed to achieve maximal blood flow under hyperemia simulation. Since absolute myocardial flow is not easy to determine, surrogates of flow are used, including flow velocities assessed by a Doppler wire (FloWire or ComboWire; Volcano, Philips Healthcare, Best, the Netherlands) or mean transit time thermodilution using a PressureWire (St. Jude Medical, MN). The major limitation of CFR is the inability to separate the epicardial and microcirculatory components to flow impedance. Hence, the hyperemic microvascular resistance, which uses phasic pressure and velocity measurements for the assessment of the microvasculature is independent of epicardial vessel stenoses and may be more accurate in reflecting microvascular function.

In a study of 21 patients undergoing CTO PCI,[60] the CFR was abnormal in >70% of patients, while also abnormal in approximately half of the non-CTO vessels, suggesting global microvascular dysfunction, although predominantly in the CTO segment. Thirty-three percent of CTO vessels demonstrated an abnormal HMR after CTO PCI, which was similar in non-CTO vessels. The dysfunction appears to be an abnormal autoregulation of microvascular tone.

We have also previously shown[61] that the distal CTO vessel increases in size by 30% on repeat angiography, after CTO PCI, which may be a reflection of early microvascular dysfunction that may be reversible. Indeed, in a study of 120 patients who underwent CTO PCI, Werner et al[62] found that microvascular dysfunction, defined as a CFR<2.0 was seen in 46% of patients immediately after CTO PCI, with the CFR improving in 20% of patients during a mean follow-up of 5 months, suggesting a transient microvascular dysfunction in some, but not all, patients with a CTO.

Consequently, the early but reversible microvascular dysfunction in CTO vessels may explain the mechanism of collateral steal, as well as indicate why pressure wire assessment in the donor vessel may not change significantly after CTO PCI (Figure 5).

CLINICAL IMPLICATIONS

The clinical situation of coronary artery disease remote from a CTO is a commonly faced scenario. Thorough and accurate assessment of lesion severity is vital, as

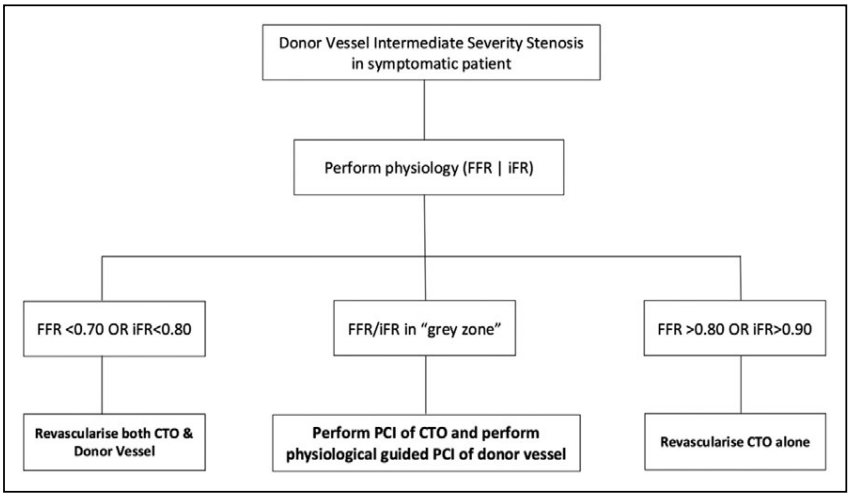

Figure 6. Proposed algorithm for assessment of donor vessel stenoses.
CTO indicates chronic total occlusion; FFR, fractional flow reserve; iFR, instantaneous wave-free ratio; and PCI, percutaneous coronary intervention.

it will dictate the appropriate management strategy and may alter surgical revascularization to either percutaneous or else medical management. Current available data suggest that there is a modest but constant increase in the donor vessel FFR of between 0.02 and 0.04 after CTO PCI.

As such, we would recommend the below strategy (Figure 6) of assessing donor vessel lesion severity. Initially, if a lesion in the donor vessel seems angiographically intermediate in severity, either FFR or iFR should be performed. If the value is above the threshold for ischemia, it would, with a relatively high degree of sensitivity, predict that even after revascularization of the CTO, and reduction in flow across the donor vessel, the lesion would remain nonischemia inducing. If the FFR or iFR is significantly ischemic, such as an FFR<0.70 or iFR<0.85, we would recommend revascularization of both vessels as the expected modest increase after CTO revascularization would still render these lesions ischemic. Moreover, revascularization of the donor vessel may be needed before CTO PCI to prevent ischemia when retrograde crossing is used via the donor vessel. In the gray zone of an FFR 0.7 to 0.8 and an iFR 0.85 to 0.9, we would recommend a multidisciplinary team discussion to determine the best approach to revascularisation, with PCI of the CTO preferred first, to not only unmask the ischemia potential of the donor vessel but also to minimize the risk of a large territory of ischemia in the setting of slow flow or procedural complication of the donor vessel.

CONCLUSIONS

Physiological assessment of a vessel remote from a CTO requires careful consideration of the physiology of collaterals, the microcirculation, as well as local physiological changes. An appreciation of this is vital to the comprehensive physiological assessment of patients with a CTO and may alter management. While studies have shown that the FFR and iFR overestimate the severity of a lesion supplying a CTO compared with if it were not, there is significant heterogeneity in this situation depending on the caliber of the collaterals (both physiological and anatomic), the rapidity of regression, chronicity of the process, as well as remaining collateral-dependent myocardium after CTO PCI. Ongoing studies looking at the mechanisms of alteration of physiology after revascularization of the CTO are vital to determine the widespread applicability of coronary physiology in this clinical scenario.

ARTICLE INFORMATION

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Chapter 9: The impact of coronary collaterals on pressure and flow during percutaneous coronary intervention for chronic total occlusion

9.1 Abstract

Background: Concurrent coronary disease in patients with a chronic total occlusion (CTO) is frequently identified during angiography. Fractional flow reserve (FFR) is a commonly used modality to assess the significance of a coronary lesion in producing ischaemia. As FFR is dependent on myocardial blood flow, it is uncertain whether a donor vessel, which supplies collaterals to a CTO, may be accurately assessed with FFR.

Methods: Patients presenting for elective CTO percutaneous coronary intervention (PCI) were included in the study. All patients underwent coronary pressure and flow assessment of the donor vessel using a Doppler wire (ComboWire XT, Phillips Volcano), prior to and following CTO PCI. Indices recorded included the FFR, coronary flow reserve (CFR), peak velocity, coronary blood flow, hyperaemic microvascular resistance (HMR) and hyperaemic stenosis resistance (HSR).

Results: 38 patients were included in the study, of which 28 (93.3%) had successful CTO PCI. 26 patients had paired coronary physiology performed, prior to and following CTO PCI. The mean age was 70.3 with 10% females. The CTO vessel was the left anterior descending artery in 13 cases (43.3%). Following successful CTO PCI, the FFR of the donor vessel increased significantly (0.86 vs 0.89, $p<0.0001$), crossing the ischaemic threshold from <0.80 to >0.80 in 42.9% of patients of patients with an initial FFR<0.80. This change in FFR was driven by a significant reduction in corrected resting coronary blood flow (179.2mL/min vs 153.0mL/min, $p<0.05$). The increase in FFR was seen irrespective of the CTO or donor vessel or the degree of stenosis in the donor vessel. 75% of patients had an abnormal CFR of the donor vessel prior to CTO PCI.

Conclusion: In patients presenting for CTO PCI, the donor vessel has a significant underestimation of FFR, which, following CTO revascularisation and collateral regression, increases by a mean of 0.03, attributable to a reduction in coronary blood flow and associated effects of microvascular dysfunction. Whether this change in coronary blood flow and therefore FFR is maintained over the long term and implications for management should be confirmed.

9.2 Introduction

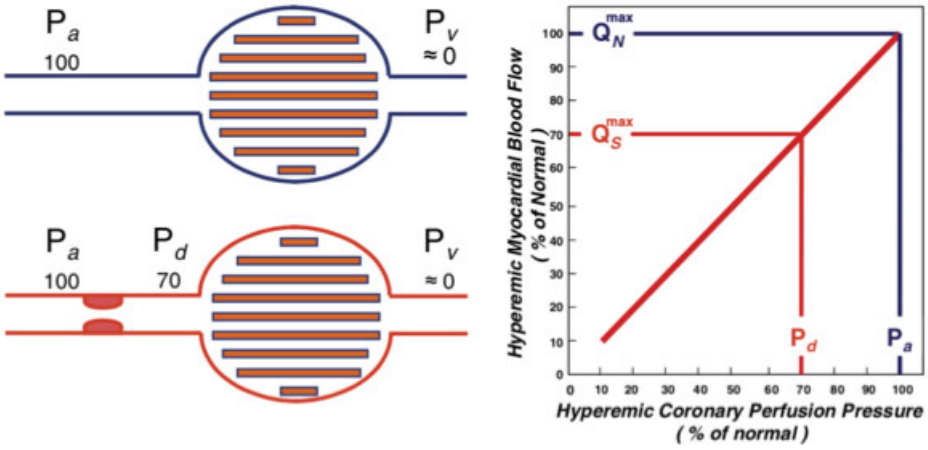
As presented in chapter 8, the presence of functional coronary collaterals results in a significant impact on pressure and flow haemodynamics in patients with a chronic total occlusion (CTO). Approximately 50% of these patients require revascularisation of a vessel remote from the CTO (1). Coronary angiography alone does not correlate with the haemodynamics of the coronary circulation to guide management strategies. The ultimate goal for management of obstructive coronary disease is to relieve flow limiting lesions and thereby improve myocardial oxygenation and perfusion. Blood flow is difficult to accurately measure within the coronary circulation, due to equipment which is challenging to accurately use and reliance on surrogate markers of flow. While pressure and flow, in the basal setting are not proportional, during maximal hyperaemia, commonly achieved through the administration of the potent vasodilator adenosine, resistance is minimal and constant. Therefore in this setting, pressure can be used as a surrogate for flow.

9.2.1 Pressure derived indices of lesion severity

9.2.1.1 Hyperaemic Indices of lesion severity - Fractional Flow Reserve

The fractional flow reserve (FFR) is a pressure derived ratio of the mean coronary pressure (P_d) at a point distal to an epicardial stenosis, relative to the mean aortic pressure (P_a) during maximal hyperaemia where FFR = P_d/P_a. Consequently, the FFR represents the fraction of normal coronary blood flow across a stenosis, with a normal value being 1.0, i.e. pressure (and flow) proximal and distal is equivalent (Figure 9.1).

Figure 9.1: Concept of Fractional Flow Reserve Measurements



When no epicardial stenosis is present (blue lines), the driving pressure P_a determines a normal (100%) maximal myocardial blood flow. In the case of stenosis responsible for a hyperaemic pressure gradient of 30mmHg (red lines), the driving pressure will no longer be 100mmHg but instead will be 70mmHg (P_d). Because the relationship between driving pressure and myocardial blood flow is linear during maximal hyperaemia, myocardial blood flow will only reach 70% of its normal value. This numerical example shows how a ratio of 2 pressure (P_d/P_a) corresponds to a ratio of 2 flows (Q_s^{max}/Q_N^{max}). It also demonstrates how important it is to induce maximal hyperaemia. P_v=central venous pressure. Adapted from (2)

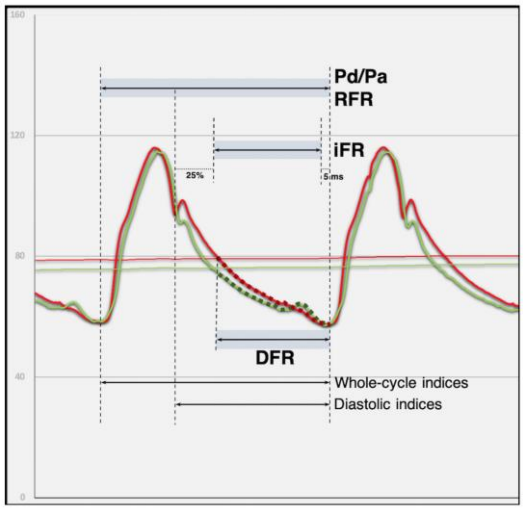
Three large scale randomised trials have been published, namely the DEFER (3), FAME (4) and FAME II (5) trials which initially showed the non-inferiority of deferral of percutaneous coronary intervention (PCI) in patients with FFR negative stenosis, and subsequently superiority of FFR guided revascularisation over angiography alone. These results have firmly established FFR as an integral tool in the assessment of coronary artery disease severity, receiving class I, level of evidence A in the European Society of Cardiology guidelines to assess the presence of ischaemia and to guide revascularisation (6).

9.2.1.2 Resting indices of lesion severity

Due to the need for administration of vasodilators, with consequent cost, patient experienced side effects and risk, there has been a move to develop resting indices of coronary artery severity assessment. The most validated of these is the instantaneous wave free ratio (iFR).

Similar to FFR, iFR relies on the proportional relationship of pressure and flow that occurs with constant intracoronary resistance. However, instead of requiring pharmacological vasodilatation to achieve this, it is measured over a diastolic wave-free period in the cardiac cycle where intracoronary resistance is naturally constant (7). iFR is calculated as the ratio of distal to proximal pressures over this wave-free period. Several large clinical studies have shown iFR is non-inferior to FFR with respect to clinical outcomes, and consequently has been increasingly adopted in clinical practice (8,9). Subsequently a number of industry specific resting indices have been suggested as equivalent to iFR, using propriety specific algorithms to detect pressure difference. The most commonly used algorithms are the resting full-cycle ratio (RFR) [Abbott laboratories, Illinois, USA], the diastolic hyperaemia-free ratio (DFR) [Boston Scientific, Massachusetts, USA] and the resting full cycle Pd/Pa. [Figure 9.2 and Table 9.1], which have been validated to a lesser extent than iFR.

Figure 9.2: Schematic representation of timing within cardiac cycle and calculation of resting indices are calculated.



DFR, diastolic hyperaemia-free ratio; iFR, instantaneous wave-free ratio; Pd/Pa, ratio of mean distal coronary artery pressure to mean aortic pressure in the resting state; RFR, resting full-cycle ratio. Adapted from (10)

Table 9.1: Resting Indices currently available

Table 1 Currently available non-hyperaemic pressure ratios (NHPRs)

NHPR	Calculation	Period of the cardiac cycle	Manufacturer	Threshold	Summary of evidence
Instantaneous wave-free ratio (iFR)	Pd/Pa calculated during the WFP within diastole.	WFP in diastole	Philips Healthcare	≤0.89	Validated by randomised prospective clinical trials: DEFINE-FLAIR and SWEDEHEART.[3 5] iFR non-inferior to FFR for MACE at 1 year.
Resting full-cycle ratio (RFR)	The lowest Pd/Pa over the entire cardiac cycle. Mean of 4–5 consecutive cycles.	Whole cycle	Abbott	≤0.89	VALIDATE RFR and RE-VALIDATE RFR both show diagnostic equivalence between RFR and iFR.[7 33] RFR correlates as highly with FFR as do other NHPR's.
Diastolic hyperemia-free ratio (DFR)	Average Pd/Pa over the approximated* diastolic period averaged over five consecutive cardiac cycles.	Diastole	Boston Scientific	≤0.89	DFR is diagnostically equivalent to iFR in multiple validation studies.[8 22] DFR correlates as highly with FFR as do other NHPR's.
Resting Pd/Pa	Resting Pd/Pa averaged over the entire cardiac cycle.	Whole cycle	Not proprietary technology	≤0.91	Resting Pd/Pa provides excellent agreement with iFR and FFR. Resting Pd/Pa has slightly lower sensitivity to stenosis severity than iFR.

*Diastole approximated as negatively sloped segment of tracing where instantaneous Pa falls below mean Pa.
FFR, fractional flow reserve; Pd/Pa, ratio of mean distal coronary artery pressure to mean aortic pressure in the resting state; WFP, wave-free period.

Adapted from (10)

9.2.2 Flow measures of lesion severity

Although flow is difficult to directly measure, Doppler wires and temperature sensor wires can be used to measure surrogates of blood flow. These flow derived indices may detect pathology in epicardial vessels, microvascular dysfunction or a combination of both.

9.2.2.1 Coronary Flow Reserve

Coronary flow reserve (CFR) is the ratio of hyperaemic blood flow (Q_{max}) to resting myocardial blood flow (Q_{rest}), where CFR = Q_{max}/Q_{rest} (11). It is a combined measure of the capacity of the major resistance components, that is, the epicardial coronary artery and the vascular bed, to achieve maximal blood flow under hyperaemic stimulation. CFR can be measured invasively by either a temperature-sensitive guide wire applying the coronary thermodilution technique or a Doppler sensor equipped guide wire measuring Doppler flow velocity (12,13).

Coronary thermodilution measurements, using a PressureWire [Abbott laboratories, Illinois, USA], requires brisk saline injections during resting and hyperaemic conditions to calculate CFR based on the average mean transit time of multiple saline boluses. Although it correlates well to absolute coronary flow in research settings (14), this method is prone to measurement errors due to sensitivity to the saline injections, as well as possible alterations to coronary haemodynamics during brisk injection (15).

The Doppler technique, assessed using a Doppler wire (FloWire® or ComboWire®, Volcano, Philips Healthcare, Best, The Netherlands) provides Doppler flow velocity values, but also allows tracking of real-time phasic flow velocity signal providing both additional information on the functional status of the coronary circulation and direct feedback with regard to signal quality, allowing more advance physiology techniques to be employed. It is, however, significantly subject to operator's experience and proper positioning of the guide wire in order to obtain a reliable flow signal (15). CFR using the Doppler wire technique is calculated as;

$$\text{CFR} = \frac{APVH}{APVB}$$

where APVH = average peak velocity at steady state hyperaemia and APVB = average peak velocity at baseline, both measured over 5 cardiac cycles and measured in cm/sec. The diagnostic accuracy of CFR has been evaluated with non-invasive ischaemia testing with a relatively high accuracy of 81% with an ischaemic value set at <2.0 as compared with myocardial perfusion scintigraphy, exercise stress electrocardiography and dobutamine stress echocardiography. CFR has the same level of accuracy as FFR in detecting ischaemia compared with these modalities (16). Furthermore, non-randomised trials have shown a significant relationship with lower CFR and poorer clinical prognosis with higher rates of adverse events (17).

The Doppler wire method of attaining CFR has the additional advantage of allowing simultaneous pressure and velocity sensors, thereby providing haemodynamic information on the physiological state of the entire coronary circulation, both epicardial and microvascular. Particularly in the CTO setting, this is imperative as previous small studies have suggested these patients have significant microvascular dysfunction (18-20).

9.2.2.2 Hyperaemic Microvascular Resistance

The hyperaemic microvascular resistance (HMR) describes the maximal vasodilatory capacity of the microvasculature, indexed for distal perfusion pressure using mean, whole cardiac cycle pressure and flow velocity (21,22). An assessment of microvascular resistance at hyperaemia is calculated as:

$$\text{HMR} = \frac{Pd}{APVH}$$

Where Pd = distal coronary pressure and APVH = average peak velocity at steady state hyperaemia. The HMR is measured in mmHg/cm/sec and is measured over 5 cardiac cycles during steady hyperaemia. This is based on application of Ohm's law, where resistance is equal to pressure divided by flow. This index is pressure dependent, such that in the context of an epicardial stenosis, HMR is higher than post percutaneous intervention in the same vessel, where local autoregulatory processes modulate microvascular tone despite hyperaemic stimulation (22).

9.2.2.3 Hyperaemic Stenosis Resistance

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.This index provides a physiological means of quantifying the impediment to maximal flow caused

exclusively by the stenosis. Like FFR, it is independent of basal haemodynamic conditions, with high reproducibility and low variability (23). It was found to have a higher diagnostic accuracy than FFR (24), and therefore provides a more refined assessment of stenosis severity. An optimal cut-off value of 0.8mmHg/cm/sec has been proposed, with values above this considered the threshold for ischaemia (24). It is calculated as:

$$\text{HSR} = \frac{Pa-Pd}{APVH}$$

All measurements are taken over 5 cardiac cycles during steady state hyperaemia and is measured in mmHg/cm/sec

9.2.2.4 Absolute Coronary Blood Flow

Absolute coronary blood flow (CBF) can be estimated if coronary diameter is known and flow velocity is measured and has been validated in animals (25) and used in clinical studies (19). CBF is calculated as:

$$\text{CBF} = (\pi r^2) \text{ x } \frac{APV}{2}$$

APV can be measured in hyperaemia or the basal setting, with the CBF measured in mL/min. Coronary blood flow is exquisitely related to oxygen demand and therefore, cardiac work (26), with an increase in heart rate (27) and systolic blood pressure (28) associated with an increase in coronary blood flow. Consequently, to correct for these factors, resting absolute coronary blood flow can be corrected for the rate pressure product [RPP] (defined as the heart rate x systolic blood pressure) (19). The corrected resting CBF is calculated as:

$$\text{Corrected CBF} = CBF \text{ x } \frac{10{,}000}{RPP}$$

9.2.2.5 Index of Microvascular Resistance

The index of microvascular resistance (IMR) assesses microvascular resistance using distal coronary pressure and thermodilution-derived flow:

$$\text{IMR} = \frac{Pd}{1/\text{Tmn}}$$

where T_{mn} is the mean transit time of a bolus of saline injected at room temperature into the coronary artery at maximal hyperaemia. A higher value suggests abnormal microvascular resistance, with differing optimal cut-offs quoted in the literature of 21.5 units (29), 23units (11) or 40 units (30). It has been shown to be an independent predictor of acute and short-term myocardial damage, an early marker of myocardial viability, LV recovery and long-term outcome in patients undergoing primary PCI (30).

9.2.3 Rationale for the study

One of the primary determinants of FFR and all pressure derived indices is the territory of myocardium subtended by the epicardial vessel. As described in chapter 8, larger territories of myocardium supplied by a vessel result in greater per unit blood flow. In the setting of a CTO, the myocardial territory supplied by a donor vessel is far greater than in the normal setting, as collaterals perfuse the area subtended by the CTO vessel. As FFR provides a vessel specific value to determine the need for revascularisation, interpretation in the setting of a CTO is difficult. In particular, this has implications for whether a patient requires revascularisation for the CTO (which is in a constant state of ischaemia) (31,32) and the donor vessel, or just the CTO alone.

Consequently, the aim of the study was to determine the impact of the presence, and subsequent regression of coronary collaterals on donor vessel coronary haemodynamics. Furthermore, to determine the prevalence of microvascular dysfunction in patients with a

CTO, and subsequent effects on measures of epicardial and microvascular physiological indices.

9.3 Methods

9.3.1 Study Population

Consecutive patients presenting for elective CTO PCI between June 2018 and October 2020 were approached to be included in the "A comprehensive evaluation of biochemical, haemodynamic, structural and cellular of collateral function in human coronary arteries in chronic total occlusions and coronary artery disease – The COLLATERAL CTO Study". This is a single centre, prospective observational study including patients presenting for elective CTO PCI as well as patients undergoing diagnostic angiography for stable coronary artery disease. Institutional ethics approval was obtained from the Northern Sydney Local Health District Human Research Ethics Committee (NSLHD HREC) prior to commencing the project. Patients with a previous coronary artery bypass graft (CABG) to either the donor or the CTO were excluded as were patients with predominantly ipsilateral or "bridging" collaterals.

9.3.2 Coronary Physiology Assessment

In patients undergoing CTO PCI, dual vascular access was obtained as per clinical indication, with an 8 French sheath in the right femoral artery and a 6 French sheath in the right radial artery. The CTO vessel was accessed with an 8F guiding catheter, while a 6F guiding catheter was used for the donor. Heparin was administered at the beginning of the procedure with 100 international units of heparin per kg intra-arterial. Additional heparin was given routinely every hour.

Prior to CTO PCI, the 6-F guiding catheter was advanced into the ostium of the donor vessel, and an intracoronary bolus of 200 mcg of nitroglycerin was administrated. A 0.014-inch guidewire equipped with both a pressure sensor and a Doppler crystal (ComboWire XT, Philips Volcano Corporation, Del Mar, California) was initially equalised with the aortic pressure, before being advanced to a location distal to the collaterals in the donor vessel. The Doppler guidewire was manipulated until an optimal and stable Doppler flow signal was obtained. Baseline readings were recorded, at which time intravenous adenosine at a rate of 140 μg/kg/hr was administered through a large bore peripheral cannula for 90 seconds to achieve hyperaemia. The FFR, CFR, HSR, HMR, APVH, APVB and heart rate were all recorded. The resting Doppler flow velocity, electrocardiographic signals, aortic pressure, and distal coronary pressure were simultaneously recorded using the ComboMap system (Philips Volcano Corporation). CTO PCI was subsequently performed as per the operator discretion. At the completion of CTO PCI, the donor vessel was re-interrogated with the ComboWire at both rest and hyperaemia.

9.3.3 Angiographic Assessment

The degree of collaterals was graded according to the Rentrop classification (33-41) and the Collateral Connection grade (CC) (42). The donor vessel was defined as the epicardial vessel from which the predominant contralateral collaterals arose. If the collateral vessels arose from a branch of a main epicardial vessel, the donor vessel was defined as the main epicardial vessel from which the branch arose. The size of the donor vessel and degree of stenosis in the donor vessel were determined using quantitative coronary angiography (QCA) program on a commercially available platform (McKesson, Irving TX, USA) using the known diameter of the guiding catheter to calibrate size. CTO PCI technical success was defined as <30%

residual diameter stenosis within the treated segment and restoration of Thrombolysis in Myocardial Infarction (TIMI) grade 3 antegrade flow.

9.3.4 Clinical Data

Past medical history and functional status was recorded prior to planned procedure through patient interviews. A history of diabetes, which is associated with microvascular dysfunction was defined as a self-reported history. Medication usage was defined as a prior history of taking medications for at least 4 weeks. Ejection fraction was documented based on echocardiography performed within 3 months of the planned CTO procedure.

9.3.5 Statistical Analysis

Categorical data was presented as number of cases and percentage of the total population. Continuous variables were firstly assessed by the Shapiro-Wilk test to ascertain normality of distribution, where normally distributed data was presented as means ± standard deviation or as medians and interquartile ranges for data not normally distributed. Comparison between pre CTO PCI and post CTO PCI was performed using the Student's paired T test where equal variances were not assumed. All tests were 2-sided, and $p < 0.05$ was considered statistically significant. Analyses were performed using SPSS (version 24, IBM, New York, New York).

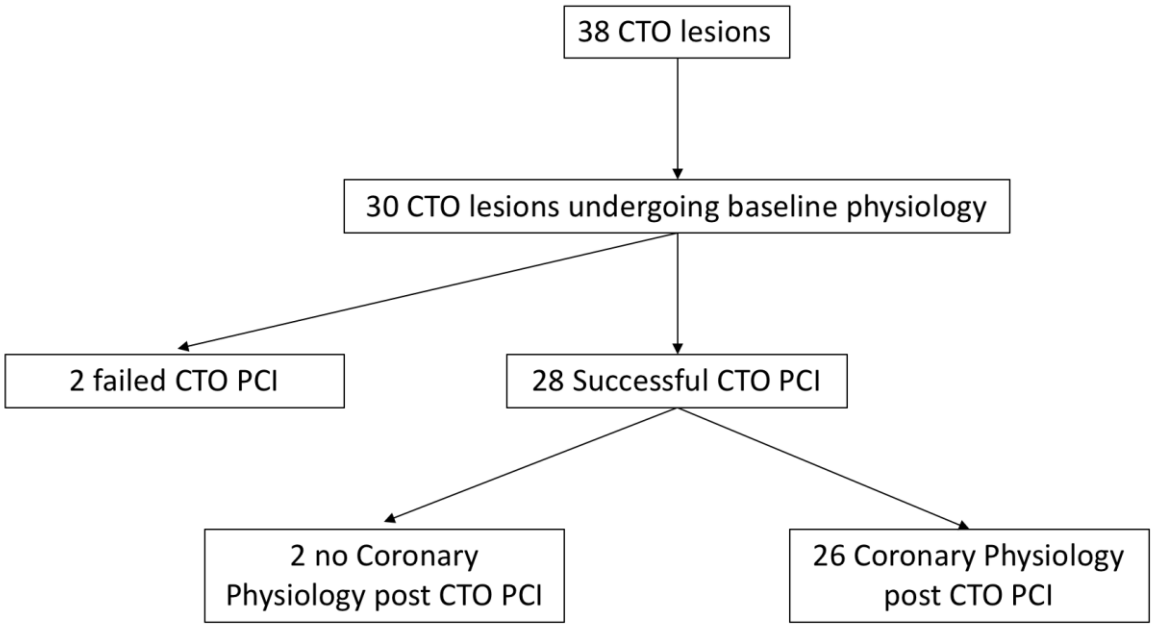
9.4 Results

9.4.1 Study Population

Over the study period, 38 patients with a CTO were recruited, of which, 30 patients had baseline coronary physiology of the donor vessel performed. 28 patients had successful CTO PCI (93.3%), with 26 patients subsequently having coronary physiology of the donor vessel. Of the 2 patients who did not have physiology repeated following CTO revascularisation, one

patient had a balloon mediated coronary perforation and pericardial effusion which was successfully treated with a covered stent. The second patient while undergoing attempt at repeat coronary physiology of the donor vessel sustained a probable guidewire induced coronary dissection requiring immediate stenting of the donor vessel. Therefore 26 patients were included in the final analysis (Figure 9.3).

Figure 9.3: Patients included in the study



The mean age of patients was 70.3 years with 10% females and a mean BMI of 29.0 kg/m^2. The CTO vessel was the right coronary artery (RCA) in 14 cases (46.7%), the left circumflex artery in 9 cases (30%) and the left anterior descending artery (LAD) in 7 cases (23.3%). The donor vessel physiologically interrogated was the LAD in 13 cases (43.3%), the LCx in 10 cases (33.3%) and the RCA in 7 cases (23.3%), with a median stenosis of 40% in the donor vessel. The median Syntax score for included patients was 15.5 Most patients had Rentrop grade 3 (50%) or grade 2 collaterals (46.7%) [Table 9.2 & 9.3].

Table 9.2: Baseline characteristics of patients undergoing CTO PCI

Female Sex *(n)*	3 (10%)
Age *(yrs)*	70.3 ± 12.2
BMI *(kg/m^2)*	29.0 ± 5.1
Heart Rate *(bpm)*	70.5 ± 13.8
Systolic blood pressure *(mmHg)*	136.3 ± 28.4
Diastolic blood pressure *(mmHg)*	71.7 ± 14.5
Past Medical History *(n)*	
Hypertension	22 (73.3%)
Hypercholesterolaemia	28 (93.3%)
Diabetes Mellitus	7 (23.3%)
Smoking History	
Non-smoker	8 (26.7%)
Ex-smoker	17 (56.7%)
Current Smoker	4 (13.3%)
Atrial Fibrillation	7 (23.3%)
Medications *(n)*	
Aspirin	28 (93.3%)
P2Y12 Inhibitor	28 (93.3%)
Statin	23 (76.7%)
ACE-I/ARB	18 (60%)
Beta Blocker	18 (60%)
Non-dihydropyridine CCB	1 (3.3%)
Dihydropyridine CCB	8 (26.7%)
Thiazide diuretic	3 (10%)
Loop Diuretic	2 (6.7%)
Aldosterone Antagonist	2 (6.7%)
Long acting Nitrate	6 (20%)
LVEF *(%)*	60 (50 – 60)
NYHA Class Symptoms *(n)*	
I	4 (13.3%)
II	15 (50%)
III	10 (33.3%)
IV	1 (3.3%)
CCS Class *(n)*	
II	5 (16.7%)
III	18 (60%)
IV	7 (23.3%)

ACE-I = angiotensin converting enzyme inhibitor; ARB = angiotensinogen II receptor blocker; BMI = body mass index; bpm = beats per minute; CCB = calcium channel blocker; CCS =Canadian Cardiovascular Society; kg = kilograms; LVEF = left ventricular ejection fraction; m=metre; mmHg = millimetres of mercury; n = number, NYHA = New York heart association.

Table 9.3: Angiographic characteristics of patients undergoing CTO PCI

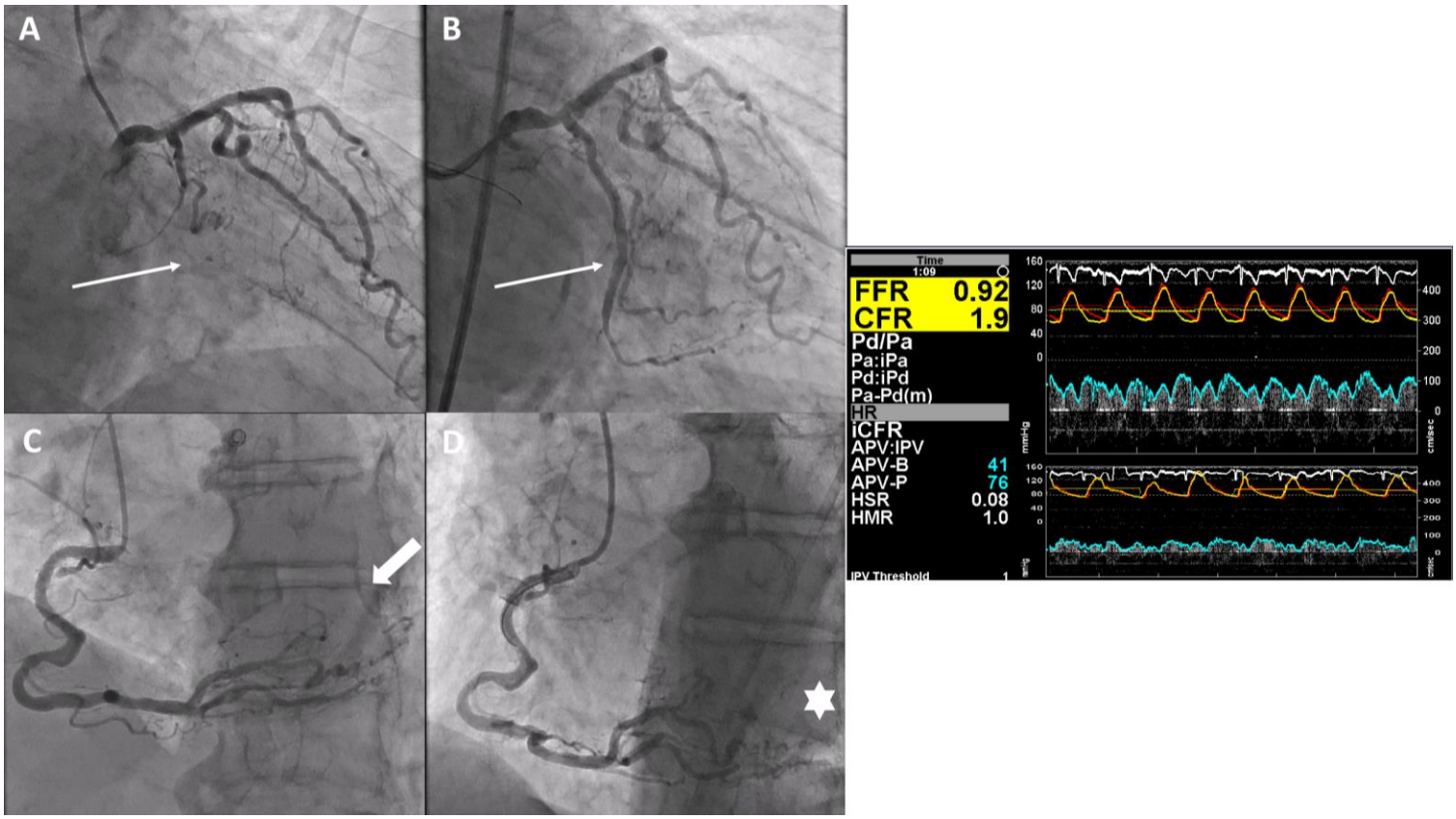
Number of CTOs *(n)*	
1	30 (100%)
>1	0 (0%)
CTO Vessel *(n)*	
LAD	7 (23.3%)
LCx	9 (30%)
RCA	14 (46.7%)
Predominant Donor Vessel *(n)*	
LAD	13 (43.3%)
LCx	10 (33.3%)
RCA	7 (23.3%)
Rentrop Classification*(n)*	
0 or 1	1 (3.3%)
2	14 (46.7%)
3	15 (50%)
CC Score *(n)*	
1	6 (20%)
2	21 (70%)
3	3 (10%)
Stenosis in donor vessel *(%)*	40 (30 – 50)
Syntax Score *(n)*	15.5 (11.1 – 21.5)
Successful CTO PCI *(n)*	28 (93.3%)
PCI Approach *(n)*	
Anterograde	24 (85.7%)
Retrograde	4 (14.3%)

CC= Coronary Collateral; CTO = chronic total occlusion; LAD = left anterior descending artery; LCx = left circumflex artery; PCI = percutaneous coronary intervention; RCA = right coronary artery;

9.4.2 Pressure and flow indices in the donor vessel

Of the 26 patients who underwent physiological assessment of the donor vessel prior to and following CTO PCI, all had FFR values recorded. 20 patients had Doppler data of high fidelity to allow interpretation of flow derived indices, while 16 of the 26 patients (61.5%) had flow indices recorded following revascularisation (Figure 9.4).

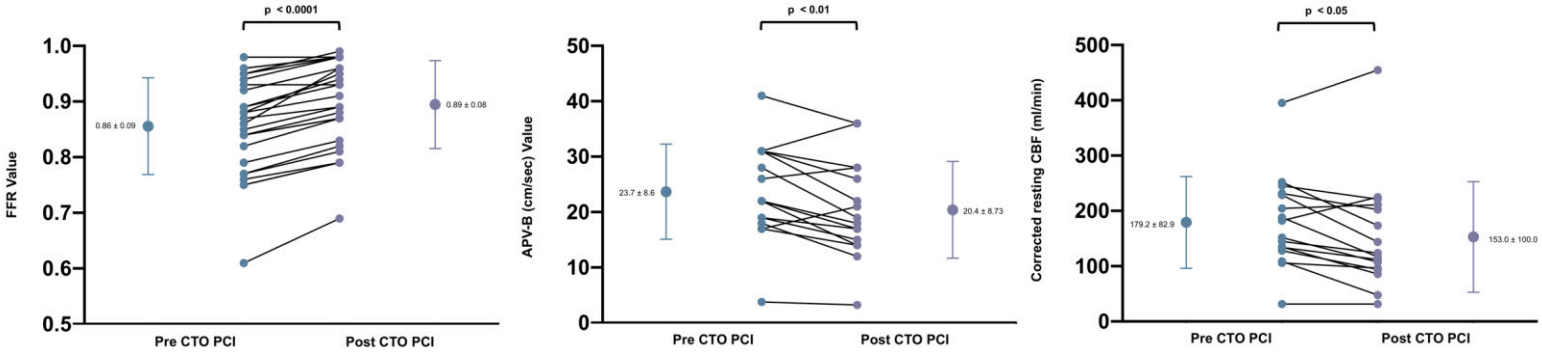
Figure 9.4: Example of pressure and flow indices measured



(A) Coronary angiogram showing a CTO of the left circumflex artery (LCx) with no contrast opacification (white arrow) distal to the site of occlusion. (B) Following CTO PCI, there is now anterograde contrast opacification of the LCx (white arrow). (C) Prior to CTO PCI of the LCx, contrast opacification of the distal LCx (thick arrow) from collaterals from the right coronary artery (RCA). (D) Following CTO PCI of the LCX, no collateral filling of the LCx (6 point star) from the RCA. Panel showing an example of the "Combomap" system showing simultaneous pressure and flow readings.

Following successful CTO PCI, the FFR of the donor vessel increased from 0.86 to 0.89 ($p<0.0001$). There was an associated decrease in the average peak velocity at baseline (23.7cm/sec vs 20.4cm/sec, $p<0.01$) and corrected resting CBF (179.2mL/min vs 153.0mL/min, $p<0.05$) (Figure 9.5). Seven patients (26.9%) had a resting donor vessel FFR below the threshold for ischaemia (FFR<0.80). Of these patients, following successful CTO PCI, 3 patients (42.9%) no longer had an ischaemic donor vessel, while 4 remained ischaemic. In patients with a baseline FFR<0.80, following successful CTO PCI, the FFR increased from 0.74 to 0.78 ($p<0.0001$).

Figure 9.5: Changes in FFR, APV-B and corrected resting CBF in donor vessel pre and post CTO PCI



[LEFT PANEL]: FFR of the donor vessel pre-CTO PCI and post-CTO PCI, showing a significant increase (0.86 vs 0.89, p<0.0001; n=26). [MIDDLE PANNEL]: Average peak velocity at baseline (APV-B) of the *donor vessel pre-CTO PCI and post-CTO PCI, showing a significant reduction (23.7cm/sec vs 20.4cm/sec, p<0.01; n = 16). [RIGHT PANNEL]: Corrected resting coronary blood flow (CBF) of the donor vessel pre-CTO PCI and post-CTO PCI showing a significant reduction (179.2mL/min vs 153.0mL/min, p<0.05; n=16).*

Prior to CTO PCI, 15 patients (75%) had an abnormal CFR (<2.0) of the donor vessel. In patients in whom both FFR and CFR was recorded, 11 (73.3%) had an abnormal CFR with a non-ischaemic FFR. Prior to CTO PCI, in patients in whom both FFR and HSR were measured, in the 5 patients with an ischaemic FFR (<0.80), all had a non-ischaemic HSR (<0.80). Following CTO revascularisation, in the patients in whom both FFR and HSR were measured, 2 patients had an ischaemic FFR value, and 1 of these patients (50%) had an ischaemic HSR value. Following successful CTO PCI there was no difference in CFR, APV-H, hyperaemic CBF, HSR or HMR of the donor vessel (Table 9.4).

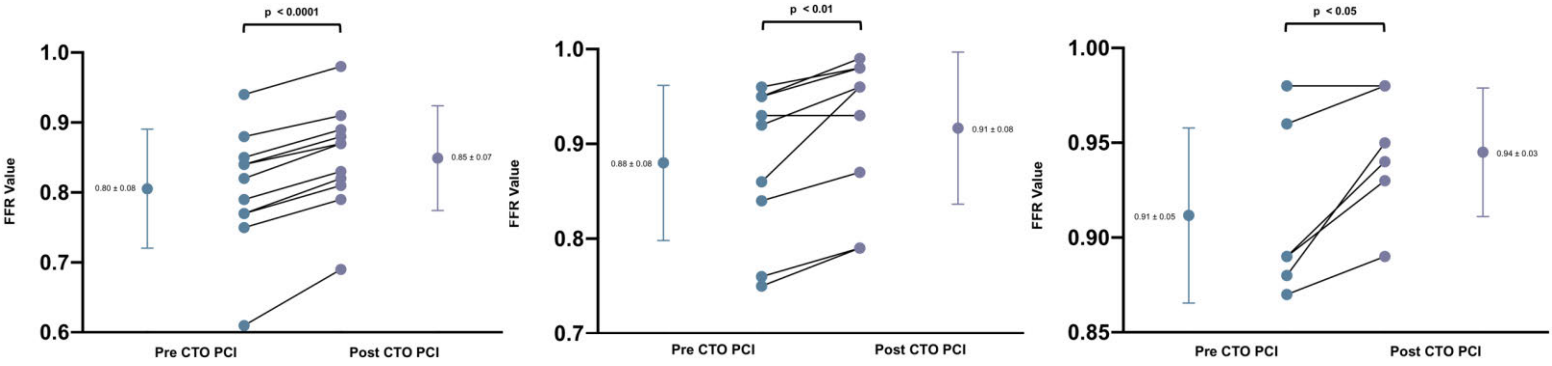
Table 9.4: Invasively measured pressure and flow indices in the predominant donor vessel

	Pre CTO PCI	Post CTO PCI	p-value
FFR *(ratio)*	**0.86 ± 0.09**	**0.89 ± 0.08**	**<0.0001**
CFR *(ratio)*	1.80 ± 0.82	1.78 ± 0.92	0.94
APV-B *(cm/sec)*	**23.67 ± 8.59**	**20.38 ± 8.73**	**<0.01**
Resting Coronary Blood Flow* *(mL/min)*	**179.2 ± 82.9**	**153.0 ± 100.00**	**<0.05**
APV-H (cm/sec)	43.50 ± 26.78	40.88 ± 12.94	0.68
Hyperaemic Coronary Blood Flow *(mL/min)*	324.6 ± 193.2	315.3 ±153.4	0.82
HMR *(mmHg/cm/sec)*	2.76 ± 2.45	3.09 ± 2.49	0.71
HSR *(mmHg/cm/sec)*	0.35 ± 0.27	0.29 ± 0.28	0.18
HR *(bpm)*	75.13 ± 13.53	79.38 ± 16.01	0.17

N = 26 for FFR values and n=16 for all other values. * Corrected for RPP
APV-B = Average peak velocity at baseline; APV-H = Average peak velocity during hyperaemia; bpm = beats per minute; CFR = Coronary Flow Reserve; cm= centimetre; FFR = fractional flow reserve; HMR = hyperaemic microvascular resistance; HR = heart rate; *HSR = hyperaemic stenosis resistance; min = minute; mL = millilitre; mmHg = millimetres of mercury; sec = second;*

In patients in whom the LAD was the donor vessel, there was a significant increase in FFR following successful CTO PCI (0.80 vs 0.85, p<0.0001) as was seen with an LCx donor vessel (0.88 vs 0.91, p<0.01) and RCA donor vessel (0.91 vs 0.94, p<0.05) [Figure 9.6].

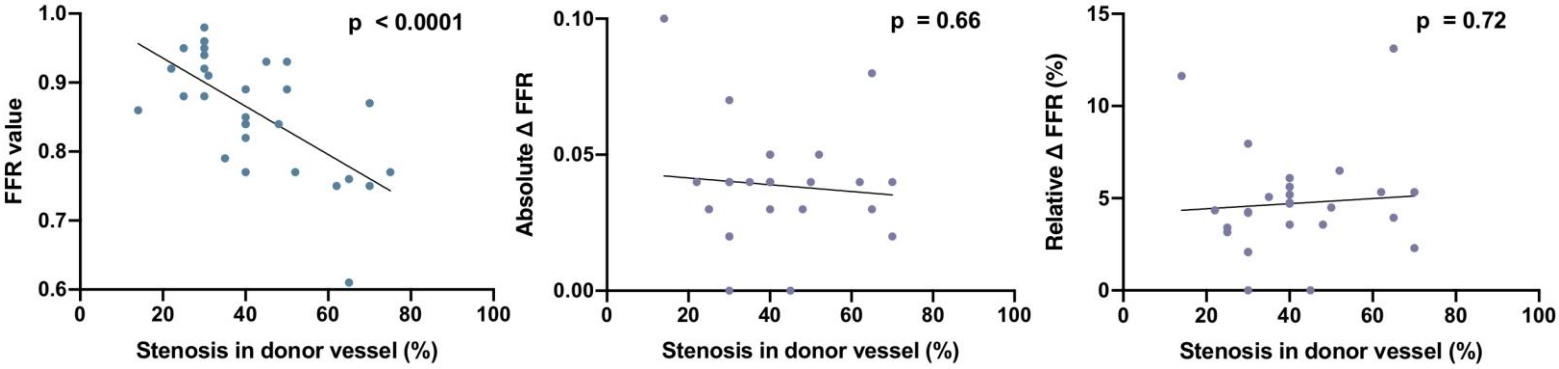
Figure 9.6: Changes in FFR, pre and post CTO PCI stratified by donor vessel



[LEFT PANEL]: FFR of the LAD pre-CTO PCI and post-CTO PCI, showing a significant increase (0.80 vs 0.85, p<0.0001; n=11). [MIDDLE PANNEL]: FFR of the LCx pre-CTO PCI and post-CTO PCI, showing a significant increase (0.88 vs 0.91, p<0.01; n=8) [RIGHT PANNEL]: FFR of the RCA pre-CTO PCI and post-CTO PCI, showing a significant increase (0.91 vs 0.94, p<0.05; n=7).

There was a significant inverse correlation between degree of stenosis in the donor vessel and FFR value prior to CTO PCI (p<0.0001). Following CTO PCI, there was no correlation between degree of donor vessel stenosis and absolute change in FFR (p=0.66) or relative change in FFR (p=0.72) [Figure 9.7].

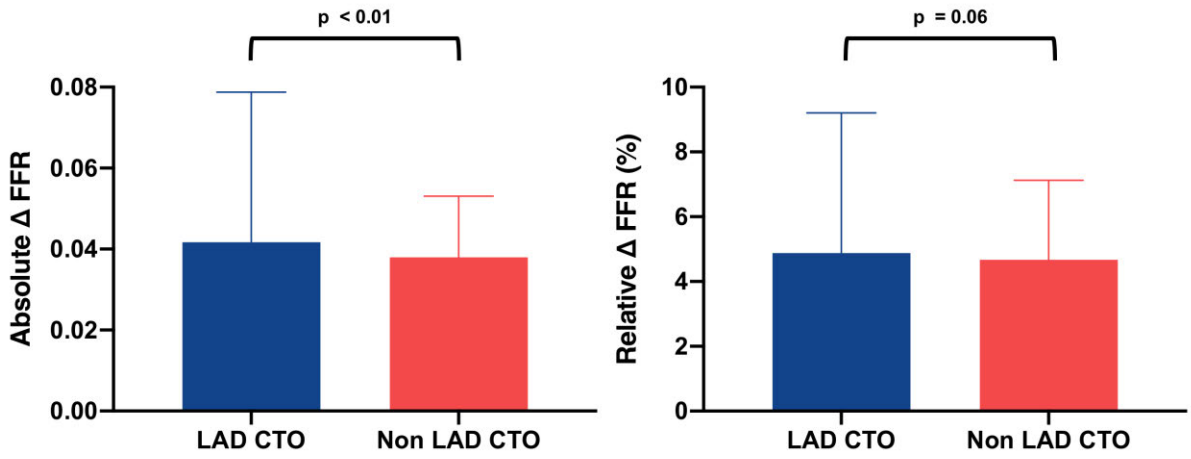
Figure 9.7: Correlation of FFR with stenosis in donor vessel



[LEFT PANEL]: Significant inverse correlation with degree of stenosis in donor vessel and FFR (p<0.0001). [MIDDLE PANNEL]: No correlation between degree of stenosis in donor vessel and absolute change in FFR following CTO PCI (p=0.66) [RIGHT PANNEL]: No correlation between degree of stenosis in donor vessel and change in FFR relative to initial FFR (p=0.72).

There was no difference in the FFR of the donor vessel with an LAD CTO as compared to a non-LAD CTO (0.89 vs 0.85, p=0.78). Following successful CTO PCI, there was a greater increase in the donor vessel FFR in patients with an LAD CTO as compared to a non-LAD CTO (0.041 vs 0.038, p<0.01), with no difference in relative change of FFR (4.9% vs 4.7%, p=0.06) [Figure 9.8]

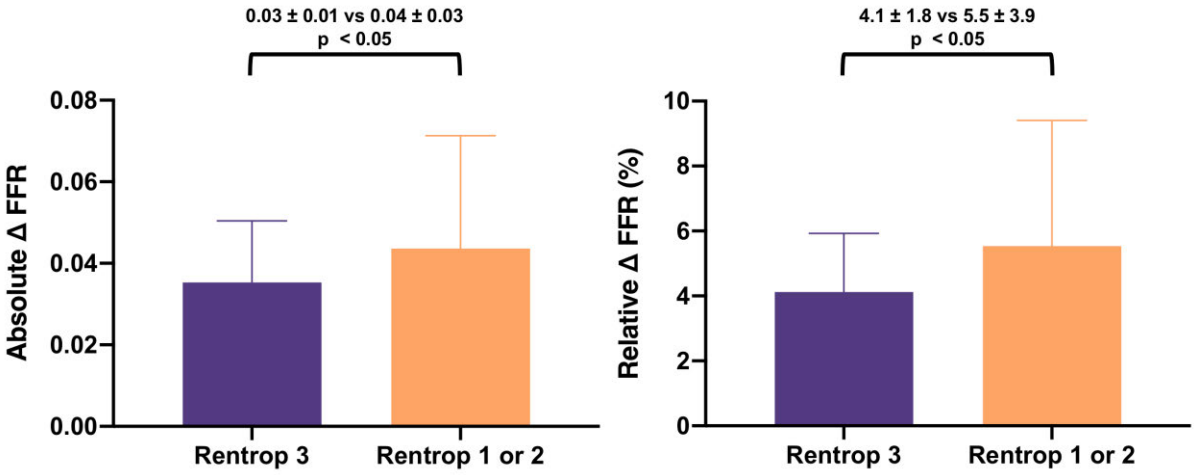
Figure 9.8: Change in FFR in donor vessel perfusing an LAD CTO and non-LAD CTO



[LEFT PANEL]: Greater increase in FFR of the donor vessel if supplying an LAD CTO compared to a non-LAD CTO (0.041 vs 0.037, p<0.01). [RIGHT PANEL]: No difference in relative change in FFR of the donor vessel if supplying an LAD CTO compared to a non-LAD CTO (4.9% vs 4.7%, p=0.06).

There was no difference in baseline donor vessel FFR in those with Rentrop grade 3 collaterals compared with those with Rentrop grade 1 or 2 (0.87 vs 0.84, p=0.44). Following successful CTO PCI, there was a smaller absolute and relative change in FFR in those with Rentrop grade 3 collaterals compared with those with Rentrop grade 1 or 2 (0.03 vs 0.04, p<0.05 and 4.1% vs 5.5%, p<0.05 respectively) [Figure 9.9].

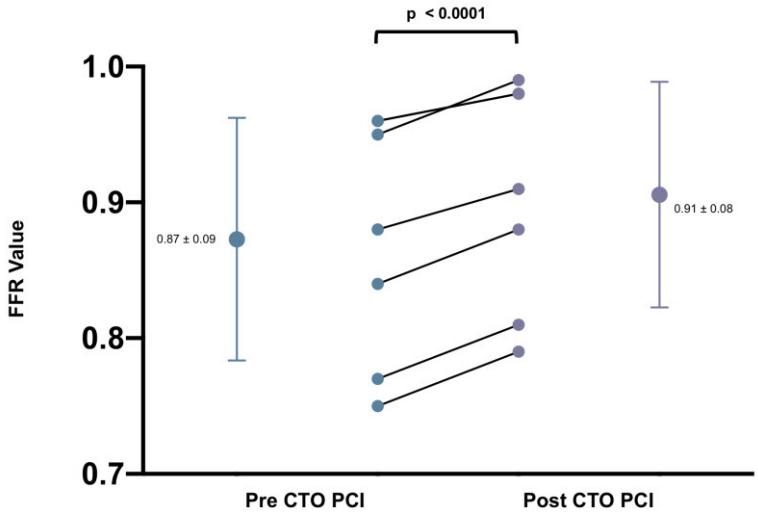
Figure 9.9: Change in FFR in donor vessel stratified by Rentrop grade



[LEFT PANEL]: Lower absolute change in FFR of the donor vessel if supplying Rentrop grade 3 collaterals compared to Rentrop grade 1 or 2 (0.03 vs 0.04, p<0.05). [RIGHT PANEL]: Lower relative change in FFR of the donor vessel if supplying Rentrop grade 3 collaterals compared to Rentrop grade 1 or (4.1% vs 5.5%, p<0.05).

Seven patients had a history of diabetes mellitus, with all 7 (100%) undergoing successful CTO PCI, with a resultant increase in the donor vessel FFR (0.87 vs 0.91, p<0.0001) (Figure 8). This was associated with a significant reduction in the corrected resting coronary blood flow (150.3mL/min vs 98.9mL/min, p<0.05). There was no difference in CFR between patients with diabetes mellitus and those without (1.4 vs 1.8, p=0.32). There was a greater increase in the absolute and relative FFR in patients without diabetes mellitus compared with those with diabetes mellitus (0.04 vs 0.03, p<0.05 and 5.0% vs 3.9%, p<0.05 respectively)

Figure 9.10: Changes in FFR in donor vessel pre and post CTO in patients with Diabetes Mellitus



9.5 Discussion

In patients undergoing CTO PCI, the presence of functional, angiographically visible coronary collaterals, results in a significant under-estimation of the FFR of the donor vessel, which following collateral regression, results in an immediate increase by a mean of 0.03. This is associated with a significant reduction in peak coronary blood velocity and resting coronary blood flow. As the use of FFR has dramatically increased in recent years due to its ease of performance and significant evidence base (43), this study identifies that in the setting of a CTO, accurate interpretation must include an expected increase in donor vessel value.

In the setting of maximal hyperaemia, the relationship between pressure and flow is essentially linear (44) and consequently situations which result in changes in flow affect FFR. A well-recognised example of this is in the setting of previous infarct, where myocardium is not viable and there is a resultant reduction in blood flow to the territory compared with normal viable myocardium (45).

Contrastingly, in the setting of a CTO, the donor vessel is perfusing a larger territory than normal, with greater coronary blood flow. As illustrated in this study, following restoration of anterograde perfusion of the CTO vessel, there is a reduction in coronary blood flow of the donor vessel, with a resultant increase in the FFR. This is seen in all donor vessels, while the change in FFR was greater when the donor vessel was supplying a CTO of the LAD. As the LAD supplies up to 50% of left ventricular myocardial mass (46), it is not surprising that in this situation there is greater coronary flow through the donor vessel, which rapidly reduces following CTO revascularisation. While baseline FFR was closely related to the degree of stenosis in the donor vessel, following CTO PCI, the change in FFR was not related to the stenosis, suggesting the territory of myocardium supplied by the vessel is a more important determinant of the FFR.

This absolute increase in FFR of 0.03, is in keeping with the results of a similar sized study of 34 CTOs (19), which found an increase of 0.028 in the donor vessel following CTO PCI, also driven by a reduction in coronary blood flow. However in another study of 34 patients (20), there was no difference in FFR of the donor vessel immediately following CTO PCI, although at 4 months the FFR increased by 0.03. In a study of 14 successful CTO PCI out of 50 attempts (47), 6 patients went from an ischaemic FFR to a non-ischaemic FFR of the donor vessel (mean change of 0.10±0.04), 3 remained ischaemic (mean change of -0.01±0.04) and 4 remained non-ischaemic (mean change of 0.002 ± 0.04). The donor vessel was the LAD in 92.9% of cases in the latter study. These findings of a similar change in FFR have dramatic implications for management of patients with a CTO, since patients in whom a donor vessel is in the so called ‘grey-zone’ for ischaemia, generally between 0.75 - 0.80, may not require revascularisation of the donor vessel, once the CTO is revascularised.

While a significant reduction in resting coronary blood flow in the donor vessel was observed, this was not the case for hyperaemic coronary blood flow, which numerically reduced, but was not significant. Similarly, the average peak velocity at baseline of the donor significantly reduced following CTO PCI, although this was not seen in the average peak velocity at hyperaemia. This finding is potentially incongruous, as FFR is a hyperaemic index. While there was a trend toward a reduction in hyperaemic flow measures, these did not meet statistical significance, which may be a marker of insufficient size and power. An alternative explanation for this may be microvascular dysfunction following PCI where there is upregulation of the adrenergic system, which can attenuate coronary flow and blunt hyperaemic response to adenosine (48). Similarly, myocardial stunning following revascularisation is associated with an elevation in left ventricular end diastolic pressure (49,50) which would further attenuate coronary blood flow.

This implication of significant microvascular dysfunction is noted immediately following CTO revascularisation (51), which may be due to plaque embolisation to the microcirculation along with oedema from trauma of entering the extra-luminal space, or coronary dissection and microperforations, all of which result in possible attenuation of adenosine mediated maximal hyperaemia. Studies have suggested that an HMR of >2.0 is consistent with microvascular dysfunction (21,52), which was present in 8 out of 20 pre CTO PCI (40%) donor vessels and 12 out of 16 (75%) post successful CTO PCI donor vessels, suggesting the recorded hyperaemic coronary blood flow may be an underestimation of the true value. However, previous studies have suggested that a reduction in FFR in the donor vessel following CTO PCI is noted 4 months following revascularisation (20), suggesting immediate post PCI microvascular dysfunction alone is unlikely to explain the significant change in FFR.

Of the 7 patients who had an FFR of the donor vessel below the ischaemic threshold, 3 (42.9%) were no longer ischaemic following CTO revascularisation, and hence collateral regression. Previous studies have suggested that 38% of patients with a CTO are referred for CABG, possibly due to concomitant multivessel coronary artery disease (53). Early graft failure, where there is occlusion of a graft within 1 year of surgery is seen in 10-20% of patients (54-56). Lower graft flow at the time of surgery, as is seen in the setting of non-haemodynamically significant stenosis is a strong predictor of graft failure (57). It is thus imperative to understand the underestimation of the FFR of a donor vessel, to identify likelihood of successful grafting and therefore appropriateness of referral for CABG.

Seventy five percent of patients prior to CTO PCI had an abnormal CFR in the donor vessel, while immediately following CTO PCI, 73.3% of patients had an abnormal CFR in the donor vessel. The CFR allows simultaneous assessment of both epicardial and microvascular function, although does not determine at which level the dysfunction lies. While the CFR was <2.0 in all patients with an ischaemic FFR, the value was abnormal even in patients with a normal FFR. This may reflect microvascular dysfunction, which is further strengthened by the finding of non-ischaemic HSR in patients with an apparent ischaemic FFR. HSR has been shown to have a higher diagnostic accuracy than FFR (24) to discriminate ischaemia as has been shown in the current study. Indeed an abnormal CFR has been independently associated with poorer prognosis even in the setting of a normal FFR (58). This finding of significant microvascular dysfunction, may explain why randomised control trials of PCI in CTO (1,59,60), which treats only the epicardial stenosis, has failed to show a consistent prognostic benefit. Future treatment strategies based on microvascular function should be considered.

There was no difference in donor vessel FFR in patients with Rentrop grade 3 collaterals or those with Rentrop grade 1 or 2 collaterals. However, following successful CTO PCI, the absolute and relative change in donor vessel FFR was greater in those with poorer collaterals (0.04 vs 0.03, $p<0.05$ and 5.5% vs 4.1%, $p<0.05$). This would initially appear counterintuitive to changes in flow, with well-developed collaterals providing more flow to the CTO, and a consequent greater reduction in flow following collateral regression. Indeed in a small series of 8 patients (61), the change in donor vessel FFR was greater in those with Rentrop grade 2 or 3 collaterals, while those with Rentrop grade 1 had no change in the FFR. However, Werner et al. previously showed that there was no relationship between Rentrop classification and collateral function, suggesting that even in anatomically poorly visualised vessels, collateral function may be significant (42), possibly explaining this finding. Furthermore, it is possible that larger collaterals may take longer to completely regress and become non-functional, explaining the smaller change in FFR in this cohort of patients. Previous studies have suggested that loss of collateral function following CTO PCI can take between 24 hours to 1 year (62,63), although others, have suggested immediate reduction and loss of collateral function (64-67), suggesting further research is required to determine when collateral function is lost (19).

Diabetes mellitus has multiple effects on the cardiovascular system, including an attenuated increase in coronary blood flow in the setting of hyperaemia (68) attributable to microvascular dysfunction (69). In diabetic patients with a reduced CFR, even in the absence of detectable epicardial coronary disease, prognosis is equivalent to that of non-diabetic patients with prior coronary events (70). There was no difference in baseline donor vessel CFR or FFR values compared with non-diabetic patients. As seen in the entire cohort, in patients with diabetes, following revascularisation there was an increase in donor vessel FFR.

However, the degree of change was lower than in patients without diabetes, suggesting that whilst this change in FFR is constant, it may be less pronounced in diabetic patients and other cohorts with microvascular dysfunction.

9.5.1 Limitations

This study provides significant and in-depth information in the interpretation of coronary physiology in the donor vessel of a CTO, however there are limitations. Firstly, the dataset included is relatively small, particularly in patients in whom reproducible and accurate Doppler flow data was achieved. The ComboWire is technically challenging to use and requires manipulation not only to detect a flow tracing, but ensure that it is in the direction of the maximal flow. This is one of the reasons that flow wires have almost entirely been superseded by pressure wires in clinical practice. Nevertheless, the results from this study are hypothesis generating and provide a basis for ongoing research. Furthermore, the repeat physiological assessment was performed immediately following CTO revascularisation, which remains controversial with respect to whether collaterals remain functional. However, there was a constant and reproducible reduction in coronary blood flow, suggesting a reduction in flow through these collaterals. Whilst further autoregulation over time may occur and further change in blood flow, a clinically relevant change in FFR was detected in almost 50% of patients immediately and is of more relevance to decisions regarding revascularisation than remote studies conducted months in the future.

9.6 Conclusion

In patients presenting for CTO PCI, the measured donor vessel FFR is significantly underestimated, which, following CTO revascularisation and collateral regression, increases by a mean of 0.03. This is attributable to a reduction in coronary blood flow through the donor vessel, irrespective of CTO vessel or donor vessel. This change is more pronounced with a CTO of the LAD, reflecting the larger territory of myocardial mass perfused by the donor vessel, and hence greater flow. This has significant implications for clinicians when determining revascularisation management strategies. The significant microvascular dysfunction identified in 75% of patients with a CTO however may have implications for the immediate interpretation of hyperaemic derived pressure indices of lesion severity. Whether this change in coronary blood flow and therefore FFR is maintained over the long term and implications for revascularisation management remain to be confirmed.

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Chapter 10: The effect of Coronary Collaterals on Endothelial, Haematological, Biochemical and Proteomic Biomarkers

10.1 Abstract

Background and Aims: The recruitment and maturation of coronary collaterals in patients with a chronic total occlusion (CTO) occurs through increased fluid shear stress as a result of progressive coronary artery occlusion, with resultant inflammation and downstream alterations in protein and gene expression. The aim of the study was to determine whether patients with angiographically confirmed coronary collaterals with a CTO had differing concentrations of biochemical, haematological and proteomic markers compared with patients without a CTO.

Methods: Patients with stable angina presenting for diagnostic angiography or planned CTO percutaneous coronary intervention (PCI) were included in the study. All patients had baseline blood collected from a peripheral vein and aortic root prior to administration of medications or PCI. Endothelial cell derived proteins fibroblast growth factor 2 (FGF-B), monocyte chemoattractant protein 1 (MCP-1) and intracellular adhesion molecule 1 (ICAM-1) were quantified using enzyme linked immunosorbent assay (ELISA). Haematological and biochemical markers were also measured.

Results: A total of 140 patients were included in the study, 84 patients with a CTO and 56 patients with stable coronary artery disease (CAD). Patients with a CTO were older (72.5yrs vs 68.6yrs, $p<0.05$), more likely to be taking aspirin (89.3% vs 75%, $p<0.05$) and an angiotensin converting enzyme inhibitor (ACE-I) or angiotensinogen II receptor blocker (ARB) (61.9% vs 41.1%, $p<0.05$). Patients with a CTO were more symptomatic with greater rates of CCS class III angina (22.6% vs 7.1%, $p<0.05$) and higher rates of NYHA class III or IV (41.7% vs 19.7%, $p<0.05$) and a higher syntax score (20.6 vs 6.6, $p<0.0001$).

Patients with a CTO had a higher plasma FGF-B (74.2pg/mL vs 64.8pg/ml, $p<0.0001$), MCP-1 (46.1pg/mL vs 22.9pg/ml, $p<0.0001$) and ICAM-1 (54ng/mL vs 40.8ng/ml, $p<0.001$) compared with patients with CAD. There was no difference in plasma concentration of FGF-B, MCP-1 or ICAM-1 in patients with multiple CTOs compared with a single CTO or with maturity of collaterals. Six months following successful CTO revascularisation and thereby collateral regression there was an increase in MCP-1 ($p<0.01$) and ICAM-1 ($p<0.001$). Patients with a CTO had a lower peripheral lymphocyte count ($p<0.05$), higher monocyte count ($p<0.05$) and higher neutrophil to lymphocyte ratio ($p<0.05$).

Conclusion: In patients with a CTO, plasma concentrations of the endothelial cell derived biomarkers, FGF-B, MCP-1 and ICAM-1 are significantly higher than in patients without coronary collaterals, suggesting their function in collateral recruitment. MCP-1 and ICAM-1 concentrations are higher following CTO PCI, signifying a role of ischaemic preconditioning and persisting collateral recruitment potential. Alterations in peripheral leukocyte count and relative changes in cell lineages reflect the importance of these cell types in coronary collateral recruitment and maturation. The implications of these findings, and their role as a clinically relevant biomarker or potential therapeutic strategy for patients with severe coronary disease requires further assessment.

10.2 Introduction

The recruitment of coronary collaterals from a primitive network of pre-existing anastomotic connections can occur rapidly, as in the setting of an acute coronary syndrome, or more progressively such as in the setting of a chronic total occlusion (CTO). The current understanding of collateral recruitment is through arteriogenesis, the maturation of preformed vascular connections, with the process dependent on an increase in shear stress, initial inflammation, alterations in gene expression and an optimal redox state, acted upon by degrees of hypoxia and ischaemia (1) (See Section II).

A number of endothelial cell biomarkers are candidates to correlate with the presence and degree of coronary collaterals, namely fibroblast growth factor 2 (FGF-B), monocyte chemoattractant protein 1 (MCP-1) and intracellular adhesion molecule 1 (ICAM-1).

FGF is a monomeric polypeptide expressed widely by a number of cells, including endothelial cells in response to shear stress, which, when bound to FGF receptors, results in fibroblast proliferation, mitogenesis, cellular migration, differentiation, wound healing and angiogenesis. There are 23 distinct FGF factors, with FGF-B or basic fibroblast growth factor (bFGF) acting as a nitric oxide dependent vasodilator, capable of inducing systemic hypotension and coronary vasodilatation (2). In-vitro and in-vivo studies have shown that FGF is associated with smooth muscle cell proliferation and angiogenesis (3), and hence may be a candidate as a biomarker for the presence of collaterals.

MCP-1 is a member of the CC-chemokine family, a potent chemotactic factor for monocytes (4). MCP-1 is induced in the vascular endothelium by an increase in fluid shear stress (5) and is involved in attracting and binding monocytes that invade the intimal space (6), which

subsequently results in collateral maturation and functionality. ICAM-1 is a surface glycoprotein which is expressed on endothelial cells, predominantly in response to inflammation (7), promoting the adhesion of monocytes, neutrophils and lymphocytes (8,9).

Therefore, concentrations of FGF-B, MCP-1 and ICAM-1, identified in the systemic circulation may correlate with the presence and degree of coronary collaterals. Haematological markers, in-particular leukocyte populations, which are related to the maturation of coronary collaterals as well as commonly measured biochemical proteins may also be potential biomarkers.

The aim of this study was to determine whether patients with angiographically confirmed coronary collaterals with a CTO had differing concentrations of biochemical, haematological and proteomic markers compared with patients without collaterals. Furthermore, the aim was to determine the effect of CTO revascularisation, and consequent collateral regression on plasma concentration of these markers.

10.3 Methods

The COLLATERAL CTO Study is a prospective, single centre observational study including patients presenting for elective CTO PCI and consecutive patients undergoing diagnostic angiography for symptomatic stable coronary artery disease (CAD) (See Chapter 1). Patients were divided into one of 2 groups; those with a confirmed CTO and those without. All patients underwent coronary angiography for clinically indications. A CTO was defined as the presence of TIMI grade 0 flow in an epicardial artery, present for at least 3 months (10) based upon historical onset of symptoms or compared to previous angiographic imaging.

10.3.1 Blood Sample Acquisition and Storage

Blood samples were collected from patients prior to their diagnostic coronary angiogram following a minimum of an 8 hour fast. Patients were instructed to take their regular medications on the day of the procedure, with the exception of metformin or anticoagulation, which were withheld for 48 hours. Venous blood was collected from the antecubital vein using an 18 or 20 gauge needle. Blood was initially stored in a fridge at 4°C. Patients subsequently underwent insertion of arterial access, either through the radial or femoral artery, as clinically indicated and planned. Arterial blood was also collected from the aortic root prior to heparin administration. Both sets of bloods were collected in a tube containing 3.2% buffered sodium citrate solution (Greiner Bio One, Austria) which were centrifuged for 20 minutes at 1,000 relative centrifugal force (RCF) at 4°C (11). Plasma was subsequently aliquoted into sterile 1.5mL Eppendorf microcentrifuge tubes (Merck & Co, New Jersey, USA) and stored immediately in a -80°C freezer. Any samples with visible haemolysis or lipaemia were excluded from analysis.

For patients who underwent successful CTO PCI, arterial blood was collected from the aortic root just prior to removal of the guiding catheter. If patients were staying in hospital overnight, a repeat venous blood sample, as described above was taken 24 hours post CTO revascularisation. Finally another peripheral venous sample was taken 6 months following successful CTO revascularisation.

10.3.2 Enzyme Linked Immunosorbent Assay

Stored plasma was used to measure the concentrations of endothelial cell markers FGF-B, MCP-1 and ICAM-1 using a commercially available enzyme linked immunosorbent assay

(ELISA) [Thermo Fisher Scientific, Massachusetts, USA]. Samples were removed from -80°C freezer and batch thawed on ice to slowly rewarm to room temperature and were mixed gently. The samples were prepared and incubated on a standard 96 well plate, before being placed in a spectrophotometer with absorbance read at 450nm to determine concentrations, using commercially available kits: FGF-B (*Human FGF-B ELISA Kit* [Invitrogen, California, USA]), MCP-1 (*Human MCP-1 ELISA Kit* [Invitrogen, California, USA]) and ICAM-1 (*Human sICAM-1 ELISA Kit* [Invitrogen, California, USA]).

Concentrations in both arterial and venous samples were performed in the first 15 patients, 7 CTO patients and 6 CAD patients. An initial analysis was performed to determine the intra-class coefficient with subsequent analysis performed on only the arterial samples. Due to the very high intraclass correlation coefficient between recordings, further analysis on subsequent patients was not performed.

10.3.3 Haematological and Biochemical Results

Peripheral venous blood, as described in 10.3.1 was also collected in a 3.5mL vacutainer tube containing spray coated silica and a polymer gel (Greiner Bio One, Austria) and 2 x 4mL vacutainer tubes sprayed with Ethylenediaminetetraacetic acid (EDTA) (Greiner Bio One, Austria). The samples collected in the EDTA tubes were analysed for haemoglobin (g/L), white cell count and differentials (x 10^9/L), platelet count (x 10^9/L) and glycosylated haemoglobin (mmol/L). The spray coated tube was used to determine, serum creatinine (umol/L), troponin I (ng/L), N Terminal pro B-type Natriuretic Peptide (NT pro-BNP) (ng/L), albumin (g/L), cholesterol (mmol/L), C reactive protein (CRP) (mg/L), low density lipoprotein (LDL) (mmol/L), high density lipoprotein (HDL) (mmol/L) and triglycerides (mmol/L).

For biochemistry results, samples were centrifuged at 4,500 RCF for 5 minutes and kept at room temperature for the duration of the analysis. An Abbott Architect c16000 instrument [Abbott laboratories, Illinois, USA] was used along with the following reagents for each test; creatinine (Jaffe colourimetric assay); albumin (Bromcresol purple colourimetric assay), cholesterol (Cholesterol esterase: cholesterol oxidase enzymatic assay); triglyceride (Lipase: Glycerol-kinase: Glycerol phosphate oxidase enzymatic assay) and HDL Cholesterol (Homogenous Accelerator Selective Detergent method). LDL was calculated using the Friedewald equation. The Abbott Architect i2000 instrument [Abbott laboratories, Illinois, USA] was used for troponin I (ARCHITECT STAT High Sensitive Troponin-I chemiluminescent microparticle immunoassay (CMIA)) and NT-pro-BNP (Alere NT-proBNP for ARCHITECT chemiluminescent microparticle immunoassay (CMIA). The Abbott Architect c8000 instrument [Abbott laboratories, Illinois, USA] was used for HbA1c (ARCHITECT Enzymatic HbA1c assay).

10.3.4 Demographic Data Acquisition

After obtaining written informed consent, patients were asked interview questions with regards to their past medical history, current medications as well as functional effect of angina and dyspnoea. These were semi quantitatively scored using the New York Heart Association (NYHA) Class and the Canadian Cardiovascular Society (CCS) angina classification, both of which are readily used clinically and validated for clinical and research use (12,13). Patients had their weight and height recorded to calculate the body mass index (BMI). Left ventricular ejection fraction (LVEF) was recorded from an echocardiogram performed within 3 months prior to the angiogram, whilst a history of valvular heart disease

was defined as at least moderate severity mitral or aortic valve stenosis or regurgitation. Baseline heart rate, systolic blood pressure and diastolic blood pressure were recorded.

10.3.5 Angiographic Assessment and Grading

The presence and degree of collaterals was graded according to the Rentrop classification (14) and Collateral Connection grade (CC) (15). The severity of coronary disease was graded using the TAXUS Drug-Eluting Stent Versus Coronary Artery Bypass Surgery for the Treatment of Narrowed Arteries (SYNTAX) score (16).

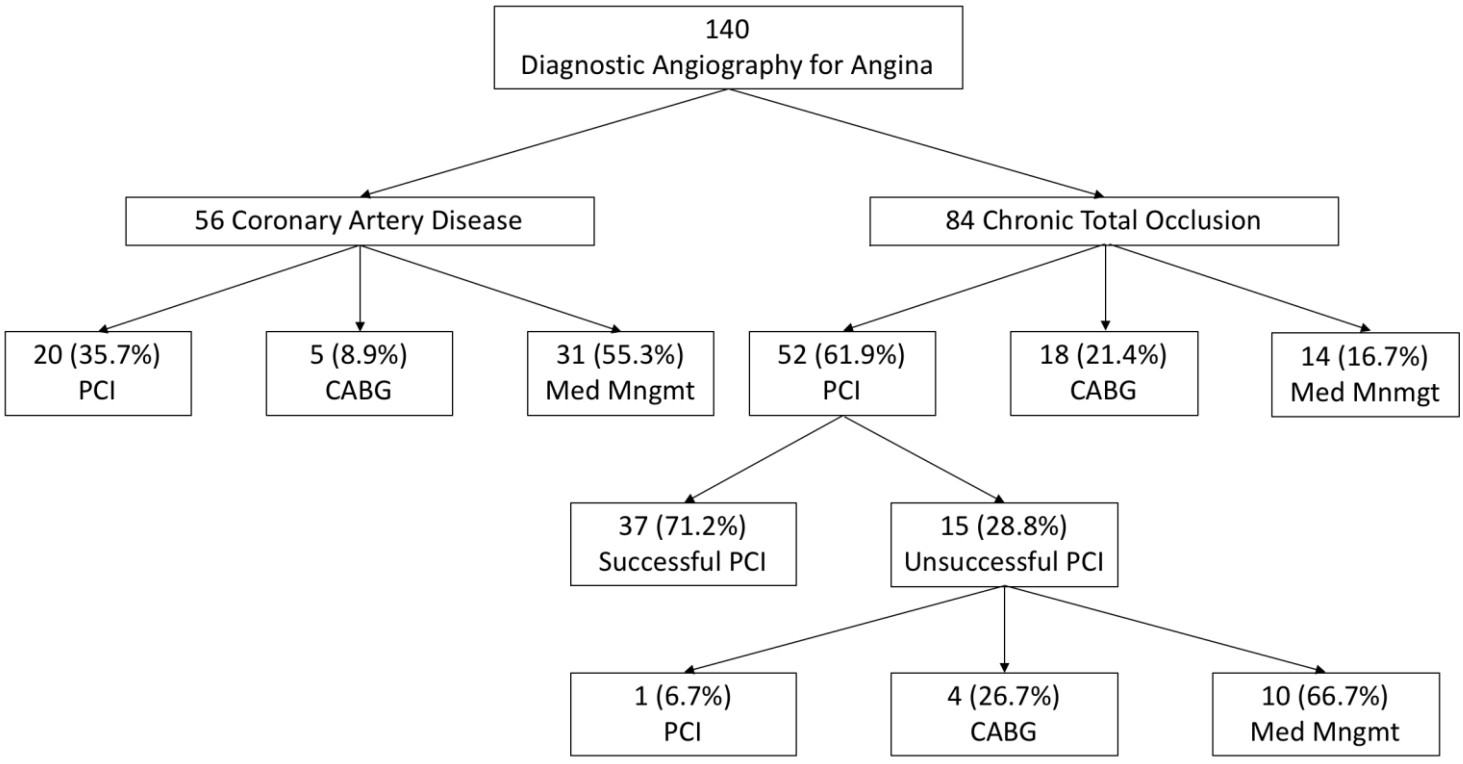
10.3.4 Statistical Analysis

Categorical data was presented as number of cases and percentage of the total population, while continuous variables were presented as means ± standard deviation for normally distributed data or as medians and interquartile ranges for data not normally distributed. Continuous data was analysed with Student's T-test for normally distributed data or the Mann-Whitney U test for not normally distributed data. Categorical variables were compared using a Chi-square test. To determine the correlation between arterial and venous samples, the intraclass correlation coefficient was analysed, where a level >0.9 was defined as excellent reliability, 0.75 – 0.9 was defined as good reliability, 0.5 – 0.75 was defined as moderate reliability while <0.5 was defined as poor reliability (17). To determine whether there was any temporal change in concentrations of endothelial cell biomarkers, an analysis of variance (ANOVA) regression between each time point was performed. All tests were 2-sided, and $p < 0.05$ was considered statistically significant. Analyses were performed using SPSS (version 24, IBM, New York, New York) and Prism 8.0 (GraphPad Software, San Diego, California).

10.4 Results

Over the study period, 140 patients who underwent diagnostic coronary angiography were included in the study. Of these, 56 patients (40%) had stable coronary artery disease (CAD), and 84 (60%) had a CTO. Of the patients with a CTO, 15 (17.9%) had multiple CTOs. Of the patients with CAD, 20 (35.7%) underwent PCI, 5 (8.6%) were referred for coronary artery bypass grafting (CABG), and 31 (55.3%) were managed medically. Of the 84 patients with a CTO, 52 patients (61.9%) underwent PCI, 18 (21.4%) were referred for CABG and 14 (16.7%) underwent medical management. Of the patients undergoing PCI, 37 (71.2%) were successful, while 15 (28.8%) were unsuccessful. The patients who had an unsuccessful CTO PCI were subsequently managed with repeat CTO PCI attempt, CABG or medical management as shown in Figure 10.1.

Figure 10.1: Patients included in the study



10.4.1 Baseline Characteristics

Baseline characteristics between patients with a CTO and CAD are shown in Table 10.1. Patients with a CTO were older (72.5yrs vs 68.6yrs, $p<0.05$) and had greater rates of peripheral vascular disease (PVD) (14.3% vs 3.6%, $p<0.05$) compared with patients with CAD. Rates of cardiovascular risk factors were similar, as were rates of valvular heart disease or atrial fibrillation. Patients with a CTO were more likely to be taking aspirin (89.3% vs 75%, $p<0.05$) and an angiotensin converting enzyme inhibitor (ACE-I) or angiotensinogen II receptor blocker (ARB) (61.9% vs 41.1%, $p<0.05$) compared with patients with CAD. Patients with a CTO were more symptomatic with greater rates of CCS class III (22.6% vs 7.1%, $p<0.05$) and higher rates of NYHA class III or IV (41.7% vs 19.7%, $p<0.05$) and had a higher syntax score (20.6 vs 6.6, $p<0.0001$).

10.4.2 Arterial and Venous Concentrations of Endothelial Cell Biomarkers

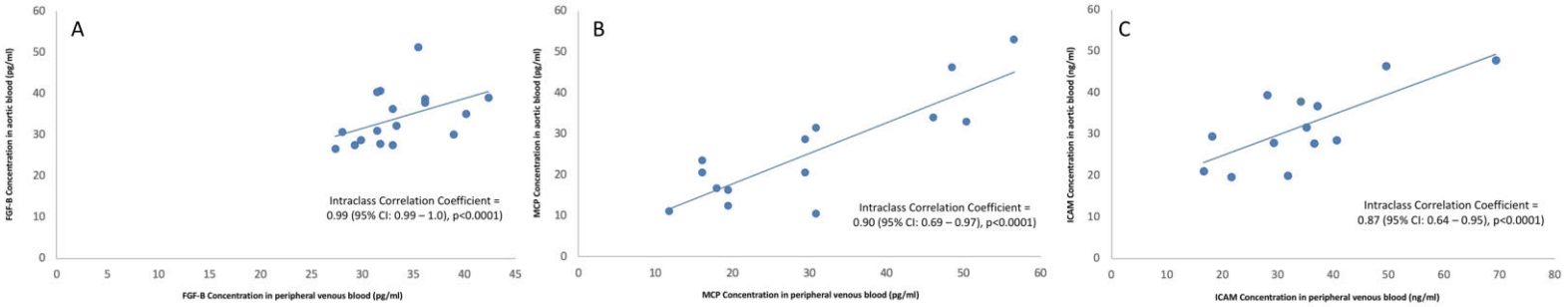
In a subset of patients, both arterial and venous samples were used to determine the concentration of FGF-B (n=18), MCP (n=14) and ICAM-1 (n=13). The intraclass correlation coefficient between arterial and venous samples for FGF-B was 0.99 (95%CI: 0.99 – 1.0, $p<0.0001$), consistent with excellent correlation. The intraclass correlation coefficient for MCP-1 was 0.90 (95%CI: 0.69 – 0.97, $p<0.0001$), consistent with excellent correlation, while the intraclass correlation coefficient for ICAM-1 was 0.87 (95%CI: 0.64 – 0.95, $p<0.0001$) consistent with good correlation (Figure 10.2).

Table 10.1: Baseline Characteristics of patients with a CTO and CAD

	CTO n = 84	**CAD n = 56**	**p-value**
Female Sex *(n)*	20 (23.8%)	20 (35.7%)	0.13
Age *(yrs)*	**72.5 ± 10.5**	**68.6 ± 11.0**	**<0.05**
BMI *(kg/m^2)*	28.1 ± 6.3	28.1 ± 5.4	0.96
Past Medical History *(n)*			
Hypertension	64 (76.2%)	42 (75%)	0.87
Hypercholesterolaemia	77 (91.7%)	48 (85.7%)	0.26
Diabetes Mellitus	24 (28.6%)	23 (41.1%)	0.12
Smoking History			0.12
Never	32 (38.1%)	31 (55.4%)	
Ex-Smoker	42 (50%)	19 (33.9%)	
Current	10 (11.9%)	6 (10.7%)	
History of PVD *(n)*	**12 (14.3%)**	**2 (3.6%)**	**<0.05**
Valvular Heart Disease *(n)*	5 (6%)	7 (12.5%)	0.17
Atrial Fibrillation *(n)*	13 (15.5%)	6 (10.7%)	0.42
Medications *(n)*			
Aspirin	**75 (89.3%)**	**42 (75%)**	**<0.05**
P2Y12 Inhibitor	52 (61.9%)	23 (41.1%)	<0.05
Statin	72 (85.7%)	42 (75%)	0.11
ACE-I/ARB	**52 (61.9%)**	**25 (41.1%)**	**<0.05**
Beta Blockers	48 (57.1%)	33 (58.9%)	0.83
Non-dihydropyridine CCB	8 (9.5%)	2 (3.6%)	0.18
Dihydropyridine CCB	18 (21.4%)	9 (16.1%)	0.43
Thiazide	5 (6%)	4 (7.1%)	0.78
Loop Diuretic	7 (8.3%)	7 (12.5%)	0.42
Aldosterone Antagonist	6 (7.1%)	3 (5.4%)	0.67
Nitrate	13 (15.5%)	5 (8.9%)	0.26
NYHA Class *(n)*			**<0.01**
I	**11 (13.1%)**	**20 (23.8%)**	
II	**38 (45.2%)**	**25 (29.8%)**	
III	**32 (38.1%)**	**10 (17.9%)**	
IV	**3 (3.6%)**	**1 (1.8%)**	
CCS Class *(n)*			**<0.05**
I	**19 (22.6%)**	**20 (35.7%)**	
II	**46 (54.8%)**	**32 (57.1%)**	
III	**19 (22.6%)**	**4 (7.1%)**	
Heart rate *(bpm)*	69.7 ± 13.0	73.5 ± 18.0	0.15
Systolic Blood Pressure *(mmHg)*	133.4 ± 24.5	132.7 ± 19.2	0.84
Diastolic Blood Pressure *(mmHg)*	**72.9 ± 11.8**	**78.5 ± 14.9**	**<0.05**
LVEF *(%)*	**60 (50 – 60)**	**60 (60 – 60)**	**<0.05**
Syntax Score	**20.6 ± 11.8**	**6.6 ± 7.4**	**<0.0001**
Successful PCI performed	37 (44.0%)	20 (35.7%)	0.33

** ACE-I = angiotensin converting enzyme inhibitor; ARB = Angiotensinogen II receptor blocker; bpm = beats per minute; CCB = calcium channel blocker; CCS = Canadian Cardiovascular Society; LVEF = left ventricular ejection fraction; n= number; mmHg = millimetres of mercury; NYHA = New York Heart Association; PVD = peripheral vascular disease; yrs = years;*

Figure 10.2: Endothelial Cell Biomarker concentrations in arterial and venous circulations

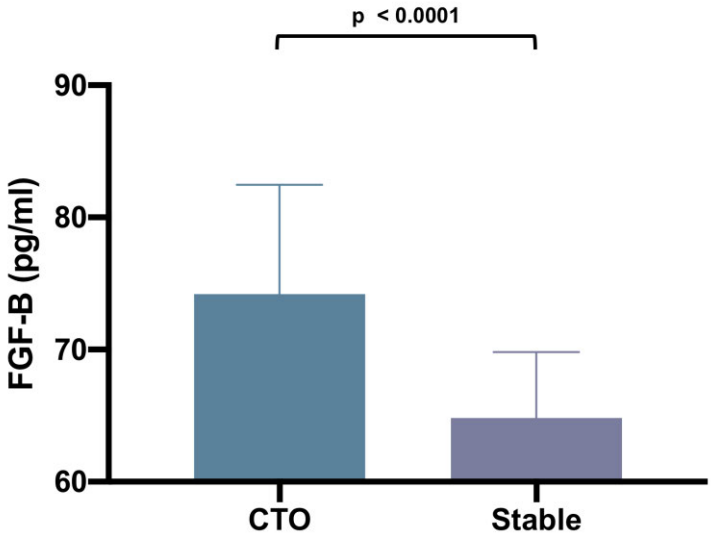


Arterial (y axis) and venous (x axis) concentrations of plasma FGF-B (A), MCP-1 (B) and ICAM-1 (C) in the same patient prior to angiography.

10.4.3 Concentrations of Endothelial Cell Biomarkers in Patients with CTO and CAD.

In patients with a CTO, the plasma concentration of FGF-B was significantly higher than in patients with stable CAD (74.2pg/mL [64.2pg/mL – 82.5pg/ml] vs 64.8pg/mL [31.7pg/mL – 69.8pg/ml], p<0.0001) (Figure 10.3).

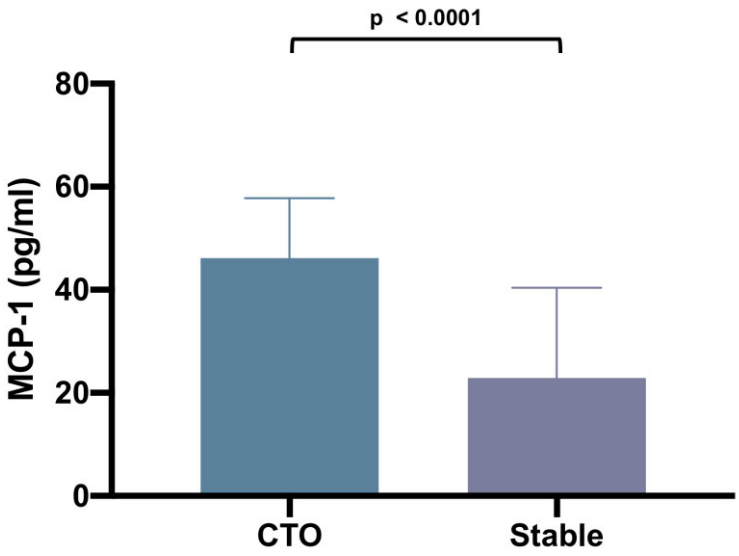
Figure 10.3: Plasma concentration of FGF-B in patients with CTO and CAD.



Data illustrated as Mean + SD illustrated for patients with a CTO and stable CAD.

In patients with a CTO, the plasma concentration of MCP-1 was significantly higher than in patients with stable CAD (46.1pg/mL [32.4pg/mL – 57.8pg/ml] vs 22.9pg/mL [17.8pg/mL – 40.4pg/ml], p<0.0001) (Figure 10.4).

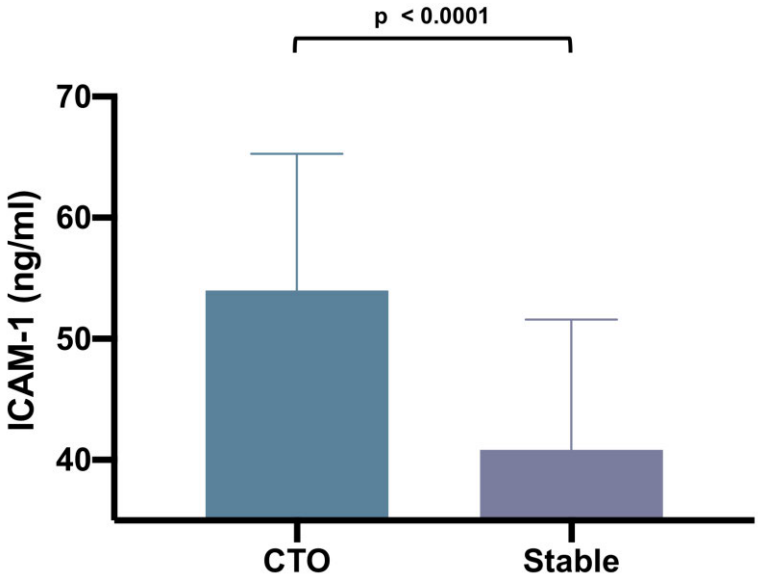
Figure 10.4: Plasma concentration of MCP-1 in patients with CTO and CAD



Data illustrated as Mean + SD illustrated for patients with a CTO and stable CAD.

In patients with a CTO, the plasma concentration of ICAM-1 was significantly higher than in patients with stable CAD (54.0ng/mL [38.2 ng/mL –66.3 ng/ml] vs 40.8 ng/mL [28.1ng/mL – 51.6 ng/ml], p<0.001) (Figure 10.5).

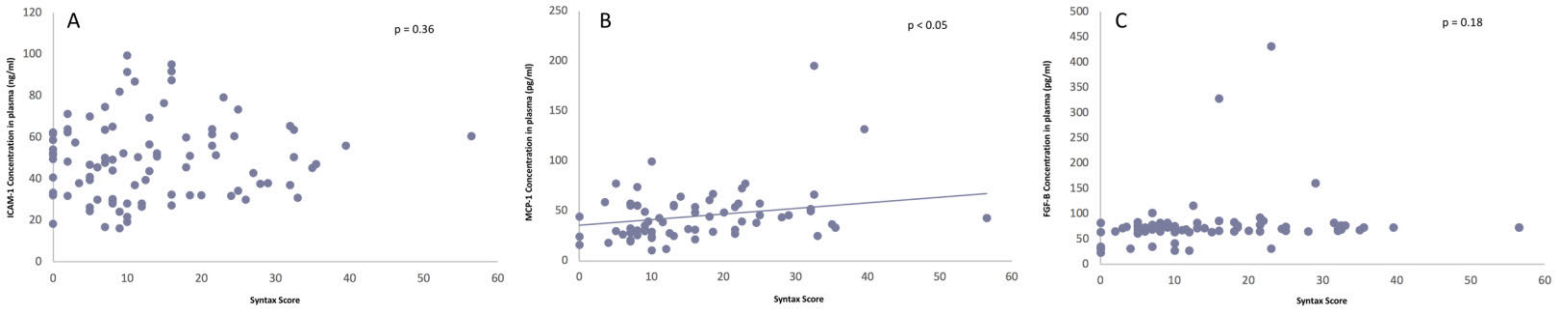
Figure 10.5: Plasma concentration of ICAM-1 in patients with CTO and CAD



Data illustrated as Mean + SD illustrated for patients with a CTO and stable CAD.

There was no association between plasma FGF-B concentration and Syntax score (p=0.18), or between plasma ICAM-1 and Syntax score (p=0.36).There was however, a direct relationship between plasma MCP-1 and Syntax score (p<0.05) (Figure 10.6).

Figure 10.6: Plasma concentration of ICAM-1 in patients with CTO and CAD

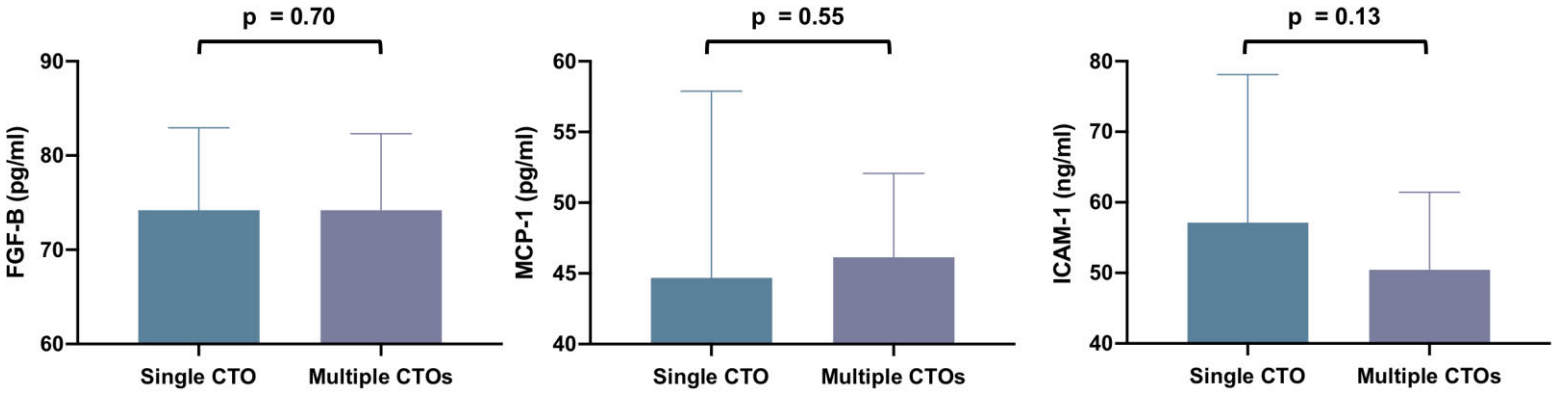


(A) No correlation between plasma FGF-B and Syntax score (p=0.18). (B) Significant linear relationship between plasma MCP-1 and Syntax Score (p<0.05). (C) No correlation between plasms ICAM-1 and Syntax score (p=0.36).

10.4.4 Plasma concentration of endothelial cell biomarkers in patients with a CTO

In patients with multiple CTOs, there was no difference in plasma concentration of FGF-B (74.2pg/mL [69.2pg/mL – 82.9pg/ml] vs 74.2pg/mL [71.1pg/mL – 82.3pg/ml], p=0.70), MCP-1 (44.7pg/mL [31.3pg/mL – 57.9 pg/ml] vs 46.1 [43.3pg/mL – 52.1pg/mL],p=0.55) or ICAM-1 (57.1ng/mL ± 21.0ng/mL vs 50.4ng/mL ± 11.0ng/ml, p=0.13) (Figure 10.7).

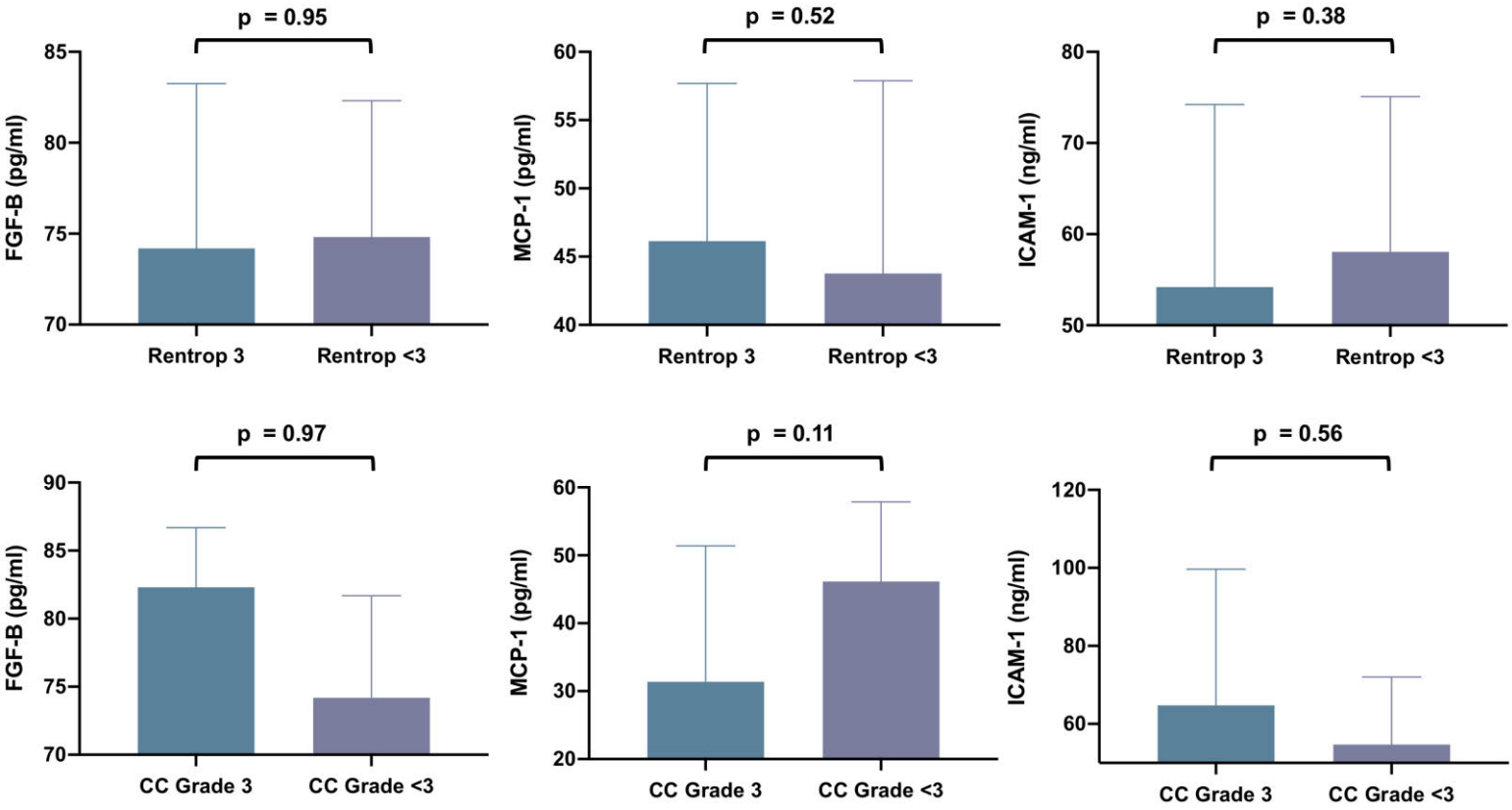
Figure 10.7: Plasma concentration of endothelial cell biomarkers relative to number of CTOs



Data illustrated as Mean + SD illustrated for patients with a CTO and stable CAD.

There was no difference in plasma concentration of FGF-B in patients with Rentrop grade 3 vs Rentrop < 3 (74.22pg/mL [69.62pg/mL – 83.22pg/ml] vs 74.8 [69.12pg/mL – 82.32pg/ml], p=0.95) or with CC grade 3 vs < 3 (82.32pg/mL [66.02pg/mL – 86.72pg/ml] vs 74.22pg/mL [69.82pg/mL – 81.72pg/ml], p=0.97). There was similarly no difference in plasma concentrations of MCP-1 in patients with Rentrop grade 3 vs Rentrop < 3 (46.12pg/mL [32.62pg/mL – 57.72pg/ml] vs 43.82pg/mL [30.42pg/mL – 57.92pg/ml], p=0.52) or with CC grade 3 vs < 3 (31.42pg/mL [23.82pg/mL – 51.42pg/ml] vs 46.12pg/mL [33.52pg/mL – 57.92pg/ml], p=0.11). There was no difference in plasma concentrations of ICAM-1 in patients with Rentrop grade 3 vs Rentrop < 3 (53.6ng/mL ± 20.9ng/mL vs 58.0 ng/mL ± 17.0 ng/ml, p=0.38) or with CC grade 3 vs < 3 (64.7 ng/mL ± 35.0 ng/mL vs 54.7 ng/mL ± 17.3 ng/ml, p=0.56) (Figure 10.8).

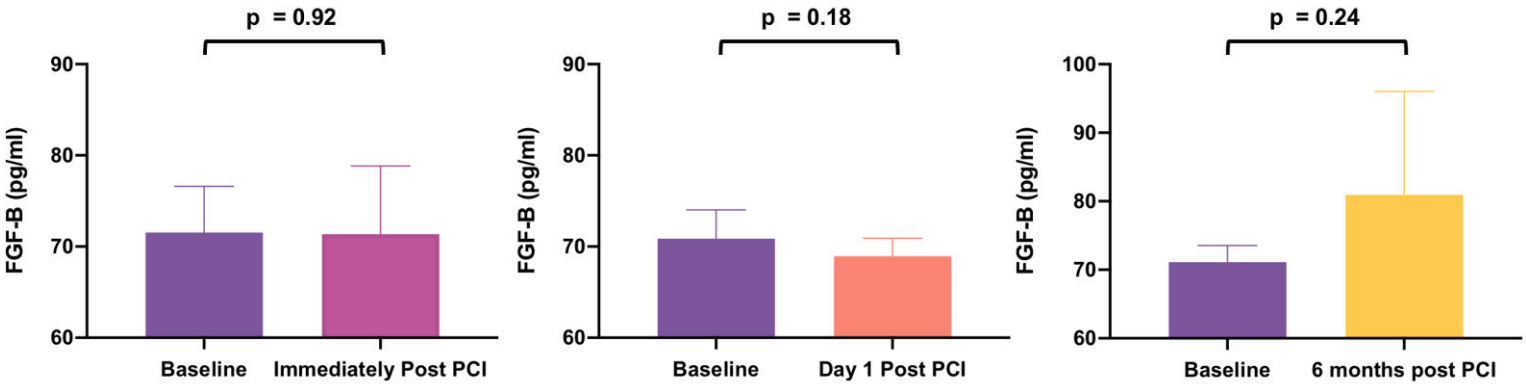
Figure 10.8: Plasma concentration of endothelial cell biomarkers stratified by angiographic assessment of robustness of coronary collaterals.



10.4.5 Plasma concentration of endothelial cell biomarkers following Collateral Regression

In patients who underwent successful CTO PCI, and hence regression of coronary collaterals, the concentration of endothelial cell biomarkers were compared in a temporal fashion. In 18 patients, immediately following collateral regression, there was no change in plasma FGF-B concentration (71.5pg/mL ± 5.1pg/mL vs 71.4pg/mL ± 7.5pg/ml, p=0.92). In 9 patients, there was similarly no change in plasma FGF-B concentration 1 day following collateral regression (70.8pg/mL ± 3.2pg/mL vs 68.9pg/mL ± 2.0pg/ml, p=0.18), while in 6 patients there was no change in FGF-B concentration 6 months following collateral regression (71.1pg/mL ± 2.4pg/mL vs 80.9pg/mL ± 15.1pg/ml, p=0.24) (Figure 10.9).

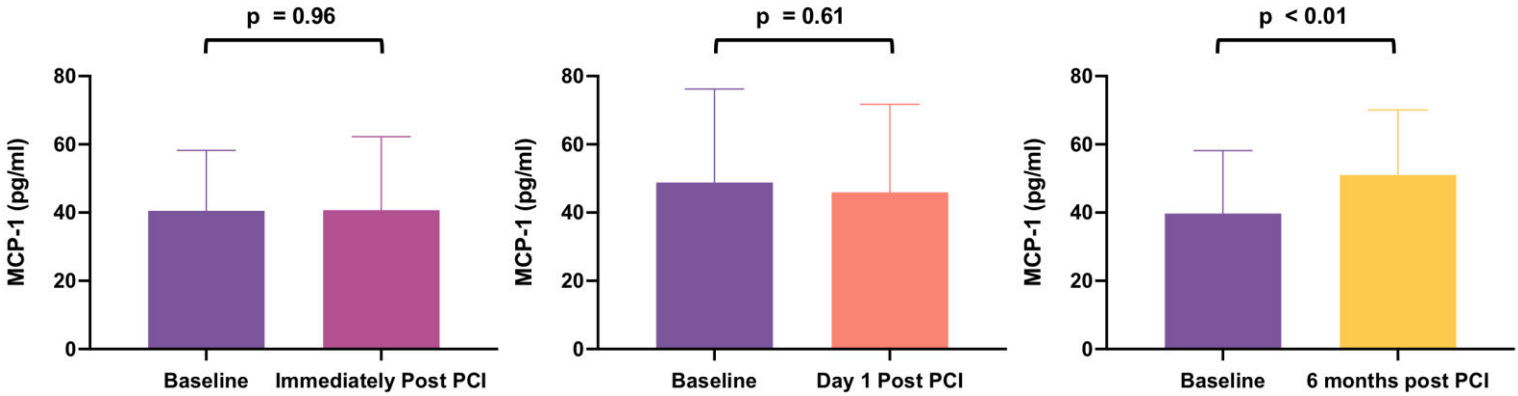
Figure 10.9: Plasma concentration of FGF-B change following collateral regression.



Data illustrated as Mean + SD illustrated for patients with a CTO and stable CAD.

In 20 patients, immediately following collateral regression, there was no change in plasma MCP-1 concentration (40.5pg/mL ± 17.7pg/mL vs 40.7pg/mL ± 21.5pg/ml, p=0.96). In 6 patients, there was no change in plasma MCP-1 concentration 1 day following collateral regression (48.8pg/mL ± 27.4pg/mL vs 45.9pg/mL ± 25.8pg/ml, p=0.61). In 18 patients there was a significant increase in plasma MCP-1 6 months following CTO PCI (39.7pg/mL ± 18.5pg/mL vs 51.1pg/mL ± 19.0pg/ml, p<0.01) (Figure 10.10).

Figure 10.10: Plasma concentration of MCP-1 change following collateral regression.

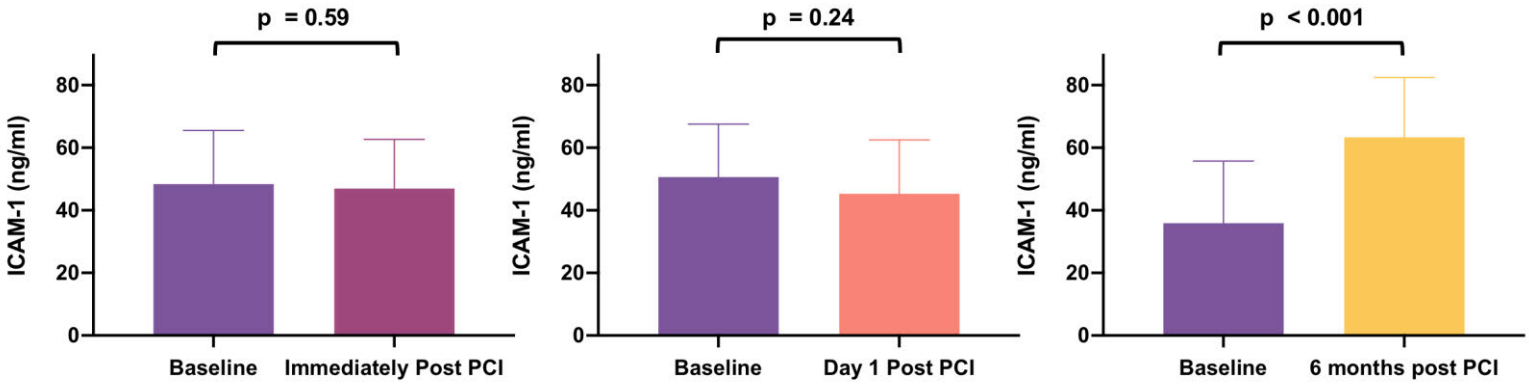


Data illustrated as Mean + SD illustrated for patients with a CTO and stable CAD.

In 21 patients, immediately following collateral regression, there was no change in plasma ICAM-1 concentration (48.3ng/mL ± 17.2ng/mL vs 46.9ng/mL ± 15.5ng/ml, p=0.59). In 8

patients, immediately following CTO PCI, there was no change in plasma ICAM-1 concentrations (50.7ng/mL ± 16.9ng/mL vs 45.3ng/mL ± 17.2ng/ml, p=0.24). In 5 patients, at 6 months following collateral regression, there was a significant increase in plasma ICAM-1 (35.9ng/mL ± 19.8ng/mL vs 63.4ng/mL ± 19.1ng/ml, p<0.001) (Figure 10.11).

Figure 10.11: Plasma concentration of ICAM-1 change following collateral regression.



Data illustrated as Mean + SD illustrated for patients with a CTO and stable CAD.

10.4.6 Concentrations of Haematological, Biochemical and Proteomic Biomarkers in patients with CTO and CAD.

With respect to haematological parameters, patients with a CTO had a lower peripheral blood lymphocyte count ($1.6x10^9$ vs $1.8x10^9$, p<0.05) and a higher peripheral blood monocyte count ($0.6x10^9$ vs $0.5x10^9$, p<0.05) compared with patients with stable CAD. Patients with a CTO also had a higher neutrophil to lymphocyte ratio (NLR) ($2.7\ x10^9$ vs $2.2\ x10^9$, p<0.05), while haemoglobin count, platelet count along with absolute neutrophil and eosinophil counts were similar between patients with a CTO and stable CAD.

In patients with a CTO, NT proBNP was significantly higher (123ng/L vs 42ng/L, p<0.0001) as was serum troponin I concentration (8ng/L vs 4.5ng/L, p<0.0001). Serum CRP was significantly higher in patients with a CTO compared with patients with stable CAD

(2.6mg/L vs 1.2mg/L, p<0.05). Serum cholesterol, LDL and HDL were all significantly lower in patients with a CTO compared with patients with stable CAD (3.1mmol/L vs 3.8 mmol/L, p<0.05; 1.6 mmol/L vs 2.1 mmol/L, p<0.05 and 0.9 mmol/L vs 1.1 mmol/L, p<0.05 respectively). Other variables were similar between both groups as outlined in Table 10.2.

Table 2: Haematological, Biochemical and Proteomic Biomarkers

	CTO n = 84	**CAD n = 56**	**p-value**
Haemoglobin *(g/L)*	135 (118.5 – 145)	133 (127.5 – 148)	0.32
WCC *(x 10^9/L)*	7.1 (5.2 – 8.4)	6.8 (5.4 – 8.4)	0.87
Platelets *(x 10^9/L)*	211.5 (173.2 – 253)	222 (181 – 250.5)	0.43
Neutrophils *(x 10^9/L)*	4.5 (3.1 – 5.5)	3.9 (3.2 – 5.4)	0.63
Lymphocytes *(x 10^9/L)*	**1.6 (1.2 – 1.9)**	**1.8 (1.3 – 2.3)**	**<0.05**
NLR	**2.7 (2.0 – 3.6)**	**2.2 (1.8 – 3.0)**	**<0.05**
Monocytes *(x 10^9/L)*	**0.6 (0.4 – 0.8)**	**0.5 (0.4 – 0.7)**	**<0.05**
Eosinophils *(x 10^9/L)*	0.2 (0.1 – 0.2)	0.2 (0.1 – 0.3)	0.38
NT Pro-BNP *(ng/L)*	**123 (54.5 – 396)**	**42 (13.5 – 153.5)**	**<0.0001**
Albumin *(g/L)*	36.6 ± 4.2	37.9 ± 3.3	0.05
Creatinine *(umol/L)*	76 (70 – 91)	74 (63.7 – 93.7)	0.59
Troponin *(ng/L)*	**8 (5 – 21)**	**4.5 (3 – 7.2)**	**<0.0001**
Cholesterol *(mmol/L)*	**3.1 (2.9 – 4.1)**	**3.8 (3.2 – 4.4)**	**<0.01**
LDL *(mmol/L)*	**1.6 (1.4 – 2.2)**	**2.1 (1.5 – 2.7)**	**<0.05**
HDL *(mmol/L)*	**0.9 (0.8 – 1.3)**	**1.1 (0.95 – 1.3)**	**<0.05**
Trig ***(mmol/L)***	1.1 (0.8 – 1.4)	1.1 (0.8 – 1.4)	0.81
CRP *(mg/L)*	**2.6 (1.1 – 6.5)**	**1.2 (0.7 – 4.2)**	**<0.05**
HbA1C *(%)*	5.7 (5.3 – 6.3	5.7 (5.4 – 6.4)	0.42

At 6 months following collateral regression, there were no changes in the concentration of haematological, biochemical or proteomic biomarkers compared with baseline (Table 10.3).

Table 10.3: Temporal changes in haematological, biochemical and proteomic biomarkers following collateral regression

	Pre CTO PCI	**Post CTO PCI**	**p-value**
Haemoglobin *(g/L)*	129.3 ± 19.1	132.4 ± 17.8	0.46
WCC *(x 10^9/L)*	6.5 ± 2.1	6.7 ± 2.4	0.75
Platelets *(x 10^9/L)*	223.7 ± 69.3	225.7 ± 68.7	0.78
Neutrophils *(x 10^9/L)*	4.2 ± 1.6	4.4 ± 1.9	0.66
Lymphocytes *(x 10^9/L)*	1.5 ± 0.5	1.5 ± 0.6	0.79
NLR	2.9 ± 0.9	3.1 ± 1.3	0.65
Monocytes *(x 10^9/L)*	0.6 ± 0.2	0.6 ± 0.2	0.82
Eosinophils *(x 10^9/L)*	0.2 ± 0.1	0.2 ± 0.2	0.69
NT Pro-BNP *(ng/L)*	257.5 ± 706.3	254.9 ± 526.5	0.96
Albumin *(g/L)*	37.0 ± 4.3	37.2 ± 8.2	0.87
Creatinine *(umol/L)*	114.9 ± 126.6	160.2 ± 232.4	0.27
Troponin *(ng/L)*	14.3 ± 25.6	10.0 ± 12.0	0.24
Cholesterol *(mmol/L)*	3.8 ± 1.2	3.6 ± 0.6	0.64
LDL *(mmol/L)*	2.0 ± 1.0	1.7 ± 0.4	0.18
HDL *(mmol/L)*	1.0 ± 0.5	1.2 ± 0.4	0.08
Trig *(mmol/L)*	1.4 ± 0.8	1.8 ± 1.3	0.18
CRP *(mg/L)*	3.7 ± 4.0	2.1 ± 2.1	0.09
HbA1C *(%)*	5.8 ± 0.9	5.9 ± 1.0	0.34

10.5 Discussion

Patients with a CTO, and hence collaterals, have distinct differences in endothelial cell, haematological and biochemical markers, which may be attributable to the underlying arteriogenesis process. The plasma concentration of endothelial cell biomarkers, namely MCP-1 and ICAM-1 are dynamic and appear to change in response to collateral regression, further suggesting their role in the process of coronary collaterals recruitment and maturation.

10.5.1 FGF-B

The plasma concentration of FGF-B was significantly higher in patients with a CTO than in those without. There was no correlation with FGF-B concentration and number of CTOs, maturity of collaterals or atherosclerotic severity as assessed by the Syntax score. Previous clinical studies have suggested that concentrations of FGF-B are higher in patients with a

CTO (18), however they did not quantify changes over time. In the present study, there was no change in the concentration of FGF-B following collateral regression. This suggests that FGF-B may be related to the degree of coronary vasodilation (19), as occurs through vascular autoregulation in the setting of a severe stenosis. This may explain the lack of change following collateral regression, as other processes may still result in vasodilatation, particularly in the setting of angina and ischaemic heart disease. Furthermore, other studies (20) have suggested that hypercholesterolaemia and endothelial dysfunction, commonly found in patients with a CTO (21-23), may antagonise the effect of exogenous FGF-B. It is thus plausible that a similar environment may affect endogenous production and synthesis of FGF-B.

10.5.2 MCP-1

Plasma MCP-1 was significantly higher in patients with a CTO compared with patients with CAD. This is similar to another study which assessed MCP-1 concentration in patients with >70% stenosis in an epicardial vessel, finding a higher concentration in patients with more robust collaterals (24), which was not found in the present study. Other research has suggested that in the setting of acute coronary syndromes, MCP-1 is elevated (25) and correlates with the presence of CAD (26). Infusion of MCP-1 in experimental animal models, resulted in increased neointima formation and a change in plaque composition towards an unstable phenotype, suggesting its effects are more complex than simply related to endothelial cell activation and collateral maturation (27). In the present study, while the concentration of plasma MCP-1 was associated with more diffuse atherosclerotic disease as assessed by the Syntax score, there was no relationship between number of CTOs or maturity of collaterals. Given one of the early steps of collateral maturation is monocyte attraction and activation, which is a predominant effect of MCP-1, it is possible this may be associated with

collateral maturity early on in formation, as would be expected in severe lesions, prior to the formation of a CTO, which would be of a longer duration (24).

Plasma MCP-1 concentrations were higher 6 months following collateral regression compared with baseline. Clinical studies have suggested that rates of AMI following CTO revascularisation are much lower than target vessel re-occlusion (28), suggesting persisting functional collaterals, or alternatively a retained potential for rapid collateral recruitment (29). MCP-1 has been implicated in ischaemic pre-conditioning (30), suggesting that in patients with the ability to recruit collaterals, a higher basal concentration may be maintained in the short to midterm, which may augment collaterals again in the setting of re-occlusion.

10.5.3 ICAM-1

Plasma ICAM-1 was significantly higher in patients with a CTO compared to patients with CAD. While animal studies have implicated ICAM-1 in the setting of peripheral vascular disease (31), there have been few studies in human CTOs. While ICAM-1 has been associated with coronary artery disease (32), in the present study, there was no correlation between plasma ICAM-1 and Syntax score, number of CTOs or maturity of coronary collaterals. One of the proposed mechanism by which ICAM-1 is believed to recruit and mature collaterals is through neutrophil chemoattraction and inflammation, which is the early phase of arteriogenesis (33,34). It is thus possible that an elevation of ICAM-1 correlates with the presence of coronary collaterals rather than the maturity of collaterals. Furthermore, similar to the results of MCP-1, at 6 months following collateral regression there was a significant increase in the plasm concentration of ICAM-1. ICAM-1 has been associated with ischaemic preconditioning (35,36), thereby suggesting a mechanism for allowing future rapid collateral recruitment in these patients.

10.5.4 Haematological Biomarkers

In patients with a CTO, peripheral blood lymphocytes were lower than in patients with CAD, while peripheral blood monocyte count was higher than in patients with CAD. There was also a significantly higher peripheral blood neutrophil to lymphocyte (NLR) ratio compared to patients with CAD.

The relationship between monocyte activation and collaterals has been suggested with the elevation in plasma MCP-1 in patients with coronary collaterals, with the absolute increase in peripheral blood monocytes reflecting this. In a study (37) of patients with at least 95% stenosis, patients with robust collaterals had a significantly lower monocyte count as compared to patients with poorer collaterals, although this was not significant on multivariate analysis. Monocytes are believed to play a crucial role in arteriogenesis through upregulation of growth factor receptors on endothelium, synthesis of inflammatory molecules and serving as precursors to endothelial cells (38). While another study suggested that it is tissue resident monocytes rather than circulating monocytes which are primarily involved in arteriogenesis (39), it is also likely that circulating mediators may upregulate haematopoietic stem cell differentiation toward monocytes.

Like monocytes, lymphocytes are key immune cells involved in arteriogenesis and inflammation. Following monocyte attraction, activation and infiltration into the primitive collateral vessel, a proportion differentiate into dendritic cells triggering the activation of antigen specific T lymphocytes associated with creation of the local inflammatory environment (40). It is thus unsurprising that peripheral lymphocytes were lower in patients with a CTO, than in patients with CAD. This may suggest that lymphocytes have migrated to the site of collateral vessels, or alternatively less haemopoietic stimulation in the setting of

collaterals. However, the implication of lymphocyte count on collateral maturation has been controversial (41), where the relative number of lymphocytes may be more relevant than an absolute number.

The neutrophil to lymphocyte ratio (NLR) has been associated with survival in multiple subtypes of malignancies (42,43) and a higher ratio is associated with poorer cardiovascular outcomes (44). Patients with a CTO had a significantly higher NLR than patients with CAD. Previous studies have suggested that in patients with well matured collaterals, the NLR is lower than in patients with poorer collaterals (45), which was also demonstrated in Section IV Chapter 6. The NLR presents a ratio of neutrophils, which are involved in the early phase of inflammation with lymphocytes, which are the involved in the regulatory and maintenance state of inflammation (46). It is possible that as patients with differing lengths of symptoms with a CTO were included, this may have included a greater proportion of relatively 'young' occlusions, thereby increasing the relative concentration of neutrophils, and hence increasing the NLR. This is further strengthened by the finding of a significantly higher serum CRP in patients with CTO as compared with patients with CAD. Previous studies have found that more well-developed collateral have lower CRP concentration (47,48), suggesting a change from the early active inflammatory phase to the later maintenance phase.

10.5.5 Biochemical Biomarkers

Patients with a CTO had a higher NT-proBNP, likely reflecting higher rates of left ventricular impairment and history of heart failure symptoms as noted in the baseline demographics compared with patients with CAD. Serum troponin I was significantly higher in patients with a CTO compared with those with CAD. As these patients presented with stable angina symptoms, this is not reflective of an acute coronary syndrome. It is known that patients with

a CTO are in a chronic state of ischaemia (49), and the higher troponin may be a marker of microvascular ischaemia, although the vast majority of patients had a troponin within the reference limit.

In patients with a CTO, the total cholesterol and LDL were lower than in patients with CAD. This may be attributable to patients with a CTO being admitted for planned PCI, and therefore more likely to have optimal medical therapy, although rates of statin usage in patients with a CTO and those with CAD were similar. Patients with higher LDL and total cholesterol are associated with poorer collateral recruitment, particularly in patients with diabetes (50). HDL was significantly lower in patients with a CTO compared to patients with CAD. HDL has long been associated with an inverse relationship with cardiovascular outcomes, attributable to reverse cholesterol transportation (51) and is an independent predictor of cardiovascular risk (52). A previous study suggested that a lower HDL was associated with poorer coronary collaterals (53), while in-vitro studies suggest that high HDL suppresses ICAM-1 secretion by endothelial cells (54), while MCP-1 also suppresses HDL (55). It is thus possible that elevated circulating endothelial derived proteins associated with collateral recruitment may reduce the HDL concentration in CTO patents.

Despite the initial correlations between collaterals and these biomarkers, there was no significant change following collateral regression. It is possible that these may take longer to show an alteration, as compared to the endothelial cell biomarkers, which would be the initial cascading event following change in blood flow and shear stress.

10.5.6 Limitations

This study, while implicating endothelial cell derived biomarkers and leukocytes with collateral recruitment, has limitations. Firstly, the comparator group, while consecutive patients with CAD, had demographic differences, which are inherent to the nature of differing severity of coronary disease. A further comparator group of patients who had medical management of their CTO was not performed, and may have confirmed the stable concentration of these biomarkers over time. Propensity matching was not possible due to the relatively small numbers, but may have allowed correction for some confounders. In patients with CTO revascularisation, repeat coronary imaging was not performed, to rule out re-occlusion, however all patients reported a sustained improvement in anginal symptoms suggesting this is less likely. Furthermore, not all patients who had successful CTO PCI had repeat serum blood test analysis, due to technical issues with obtaining or interpreting samples, or drop out from the study. This may have implications on introducing bias, and future studies should attempt to increase the number of patients included in the study.

10.6 Conclusion

In patients with angiographically confirmed coronary collaterals in the setting of a CTO, concentrations of the endothelial cell derived biomarkers, FGF-B, MCP-1 and ICAM-1 are significantly higher than in patients without collaterals. These markers are found in similar concentrations in both the peripheral venous and arterial circulation, and are markers of the presence of collaterals rather than their maturity, suggesting a role in the initiation and maintenance phase of collateral recruitment. The concentrations of MCP-1 and ICAM-1 are significantly higher following CTO PCI, suggesting a role of ischaemic preconditioning and retained collateral recruitment potential. This may explain the low rates of acute myocardial infarction following revascularisation in this cohort. Alterations in peripheral leukocyte count

and relative changes in cell lineages reflect the importance of these cell types in coronary collateral recruitment and maturation. Whether these proteins may be used as a clinically relevant biomarker or potential therapeutic strategy for patients with severe coronary disease requires further assessment.

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SECTION VI: CORONARY COLLATERALS IN AN ANIMAL MODELS

Chapter 11: Overview of Animal Models for investigation of Coronary Chronic Total Occlusion

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Short Review

Animal chronic total occlusion models: A review of the current literature and future goals

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ABSTRACT

Coronary chronic total occlusions (CTOs) are commonly found in patients undergoing coronary angiography and is associated with poorer prognosis than in those patients with other forms of stable coronary artery disease. As such, with an increasing appreciation of this clinical entity, there is a need to identify, firstly the pathophysiological process driving its formation, as well as new percutaneous strategies for revascularisation with long term durability and improved outcomes. An appropriate, reliable and reproducible animal model is vital for both of these objectives.

We review the prevalence of spontaneous collaterals in different species, as well as review the current literature with respect to animal models of CTOs, and compare and contrast the advantages and disadvantages of these differing models. Whilst both extrinsic compression models and endoluminal procedures may create situations analogous to a CTO in a human, the ideal animal model of a CTO will include an occluded artery, functional collaterals and a viable myocardium. This would allow study of the process driving collateral formation and arteriogenesis as well as percutaneous intervention strategies for both acute and long term benefits.

1. Introduction

A coronary chronic total occlusion (CTO) is characterised by significant atherosclerotic plaque burden within an artery resulting in complete (or near complete) occlusion of the vessel present for at least 3 months [1]. The incidence of a CTO has been shown to be almost 20% in patients presenting for non-urgent coronary angiography [2] and 6.6% of those presenting with an ST elevation myocardial infarction [3]. The presence of a CTO is independently associated with greater mortality and poorer prognosis [4]. Current success rates of CTO percutaneous coronary intervention in the setting of experienced operators is 91% [5], with rates of restenosis or re-occlusion 20% at 1 year, depending on stent type and PCI strategy [6]. Consequently, this remains one of the most challenging management dilemmas in modern interventional cardiology.

Animal models have been pivotal in the advancement of interventional cardiology over the past 40 years, particularly since the advent of catheter based technologies. Initial research into drug eluting stents [7,8], bifurcation stenting strategies [9], mechanisms of in stent restenosis [10], bioresorbable scaffolds [11] as well as the first transcatheter heart valves [12] (precursor to transcatheter aortic valve implantation) were all conducted in animal models. As such, a reliable and reproducible animal model of a coronary CTO is vital to appreciate not only the underlying pathophysiological processes of CTO and collateral formation, but also to trial potential therapeutic modalities and approaches to revascularisation.

In this paper, we will review the existing animal CTO models in both coronary and peripheral circulations and how these may be utilised to gain a better understanding of the disease process and formulate novel interventional approaches.

2. Human CTO histopathology

The histopathological process of CTO progression in human coronary arteries, whilst incompletely understood, is dependent on the duration of the CTO. The vast majority of data pertaining to the pathophysiology of human CTO formation is based on post-mortem data, rather than consecutive in-vivo assessment. The most commonly accepted process involves an acute occlusive thrombus, rather than gradual luminal obliteration by atheromatous progression [13]. The acute thrombus subsequently develops into an organised thrombus, which is more rigid than fresh thrombus, with a dense concentration of collagen

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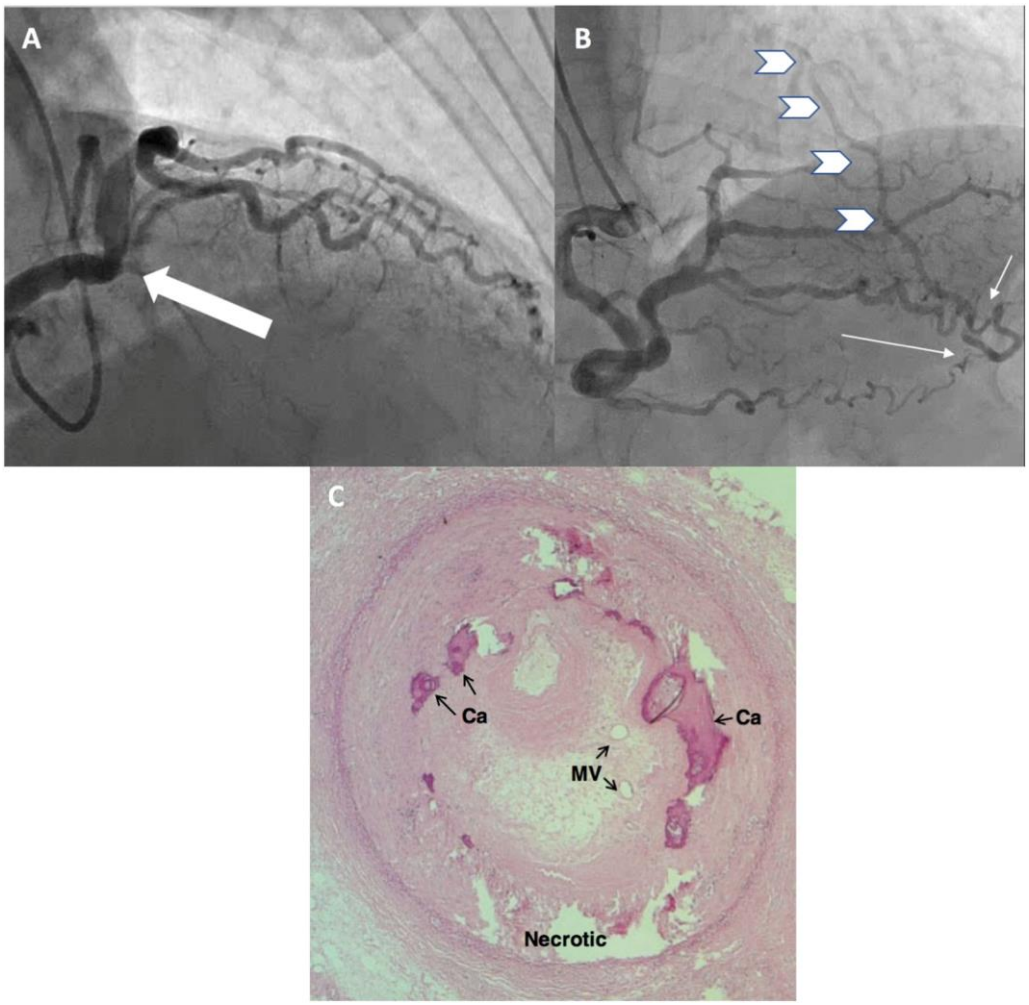

Fig. 1. Angiographic and histological images of a CTO
A & B: Angiographic images of a patient with a CTO of the proximal left anterior descending artery (LAD). (A) The occluded LAD proximal stump is seen when injecting radio-opaque contrast into the left coronary system. (B) Retrograde filling of the occluded LAD (thick arrows) via collaterals (thin arrows) is seen with injection of radio-opaque dye in the right coronary artery. C. Hematoxylin-eosin stained human CTO, demonstrating extensive collagen-rich fibrous tissue, several patches of calcification (Ca), small microvessels (MV), and a large necrotic area.
Adapted from [49].

rich fibrous tissue at the proximal and distal ends of the lesion, referred to as proximal and distal fibrous caps [14]. Early occlusions consist of microchannels within the organised thrombus, whilst CTOs of shorter duration consist of organised thrombus with necrotic core, extracellular matrix, smooth muscle cells and lipid deposits with fewer calcified lesions [15,16]. As the CTO gets older, the intimal plaque gets harder and more dense calcium formations occur without microchannels and severe negative remodelling [17] (Fig. 1). Similarly, in native vessel CTOs which have undergone coronary artery bypass grafting (CABG), there is severe calcification and moderate negative remodelling [18], whilst the presence of the bypass graft itself may accelerate CTO formation, with 43.6% of patients developing a new CTO following CABG at 1 year [19]. Whilst there is some understanding of the mechanism of the occlusion of the culprit vessel, the recruitment of collaterals, which prevent an early myocardial infarction and hence maintain a viable myocardium, and their subsequent development and maturation, is an area of ongoing research.

3. Rationale for an animal model & characteristics

Given the incidence and prognostic effects of the presence of a CTO, an appropriate animal model is indicated for a number of reasons. Broadly, these may be divided into 5 aspects;

(i) The natural history of a CTO
(ii) Pathophysiology of the CTO plaque formation
(iii) Pathophysiology of collateral formation, function and recruitability
(iv) Assess efficacy of treatment strategies – percutaneous coronary intervention (PCI) and pharmacological agents to increase collateral formation and function.
(v) Long term assessment and outcomes of a CTO and its intervention on both coronary arteries as well as the territory of myocardium supplied.

As spontaneous formation of atherosclerosis is rare in animals [20], and spontaneous CTO formation has not previously been described, interventions are required to create such a model. This results in confounders based on the intervention, however does allow study of the effects of such an occlusion. Furthermore, once a model is created, therapeutic interventions may be trialled. This would include pharmacological agents to either improve collateral formation, function and

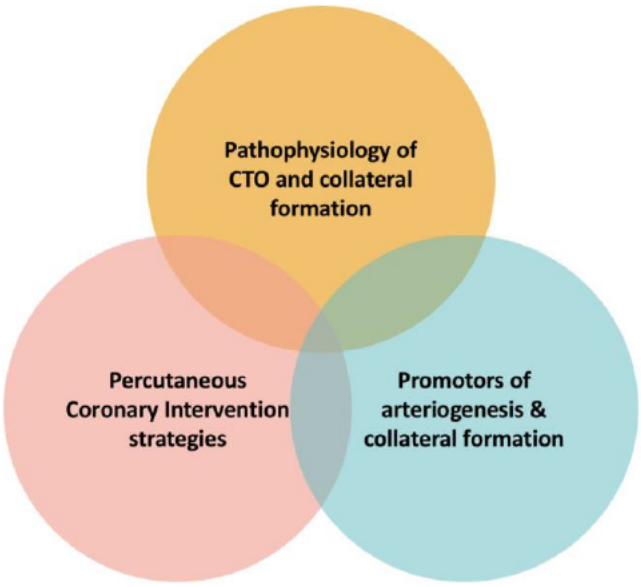

Fig. 2. Inter-species differences in collateral flow.
Adapted from [24].

recruitability or else different strategies for coronary intervention with angioplasty. Hence, an ideal animal model should include an occluded artery, functional collaterals and a viable myocardium, which would allow study of the process driving collateral formation and arteriogenesis as well as percutaneous intervention strategies for both acute and long term benefits (Fig. 3).

4. The coronary collateral circulation in animals

In humans, spontaneous coronary collaterals with sufficient collateral flow to prevent ischaemia following coronary occlusion are found in 20–25% of patients with normal coronary arteries, and 28% of those with coronary artery disease [21]. The prevalence of spontaneous coronary collaterals in animals, varies greatly amongst different species. In pigs, there are few spontaneous collaterals, and those that exist are located endomurally and subendocardially [22,23]. In contrast, in canines, there are extensive vascular communications between epicardial branches [22]. This protective occurrence, manifests as a lower mortality rate in acute myocardial infarction models compared with pigs. Maxwell et al. [24] utilised radiolabelled microspheres to determine the pre-existing collateral circulation in 8 different animals following occlusion of a major epicardial vessel to determine the relative blood flow to the non-ischaemic area of the myocardium. They found that in Guinea pigs, there was no zone of relevant underperfusion (i.e. a complete collateral circulation). The relative flow, as a percentage of normal, non-ischaemic flow was 15.9 ± 1.8% in dogs, 11.8 ± 1.1% in cats, 6.1 ± 0.7% in rats, 2.4 ± 0.6% in ferrets, 2.1 ± 0.3% in baboons, 2.0 ± 0.5% in rabbits and 0.6 ± 0.2% in pigs (Fig. 2).

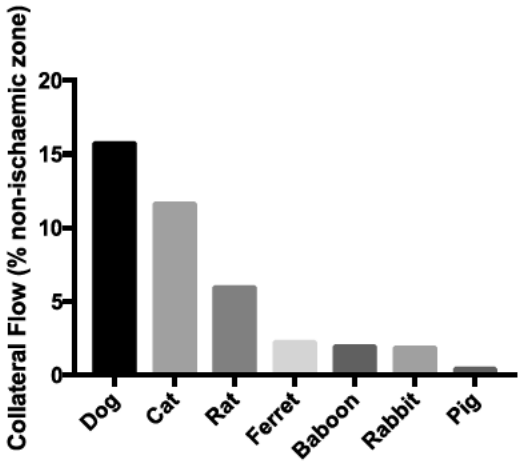

Fig. 3. Components of an ideal animal model of CTO.

5. Animal models of chronic total occlusions

To study the pathophysiological basis of CTOs, both coronary as well as peripheral animal models have been developed. To summarise these existing models, we performed a PubMed and Medline search including the terms "animal" "coronary" and "chronic total occlusion" to review existing and relevant papers. Whilst generalised percutaneous treatment strategies may be tested in either coronary or peripheral CTOs, the pathophysiological factors driving coronary CTO formations are likely to be varied, and hence would require a coronary model. Broadly, the methods of achieving this, irrespective of species studied, may be categorised as either progressive extrinsic compression, or else endoluminal intervention (Table 1).

5.1. Coronary CTO models

5.1.1. Extrinsic compression

In the method of extrinsic compression, the aim is to create progressive occlusion of a vessel, so as to reduce anterograde myocardial blood flow and allow recruitment of collateral vessels before complete coronary occlusion, thereby preventing (or limiting) myocardial infarction. The process of decrease in anterograde blood flow in the vessel results in increased blood flow and shear stress on the collateral circulation from the donor vessel, activating the process of arteriogenesis [25] and hence maturing the collateral circulation to become functional. Operschall et al. [26] utilised an ameroid constrictor in the coronary circulation of the rabbit to create a CTO model. An ameroid constrictor is a cylindrical device made up of an inner ring of casein (a hygroscopic substance that slowly expands as it absorbs fluid) surrounded by a metallic sheath. As the casein layer expands, it causes progressive external compression, until there is obliteration of the vessel lumen (Fig. 4). An ameroid constrictor was applied to the left circumflex (LCx) artery, with animal euthanasia at day 21. There was a 21.6% rate of early death, whilst in those that survived, 88% had coronary occlusion. Of these, corrosive casts showed that 4 out of 7 had vascular connections suggesting retrograde collateral flow. In the 3 others, either there was selective collateral recruitment or issues with the casting process. Radiolabelled microspheres illustrated an increase in coronary blood flow back to baseline flow (prior to occlusion) in the epicardium, but remained 50% of baseline in the endocardium, illustrating collateral maturation is greater in the epicardium compared to the endocardium.

Toyota et al. [27] utilised a method of repetitive extrinsic compression to promote and recruit coronary collaterals in the rat. In the model, rats had implantation of a mini-pneumatic snare occluder to the left anterior descending artery (LAD), following which a protocol of repetitive brief inflations were carried over 10 days. Following the end of the protocol, the rats were euthanised and coronary blood flow was measured using radiolabelled microspheres, as well as micro CT to image vascular connections. They demonstrated that repetitive myocardial ischaemia created a 6 fold increase in collateral flow from baseline and a 3 fold increase in the number of arterial-arterial anastomoses compared to baseline. This method has successfully been repeated by Hattan et al. [28], Rocic et al. [29] and Reed et al. [30]. Whilst this process recruited collaterals during brief occlusion of the LAD, a CTO was not created per se, as the ability of these collaterals to

Table 1
Published animal CTO models.

Reference	Method of occlusion	Early death	Animal Sacrifice	Method of confirmation	Vessel	Successful CTO	Histology
Rabbit							
Operschall et al. [26]	Ameroid constrictor	11/51 (21.6%)	21 days	Angiography, corrosion plastic cast, myocardial blood flow by radiolabelled microspheres, histology	LCx	7/8 (88%) by angiography 7/8 (88%) by cast	Mean infarct size 9%
Pig							
Suzuki et al. [35]	Bioabsorbable polymer sponger	3/6 (50%)	4 weeks	Angiography and histology	LAD	3/3 (100%)	Microcalcifications, microvascular channels and elastic tissue in occluded segment.
Sim et al. [34]	Copper Stent & bioabsorbable polymer	13/20 (65%) 4/20 (20%)	5 weeks and 4 weeks	Angiography and histology	LAD	7/7 (100%) and 16/16 (100%)	Fibrosis, necrosis, organised thrombus and inflammation, particularly in close proximity to the copper wire; gradual absorption of the $_{L}$-PLA polymer and formation of thrombus and fibrotic tissue
Song et al. [32]	Copper plated stent	1/18 (6%)	1 week, 4 weeks and 8 weeks	Angiography and histology	LCx	14/17 (82%)	Early group: Red thrombus and intimal fibrin with prominent inflammation; Intermediate group: organised thrombus with vascularised intima and calcification around the stent struts; Late group: Collagenous stroma and more organised calcification
Prosser et al. [36]	Oxygen enhanced bioabsorbable polymer	2/14 (14%)	Day 10 and 28	Angiography and histology	Various	12/12 (100%)	PLA completely reabsorbed with the occlusion consisting of fibrothrombotic lesions with microchannels and prominent adventitial arterioles.
Suzuki et al. [38]	Bone chips & absorbable gelatin sponge	8/20 (40%)	28 days	Angiography, histology and intravascular ultrasound (IVUS).	LAD, RCA & LCx	10/12 (83%)	Inflammatory cells and microvessel channels with moderate disruption of the internal elastic lamina, external elastic lamina and medial wall
Fefer et al. [37]	Collagen plug (angioseal)	9/26 (34.6%)	6 weeks and 12 weeks	Angiography, MRI, 3D spin CT, micro-CT imaging, histological analysis	LAD	15/17 (88.2%)	Early: Intense inflammatory reaction with prominent macrophage infiltration and a proteoglycan and collagen rich matrix Late; Less inflammation with a densely packed collagen and elastin rich matrix
Rat							
Toyota et al. [27]	Repetitive ischaemia via extrinsic compression	Nil	10 days	Myocardial blood flow radiolabelled microspheres, functional assessment, micro-CT	LAD	100% increase in collateral blood flow	N/A

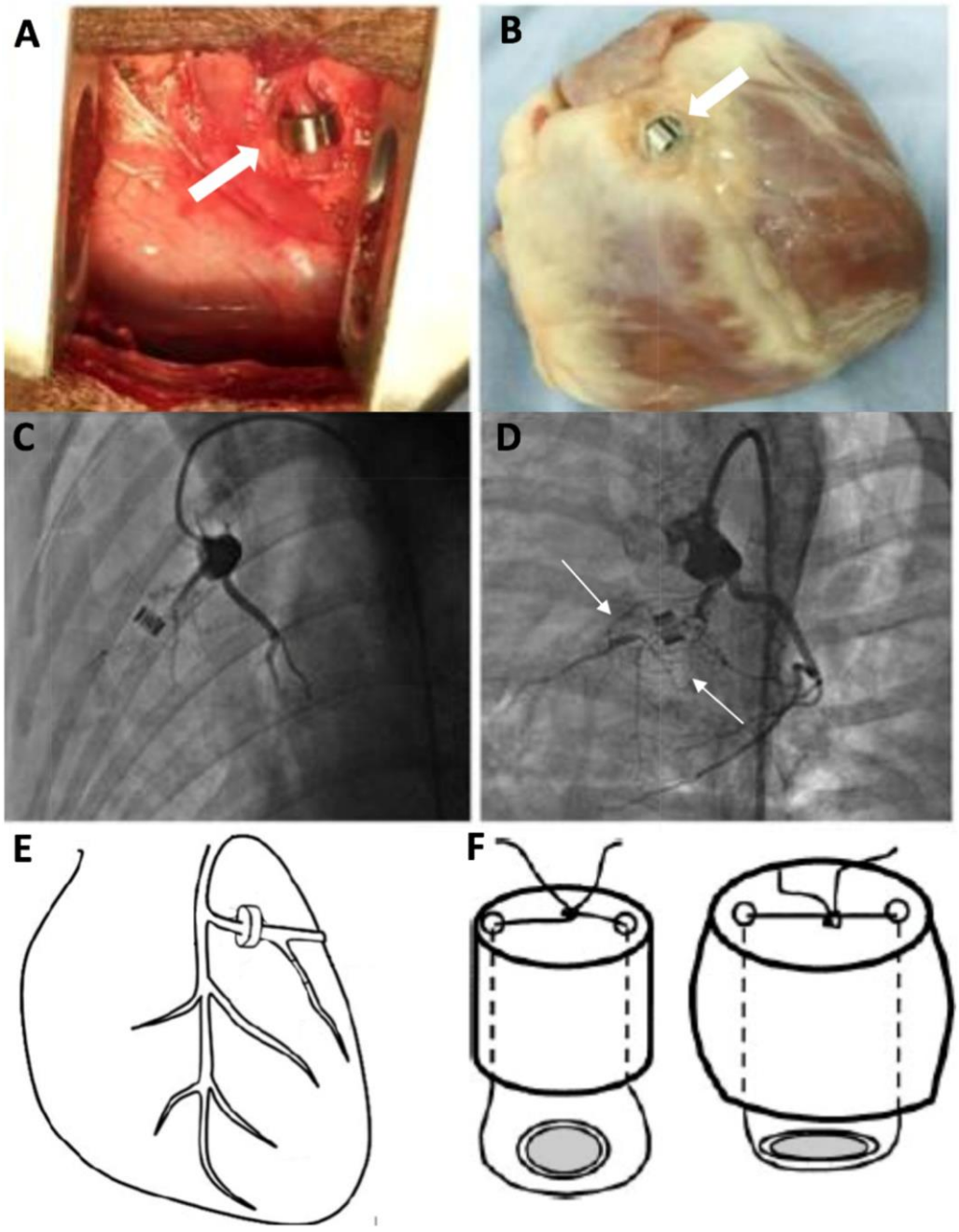

Fig. 4. Extrinsic compression model of a coronary CTO using an ameroid constrictor.
Implantation of the hydroscopic ameroid constrictor on a coronary artery within the chest cavity (A) and after explantation of the heart (B). Angiographic image of the ameroid constrictor showing occlusion of the coronary artery (C) with development of collaterals (thin arrows) filling the distal vessel beyond the ameroid constrictor (D). (E) Schematic of the ameroid constrictor implantation with (F) schematic of implantation illustrating after the suture is passed deep to the intramyocardial coronary artery and tied to the ameroid constrictor, with progressive swelling of the constrictor, the coronary artery is progressively obstructed. Adapted from [26,50].

prevent infarction with prolonged occlusion was not assessed.

Interestingly, Cohen et al. [31] attempted a similar process of repetitive myocardial ischemia with a protocol of intermittent inflation of a balloon encircling a superficial branch of the left coronary artery in the rabbit. However, in this model, there was no appreciable increase in collateral coronary blood flow, suggesting this process may either be species dependent or else highly operator dependent.

5.1.2. Endoluminal approach

Given the use of extrinsic compression renders the model unsuitable for studying revascularisation techniques, endoluminal approaches of creating a CTO have also been studied.

Song et al. [32] utilised copper stent implantation to induce a coronary CTO in a pig model. Copper stents have been shown to produce an intense inflammatory reaction and gradual obstruction of porcine arteries [33] and hence have been investigated in this setting. Stents were modified so that the copper coating was only present on the

abluminal surface, to minimise acute stent thrombosis, and implantation only into the left circumflex artery was chosen, resulting in a low early mortality rate of 5.6%. Animals were euthanised at differing time points. Animals euthanised after 1 week showed 100% occlusion, with 12 out of 14 vessels showing bridging collaterals. Histological assessment in those > 1 week showed organised thrombus with vascularised intima and calcification around the stent struts. In the late group (8 weeks) the inflammatory changes and intimal smooth muscle cells were less but more collagenous stroma and more organised calcification. Sim et al. [34] also utilised copper stents in their pig CTO model, inserting stents into the LAD rather than the circumflex, which may explain the higher early mortality rate of 65% with 7 animals euthanised at 5 weeks. All animals had an occluded LAD with evidence of a collateral circulation with histological assessment yielding fibrosis, necrosis, organised thrombus and inflammation, particularly close to the copper wire.

Bioabsorbable polymers have also been used in endoluminal models of CTO formation. Sim et al. [34] used a preparation of levo-polyactic acid polymer ($_L$-PLA) and inserted it into the distal LAD of pigs. 20% of pigs died as a result of ventricular fibrillation (VF) during the procedure, whilst the remaining 16 pigs all demonstrated total occlusion of the distal LAD with collateral circulation. Histopathological assessment showed gradual absorption of the ($_L$-PLA) polymer by 4 weeks. Suzuki et al. [35] created bioabsorbable sponges made of poly($_L$-lactide) (PLA), polyglycolide (PGA) or poly(DL-lactide-co-glycolide) coated in an apatite layer. These bioabsorbable sponges were implanted into the LAD of pigs, with 50% dying within 24 h. Of the remaining 3 pigs, after 4 weeks, all developed rich collaterals, however histological assessment demonstrated that the PLA and PGA sponges had not completely reabsorbed. Prosser et al. [36] treated PLA sponges in an oxygen environment before coronary implantation. 12% died during the procedure of VF with all of the remaining 12 developing a CTO with complete PLA reabsorption. Fefer et al. [37] used a commercially available collagen plug (Angio-Seal® Terumo IS Somerset, NJ, USA) in the LAD. 34.6% had an early death, with 15 of the remaining 17 (88%) pigs developing a successful CTO. Histological assessment revealed at 12 weeks, the occluded segment was composed of densely packed collagen and elastin rich with an inflammatory reaction. Suzuki et al. [38] attempted to create increased calcification within a CTO by injecting bone chips (harvested from the ribs of previously euthanised pigs) and a reabsorbable gelatine sponge in the coronary arteries. 40% died early with 10 of the remaining 12 (83%) developing an occluded artery with either contralateral or bridging collaterals.

5.2. Peripheral CTO models

Given the relative size of the coronary arteries, particularly in smaller animals, the peripheral circulation has been suggested as an alternative model to assess the pathophysiological process of CTO formation. As with the coronary circulation, both extrinsic compression, as well as endoluminal approaches have been described in the formation of a CTO.

Suzuki et al. [35] created a peripheral CTO model in pigs with implantation of bioabsorbable sponges into the femoral arteries. All 7 pigs had successful formation of a CTO with rich collaterals. Kim et al. [39], addressing the high rates of acute stent thrombosis with copper stents, coated these stents with a thin layer of PGA, aiming to facilitate gradual contact of copper to the vascular endothelium, achieved through progressive reabsorption of the PGA. Six stents were inserted into the femoral arteries of 3 pigs followed by euthanasia at 5 weeks. Five out of the 6 arteries developed a successful CTO, with most developing bridging collaterals. Zhu et al. [40] utilised a novel technique of inducing calcification within the occluded CTO, whereby polycaprolactone (PCL) scaffolds (a biodegradable polymer) coated with the growth factor TGFB1 and seeded with primary human osteoblasts (HOB) were inserted into New Zealand white rabbit femoral arteries. 17 out of 18 rabbits developed a successful CTO, with animals euthanised at 10 days showing the greatest extent of calcification, whilst those at 28 days had less calcification. The inflammatory milieu within the CTO also changed with early findings of lymphocytes and leukocytes near the stent struts, maturing to leukocytes within the adventitia and fibroblasts. At 28 days, leukocytes seen close to the stent struts became mixed with fibroblasts and infiltrated into the occlusion sites with microvessels from the adventitia migrating into the lumen.

Murphy et al. [41], in one of the earlier successful animal CTO models used varying combinations of multiple injury processes to induce occlusion. The femoral arteries of New Zealand white rabbits were subjected to gas drying with carbon dioxide following temporary proximal vessel occlusion, injection of bovine thrombi or injury by serial transverse clamp injury using a needle holder. Seventeen of 34 arteries demonstrated CTO formation with bridging collaterals, with the highest success rates with gas drying, thrombin injection and mechanical injury (78%), followed by gas drying and thrombin injection (60%). Histological assessment demonstrated atherosclerotic plaque with lipid laden cells and thrombus, although fibromuscular cells were also noted. Of note, there was no microcalcifications or evidence of neovascularisation within the occluded segment. Strauss et al. [42] used a similar method of thrombin injection to create a successful CTO model.

Nikol et al. utilised 3 different endoluminal approaches to create a femoral artery CTO; implantation of detachable angioplasty balloons supported by platinum coils, implantation of coils alone and specifically manufactured blind ended grafts. At 6 months, 100% of those implanted with blind ended stents remained occluded, whilst 33% of the detachable angioplasty balloons were occluded whilst all of the coil only group spontaneously recanalised.

5.3. Murine models

The mouse has become the most important, and widely used laboratory species for research, particularly in cardiovascular and metabolic disease research, owing to the availability of numerous transgenic strains, low cost, ease of housing and care as well as growing researcher familiarity. However, there are numerous physiological differences between the hearts of mice and humans, including differences in the action potential of cardiomyocytes, whereby mice cardiomyocyte action potentials have a short duration without a plateau phase as seen in humans, a five-fold higher resting heart rate than humans, and differences in active ion channel transporters [43,44].

Despite these differences, there is increasing work to create a reliable, and reproducible coronary CTO model in the mouse, although this is in its relative infancy and requires further investigation. There has been some initial success with the extrinsic compression models described above in rat models, with implantation of a mini pneumatic snare on a coronary artery during an open chest procedure, with intermittent repetitive occlusive ischaemia [45]. Other models of acute coronary ligation have demonstrated that whilst neo-collateral formation does occur, with size and numbers depending on different genetic strains, a large myocardial infarction still occurs, which precludes this method for studying human CTOs, although may have merit for investigating recruitment of acute collaterals [46].

5.4. Quantifying collaterals and CTOs

Once a potential CTO model is created, quantification of the collateral supply, both qualitatively and quantitatively may be done in a number of ways, to determine its success. These include direct visualisation using angiographic assessment or CT angiography, perfusion imaging, histological assessment and plastic mouldings; each with inherent advantages and disadvantages. In general, quantitative assessment requires explantation of the heart model, obviating the ability to test therapeutic interventions, whilst qualitative assessment allows

Table 2
Comparison between extrinsic compression and endovascular approaches to CTO formation.

	Extrinsic compression	Endovascular
Formation	Progressive occlusion	Acute occlusion
Technical difficulty	+	+ +
Rodent model	Yes	No
Early mortality	Low	High
Histopathology	Unable to assess	Organised thrombus with microchannels
Identify systemic markers/promotors of collaterals	Yes	Yes
Trial therapeutic revascularisation strategies (i.e. stent)	No	Yes

testing of such therapeutic options, although does not allow detailed analysis of the form and function of collaterals.

Ex-vivo 3D spin angiography and CT may be performed on excised hearts with perfusion of a silicon based gel into the coronary vasculature whilst the use of micro-CT allows assessment of the excised heart and vessels to a level of assessment of < 30 μm [37]. Histological assessment of the excised heart allows exquisite assessment of the structure of the collaterals, the presence of calcification, microchannels and thrombus within the CTO as well as the inflammatory response to the method of CTO induction. Corrosion casts [26] of the heart model involves flushing of blood from the microcirculation followed by direct infusion of a plastic mixture into the coronary arteries, before allowing the mixture to harden. The heart tissue is then macerated in sodium hydroxide to reveal the cast. Given the delicate nature of the collaterals, and the fact that they are often dynamic, opening only during significant flow from the contralateral (donor) vessel, meaning that smaller vessels may not be well visualised. Perfusion may be detected using radiolabelled microspheres, whereby during occlusion of a vessel, a radiolabelled microsphere is injected into the coronary circulation, which will not perfuse the tissue subtended by the artery of interest (this will be the so-called “area at risk”). Following formation of a CTO, a different radiolabelled isotope will be infused into the vasculature (during occlusion of the vessel, if not already occluded). After explantation of the tissue, and relative to the concentration of radiolabelled isotopes in the systemic circulation, relative blood flow to the at-risk area may be determined, and hence assess the collateral circulation functionally [26]. Whilst allowing functional assessment, this method does not allow anatomical classification, whilst again, requiring explantation of the heart.

6. Discussion

The ideal animal model for assessment of the pathophysiological basis of CTO and collateral formation and to evaluate the impacts of therapeutic modalities, should have a number of attributes. The model should be in an animal that reflects human biology, easy and reliably reproduced which mimics the histopathological features human CTOs, in a manner that allows intervention to be performed. The process should be such that researchers can quickly learn the skills required to create this model. As animals do not develop coronary artery disease and plaque rupture, both endoluminal and extrinsic methods of creating a CTO model can provide some of these features. The extrinsic compression models have the advantage of being technically less challenging with fewer early deaths. However, as these methods disrupt the natural architecture of the vessel wall, they are not conducive to assessment of percutaneous treatment strategies. Furthermore, they artificially obliterate the vessel lumen which does not mimic local vasomotor changes that occur with endothelial dysfunction and CTO formation.

Endovascular approaches of coronary CTO models have a relatively high early mortality rate, but in those that survive, the CTO mimics the human CTO model more closely, with organised thrombus with fibrous tissue, microcalcifications and microchannels. However, the presence of either a stent, or else other material within the lumen may not be widely applicable to all CTOs with respect to revascularisation. The adjunctive role of anti-arrhythmic therapy whilst forming these models is not standardised, and may minimise early arrhythmia induced mortalities (Table 2).

Peripheral CTO models, whilst easier to perform with less early morbidity and mortality, do not readily correlate with local biochemical and vasomotor changes which are unique to the coronary circulation and cannot reliably be used in the further investigation of coronary CTOs.

The wider implications of these CTO models, as with research in all animal models must however be tempered with caution. In numerous situations, pharmacological research in animals and humans has been discordant, often raising doubt about the wider applicability of animal research in humans [47]. Of note, with the above reviewed animal models, numbers are small, with no specific mention of blinding or independent assessors to determine success of a model, raising the possibility of bias and positive reporting. Furthermore, there are inherent differences and variation in naturally occurring coronary collaterals between species as previously described, and the above models may simply reflect this natural variation, rather than true stimulation of neo-collateralisation.

Nevertheless, the field of interventional cardiology has benefited immensely with the use of animal models, and the early suggestions of these animal models are that whilst a “gold standard” has yet to be found, there are encouraging signs. Already catheter techniques, initially trialled in animals are now available for use for interventional cardiologists for CTO percutaneous coronary intervention [48]. However, a process of rigorous scientific review must be maintained for animal models of CTOs, which will ultimately allow further advancement of disease process understanding and treatment modalities.

7. Conclusion

A reliable and reproducible animal model of a coronary CTO may be created either with extrinsic or endovascular approaches, both of which have inherent limitations. Assessment of the collaterals may either be done quantitatively or qualitatively, but often requires explantation of the heart, obviating its ability to be used for therapeutic trials. With an ever increasing interest in the management of CTOs in humans, newer models, more closely mimicking the human disease process should be continued to be investigated.

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Chapter 12: Repetitive occlusive ischaemia in a rat model to recruit coronary collaterals

12.1 Abstract

Background and Aims: The process by which coronary collaterals mature and become functional in the setting of coronary artery occlusion remains uncertain. In particular, what role chemoattractant proteins, inflammatory cytokines, growth factors and transcriptional factors play, remains uncertain.

Methods: Male Sprague-Dawley rats (350-400g) were assigned to either undergo implantation of a balloon occluder, which when inflated resulted in complete occlusion of coronary blood flow to the left anterior descending artery (LAD) or to a sham procedure. Following a 10 day protocol of repetitive occlusive ischaemia, all rats underwent ligation of the LAD and were euthanised. Tissue protein and mRNA concentrations in the two groups were determined through Western Blot, Enzyme Linked Immunosorbent Assay (ELISA) and quantitative real time polymerase chain reaction (qPCR).

Results: 14 rats underwent balloon occluder implantation while 18 rats were in the control group. Rats who underwent repetitive balloon occlusive ischaemia had a significantly greater territory of myocardium perfused (77.1% vs 49.1%, $p<0.001$) following LAD ligation compared to the control arm, driven by an increase in perfusion at the apex (73.8% vs 37.8%, $p<0.0001$). In rats with increased collateral perfusion, there was a significantly higher tissue protein expression of intracellular adhesion molecule 1 (ICAM-1) (94.6% vs 23.5%, $p<0.01$), vascular cell adhesion molecule 1 (VCAM-1) (108.5% vs 21.7%, $p<0.001$), fibroblast growth factor 2 (FGF-2) (104.2% vs 55.2%, $p<0.05$) and early growth response 1 (EgR-1) (58.5% vs 19.5%, $p<0.01$) relative to glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Tissue expression of granulocyte-macrophage colony stimulating factor (GM-CSF) by ELISA was also greater in rats with collateral perfusion (3752pg/mL vs 206pg/ml, $p<0.01$). There were

no differences in tissue expression of tumor necrosis factor alpha, interleukin 6 or interleukin 1ß between groups.

Conclusion: In a rat model, repetitive occlusive ischaemia results in an increase in myocardial perfusion in the territory subtended by the epicardial artery as a result of increased collateral blood flow. This increase in myocardial perfusion is associated with an increase in chemoattractant proteins, growth factors and regulatory proteins, while inflammatory cytokines do not appear to be associated with an increase in collateral perfusion. The mechanistic effect on collateral size and number as well as further assessment of causative processes should be investigated.

12.2 Introduction

The pathogenesis by which coronary collaterals are recruited and mature remains uncertain, with the initiating biomechanical alteration of coronary occlusion resulting in increase in fluid shear stress through primitive collateral connections. Maturation of pre-existing primitive collaterals involves the addition of new vascular components (endothelium, smooth muscle and fibroblasts) via mitosis, migration, tissue remodelling, matrix degradation, and differentiation (1-3) (See section II). Animal models (See Section VI, Chapter 11) provide a unique opportunity to experimentally induce collaterals and determine on a tissue and cellular level the mechanism by which collaterals are recruited.

The finding of an inflammatory milieu with inflammatory cells including monocytes playing a predominant role in the maturation of collateral arteries suggests a significant role for chemoattractant proteins in arteriogenesis, including intracellular adhesion molecule 1 (ICAM-1) (4), vascular cell adhesion molecule 1 (VCAM-1) (5) and monocyte chemoattractant protein 1 (MCP1) (6). Whilst inflammatory cells are found at the site of collateral growth (7), whether there is a role for potent inflammatory cytokines such as interleukin 6 (IL-6), interleukin 1ß (IL-1ß) and tumour necrosis factor-α (TNF-α) is unknown. Growth factors, either secreted by ischaemic myocardium or inflammatory cells also play a pivotal role in collateral maturation (8). Growth factors previously implicated with collateral growth include; vascular endothelial growth factor (VEGF) (9), transforming growth factor-β (TGF-β) (10), granulocyte-macrophage colony stimulating factor (GM-CSF) (11) and fibroblast growth factor 2 (FGF-2) (12-15). Finally, the role of the so called master regulator early growth response 1 (EgR-1) and hypoxia inducible factor 1 alpha (HIF-1α) are also uncertain.

The aim of this study was to determine protein and mRNA expression of growth factors, cytokines and regulatory chemokines, following experimental recruitment of coronary collaterals in a rat model, using repetitive occlusive ischaemia. Furthermore, the aim was to determine the magnitude of collateral perfusion which could be induced in this rat model.

12.3 Methods

12.3.1 Rat Experimental protocol

Ethics approval to use animals was obtained from the Northern Sydney Local Health District Animal Ethics Committee (NSLHD-AEC), which conformed to the *Australian code for the care and use of animals for scientific purposes* (16). 10 week old Male Sprague-Dawley rats (350-400g) were allowed to acclimatise to the animal facility for 1 week, with free access to food and water, with daily cleaning and changes of cage housing. Following acclimatisation, rats underwent either implantation of a balloon occluder on the anterior cardiac surface or a sham procedure.

12.3.1.1 Anaesthesia

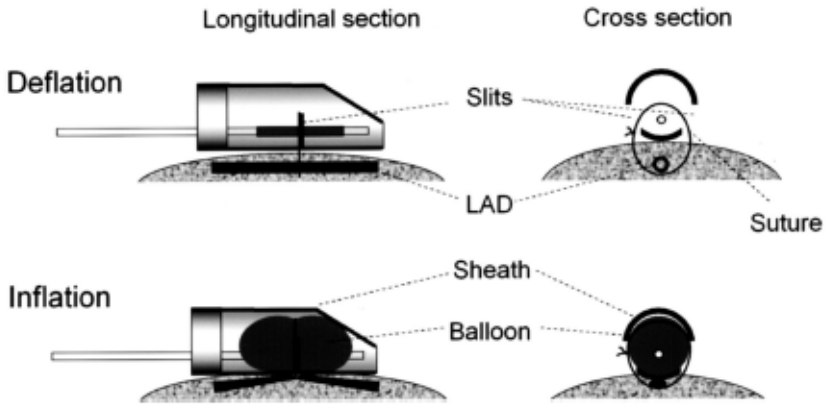
Anaesthesia was induced in an induction chamber using 5% (v/v) vaporised isoflurane. The rat was subsequently positioned supine, and endotracheal intubation performed using an 18 gauge cannula tube under direct visualisation of the vocal chords. Body temperature was maintained at 37°C using an electrical heat mat. Anaesthesia was maintained with 2% (v/v) isoflurane and the animal ventilated at a rate of 80 breaths per minute with a tidal volume of 1.5 mL per 100 g body weight, using a small animal ventilator.

12.3.1.2 Surgical Implantation of Balloon Occluder

Prior to surgery, rats had intramuscular injection of lignocaine (10mg/kg) in the hindlimb. The rat thorax was shaved and cleaned with antiseptic solution. Subcutaneous buprenorphine (0.05mg/kg) was administered near the site of incision immediately prior to surgery. A

thoracotomy was performed at the left 5th intercostal space with an incision parallel to the ribs, with subsequent exposure of the heart using a self-retaining retractor. The pericardium was entered using sharp forceps. The left anterior descending artery (LAD) was identified and a balloon occluder was implanted using a 6-0 prolene suture passed around the LAD and attached to the sheath, securing the occluder to the heart. The balloon occluder (*Mini pneumatic snare occluder;* DocXS Biomedical Products, California, USA) is a customised balloon measuring 7mm, made of a soft latex membrane which is sufficiently pliable to give negligible physical force on the coronary artery during balloon deflation (17). The balloon was connected to a polyethylene tube which was exteriorised to the dorsum of the rat, tracking subcutaneously under the skin. The balloon was inflated through the externalised tubing with 0.2-mL of air, resulting in occlusion through 2 mechanisms - "crimping" the LAD toward upward/outside and compressing the LAD by the inflated balloon/sheath (Figure 12.1).

Figure 12.1: Schematic Drawing of the Balloon Occluder



Schematic of the balloon occluder and its actions. Top, Cross-sectional and longitudinal views when the balloon is deflated. Bottom, Views during inflation. The artery is patent when the balloon is deflated, but during inflation, a snare situated underneath the artery is pulled "upward", producing the coronary occlusion. Adapted from (17)

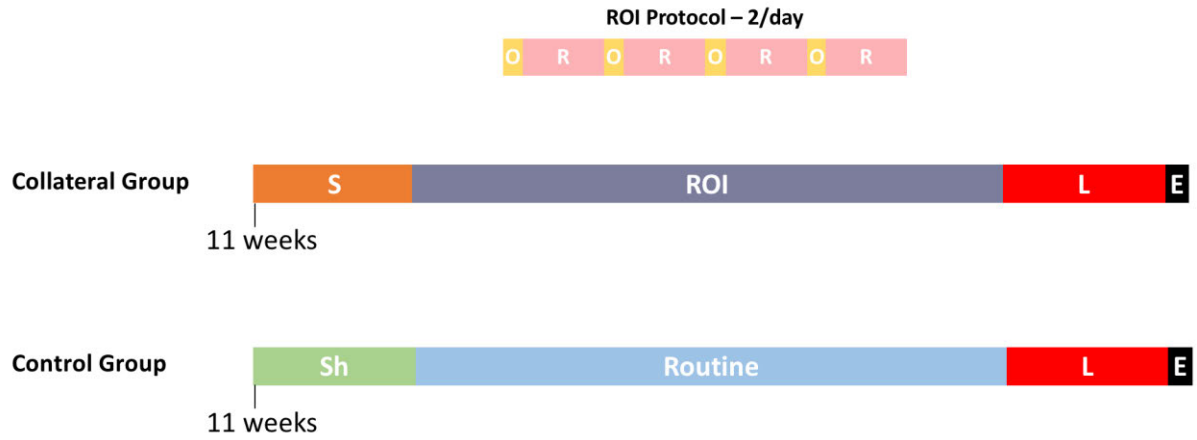
Confirmation that the occluder was functional, i.e., producing myocardial ischemia from coronary occlusion, was determined by observation of blanching and hypokinesis of the left

ventricle (LV) during inflation. Following successful occluder implantation, the chest cavity was closed, and the thoracic cavity was evacuated of air. For the sham arm, rats had a lateral thoracotomy performed and opening of the pericardium, followed by closure of the chest cavity. All rats were given cephazolin 50mg/kg subcutaneously (SC) immediately following surgery (18). Rats were observed in a recovery cage for 2 hours and then transferred to the animal care facility. For 3 days after the surgery, buprenorphine 0.05mg/kg SC was administered twice daily, with additional doses given based on behaviour.

12.3.1.3 Repetitive Occlusive Ischaemia Protocol

The day after surgical implantation of the pneumatic occluder, rats in the experimental arm underwent the protocol of repetitive occlusive ischaemia. This involved inducing anaesthesia as described in 12.3.1.1, followed by 40 seconds of occlusion, followed by 20 minutes of recovery for 4 cycles, 2 times a day. This protocol was continued for 10 days, based on similar protocols previously published (17,19-21). The LAD was occluded manually by remote inflation with 2mL of air through the externalised catheter attached to the balloon occluder (Figure 12.2).

Figure 12.2: Protocols of Rats undergoing repetitive occlusive ischaemia or sham procedure



On day 1, rats in the collateral groups have surgery (S) to have balloon occluder implanted, while rats in the control group have a sham procedure (Sh). For 10 days, rats in the collateral group have twice daily protocol of 40 seconds of balloon occlusion, followed by 20 minutes of recovery for 4 cycles. On day 12, all rats have ligation (L) of the LAD for 1 hour followed by euthanasia (E).

12.3.1.4 Induction of subacute collaterals during prolonged artery occlusion

After the 10 day repetitive occlusive ischaemia protocol all rats underwent prolonged occlusion of the LAD. All rats had induction of general anaesthesia as described in 12.3.1.1, followed by repeat thoracotomy and visualisation of the heart, as described above. The LAD was identified and ligated using a 5-0 prolene suture (Ethicon, New Jersey, USA) which was passed through the myocardium deep to the vessel and through a snare. In rats who had undergone the repetitive occlusive ischaemia protocol, the suture was placed immediately proximal to the occluder, while in sham rats the suture was placed at an approximate level of the mid vessel, corresponding to a similar location in both groups. Occlusion was confirmed with blanching and hypokinesis of the ventricle.

12.3.1.5 Injection of Evans Blue Dye and Sacrifice

In a subset of 15 rats, following 1 hour of artery ligation, a 26 gauge needle was used to inject Evans Blue Dye (Sigma-Aldrich, Missouri, USA) into the hepatic vein, until the eyes, ears,

nose, and paws were confirmed to be blue ensuring adequate dye for perfusion of the collateral dependent zone (22). The rats were euthanized and the heart was excised and washed in Phosphate-buffered saline (PBS). The heart was wrapped in plastic wrap, placed in a container and kept on dry ice. The semi frozen heart was sliced transversely into 1 to 2mm thick slices and stored in formalin

12.3.2 Collateral Perfusion Quantification

Following preparation (see 12.3.1.5) all slices were examined and digitally photographed. Perfused myocardium appeared blue, while non-perfused myocardium appeared unstained red. An automated program (ImageJ (version 1.51q, Java based software program, NIH)) using colour threshold was used to determine the perfused territory as a percentage of the entire sliced segment. As any perfusion in the myocardial territory supplied by the occluded vessel must be through collaterals, this was defined as the collateral dependent zone. After analysis of each slice, comparison was made between total rat hearts based on averaged perfusion per slice, as well as comparison between perfusion at the apex, and perfusion in the mid ventricular cavity.

12.3.3 Enzyme Linked Immunosorbent Assay (ELISA)

Enzyme-linked immunosorbent assay (ELISA) kits were used to determine the concentration of tissue vascular endothelial growth factor (VEGF) (VEGF-A Rat ELISA Kit, Thermo Fisher Scientific, Massachusetts, USA), interleukin 6 (IL-6) (Rat IL-6 ELISA Kit Thermo Fisher Scientific, Massachusetts, USA), interleukin 1 beta (IL-1ß) (Rat IL-1ß ELISA Kit Thermo Fisher Scientific, Massachusetts, USA), granulocyte-macrophage colony-stimulating factor (GM-CSF) (Rat GM-CSF ELISA Kit Thermo Fisher Scientific, Massachusetts, USA) and transforming growth factor beta 1 (TGF-β1) (Rat TGF-ß1 ELISA Kit Thermo Fisher Scientific, Massachusetts, USA). For quantification of protein in cardiac tissue, following

heart explantation, cell lysis buffer was added to a slice of the apex and homogenised by placing Zirconium oxide beads in a tube and placing in a tissue disruptor for 5 minutes. The tubes were placed in a centrifuge at 4°C at high speed and the supernatant was aliquoted into another tube. The concentration of supernatant was checked with a Bicinchoninic acid assay (BCA).

12.3.4 Quantitative Real Time Polymerase Chain Reaction (PCR)

A sample of apex was excised and washed with ice-cold PBS and "snap-frozen" in dry ice. Five hundred microlitres of Lysis solution/2-ME mixture was added to the frozen tissue and homogenised in a flat bottom DNase, RNAse free cell culture tube on ice with TissueRuptor (Qiagen, USA). Total RNA was isolated using the Gen elute Mammalian Total RNA Mini Prep kit (Sigma-Aldrich, Missouri, USA). Concentration of total RNA was measured from 2µl of the extracted RNA sample using NanoDrop 1000 Spectrophotometer (Thermofisher Scientific, Massachusetts, USA). RNA was reverse transcribed to 1µg First-strand complementary DNA (cDNA) using Tetro cDNA Synthesis Kit (Bioline, London, UK) in accordance with the manufacturer's instruction. cDNA was stored at -20°C. Messenger RNA (mRNA) sequences were obtained from Refseq along with published mRNA records from the Oryctolagus cuniculus genome in GenBank. Oligonucleotide primer pairs were designed using Primer3 primer design software (http//fokker.wi.mit.edu/primer3). Amplified product specificity was confirmed using the online web application Primer-BLAST (http://www.ncbi.nlm.nih.gov/tools/primer-blast/). Oligonucleotides were synthesised by Sigma Genosys (Sigma Aldrich, St. Louis, Mo. USA). The primers used for qPCR were vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), early growth response-1 (EgR-1), monocyte chemoattractant protein 1 (MCP-1), hypoxia

inducible factor 1 alpha (HIF-1α), fibroblast growth factor 2 (FGF-2), tumor necrosis factor alpha (TNF-α) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH)

Quantitative Real time Polymerase Chain Reaction (qPCR) was performed with the cDNA samples and RT-PCR mixture prepared using SensiFast SYBR HI ROX Kit (Bioline, London, UK) in accordance with the manufacturer's instruction. Initially, serial dilution of cDNA sample were done and RT-PCR was run to generate a standard curve to estimate the efficiency of the primers. RT-PCR was performed in an ABI Prism 7900 HT Sequence Detection System (Applied Biosystems, Foster City, CA). Reactions were performed in triplicate and were analysed by relative quantitation using RQ Manager software, version 1.2 (Applied Biosystems). All gene concentrations were standardised relative to a ubiquitous protein; GAPDH.

12.3.5 Western Blot

Protein was extracted from tissue as described in 12.3.3. Protein quantification was performed by a BCA protein estimation kit (Thermofisher Scientific, Massachusetts, USA) in accordance with the manufacturer's instruction. Samples were prepared for electrophoresis by mixing the protein sample with 10x Dichlorodiphenyltrichloroethane (DDT) (Life Technologies, Carlsbad, California, USA) and 4x gel loading buffer (Life technologies) and heating samples at 70ºC for 5 min. Equal amount of protein was resolved using Bolt Bis-Tris gel (Life Technologies) for 1h at 150V. Depending on the separation required, either MES (Life Technologies) or MOPS buffer (Life Technologies) was used as running buffer. Proteins were transferred to Hybond Nitrocellulose membranes (Amersham Pharmacis Biotech, Bucks, UK) using Bolt transfer buffer (Life technologies) by wet transfer method. Membranes were blocked in Tris-buffered saline containing 0.5% Tween-20 (TBST) (Thermofisher Scientific) in either 5% skim milk or 5% BSA (for phospho-proteins) for 1 h

and then incubated overnight at 4ºC with the primary antibodies. The Primary antibody concentrations and the host species are shown in table 12.1.

Table 12.1: Primary and Secondary Antibodies used for Western Blot

Antibody	**Source**	**Dilution**	**Blocking buffer**	**Company**
Egr-1	Rabbit	1:500	5% skim milk	Santa Cruz (Dallas, Texas, USA)
VCAM-1	Mouse	1:1000	5% BSA	Abcam (Cambridge, UK)
FGF	Rabbit	1:10000	5% Skim Milk	Thermo Fisher Scientific (Massachusetts, USA)
HIF-1a	Rabbit	1:1000	5% Skim Milk	Abcam (Cambridge, UK)
GAPDH	Mouse	1:1000	5% skim milk	Santa Cruz (Dallas, Texas, USA)
Anti Rabbit	Goat	1:5000	5% skim milk/5% BSA	Santa Cruz (Dallas, Texas, USA)
Anti Mouse	Goat	1:5000	5% skim milk/ 5% BSA	Santa Cruz(Dallas, Texas, USA)

Membranes were washed 3 times for 10 minutes per wash with TBST and incubated with 1:5000 horseradish peroxidase conjugated secondary antibody in either 5% skim milk or 5% BSA (for phosphor proteins) for 2 hours at room temperature. Membranes were then washed a further three times for 10 minutes per wash with TBST. Proteins were visualised using Supersignal West Pico Chemiluminescent substrate (ECL) kit (Thermofisher) in LAS 4000 machine. GAPDH was used as a loading control and semi-quantitative analysis was performed using ImageJ (version 1.51q, Java based software program, NIH).

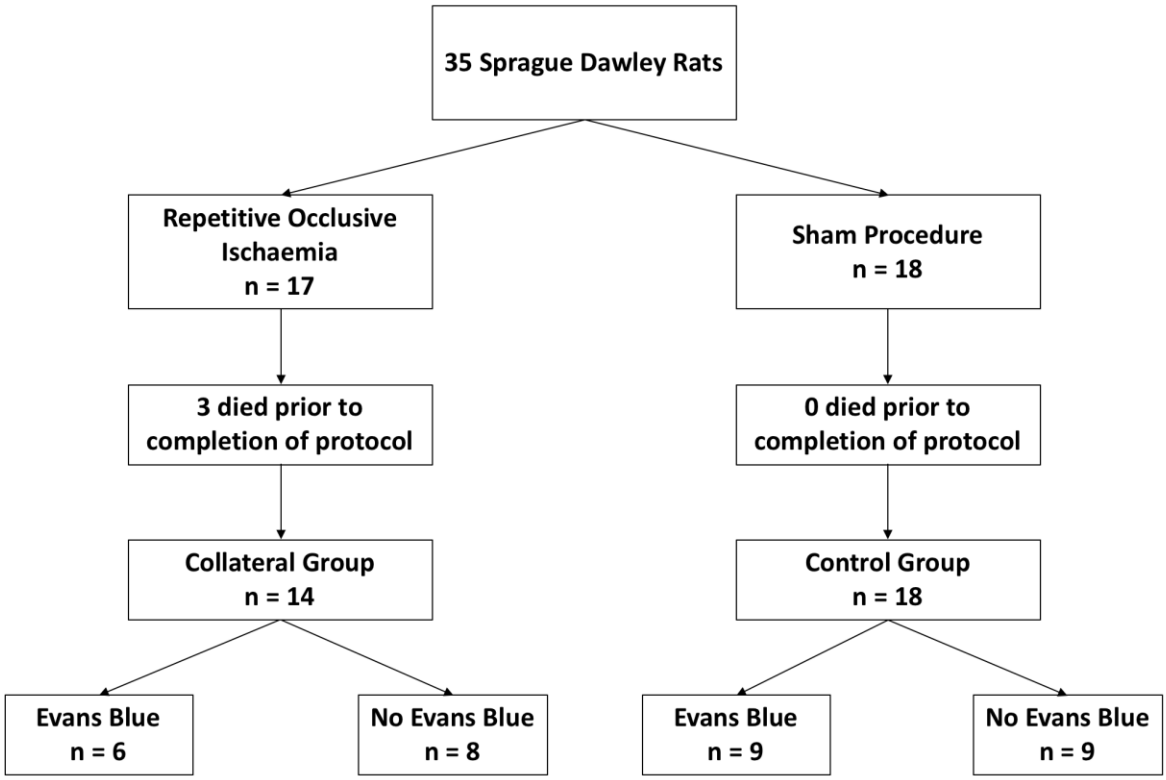
12.3.6 Statistical Analysis

Data was presented as means ± standard deviation (SD) for normalised data. Comparison between two groups was performed using two-tailed Student's T-test with Welch's correction. Analysis was performed using Prism 8 (GraphPad Inc, San Diego, CA, USA). A p-value of <0.05 was considered statistically significant.

12.4 Results

35 rats were included in the study, with 17 assigned the repetitive occlusive ischaemia protocol and 18 assigned to the sham procedure. 3 rats in the repetitive occlusive ischaemia protocol died prior to completion of the protocol, with 1 dying prior to intervention of uncertain cause, 1 dying during implantation of the balloon occluder and 1 dying on day 1 of the balloon occlusion protocol. Consequently there were 14 rats in the collateral group, and 18 rats in the control group. For the quantification of collateral perfusion, 6 rats from the collateral group and 9 from the control group had Evans Blue Dye injected prior to euthanasia (Figure 12.3).

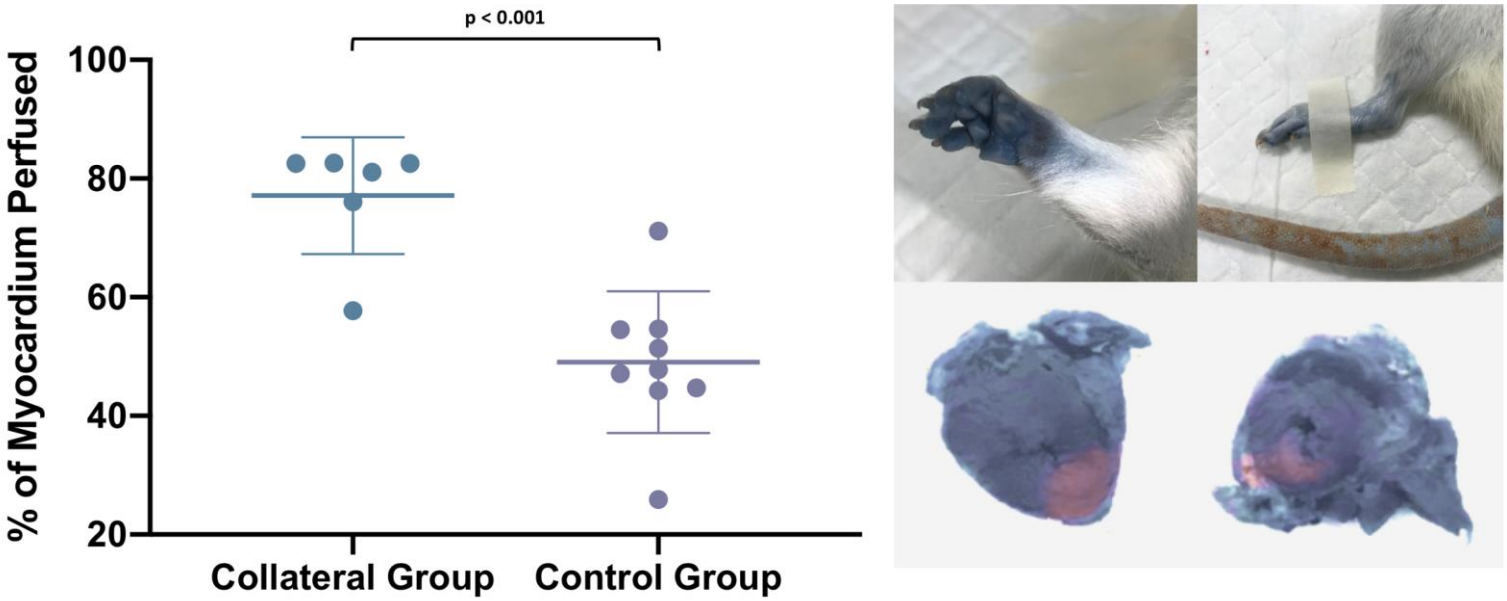
Figure 12.3: Study Protocol of rats undergoing procedures.



12.4.1 Myocardial Perfusion

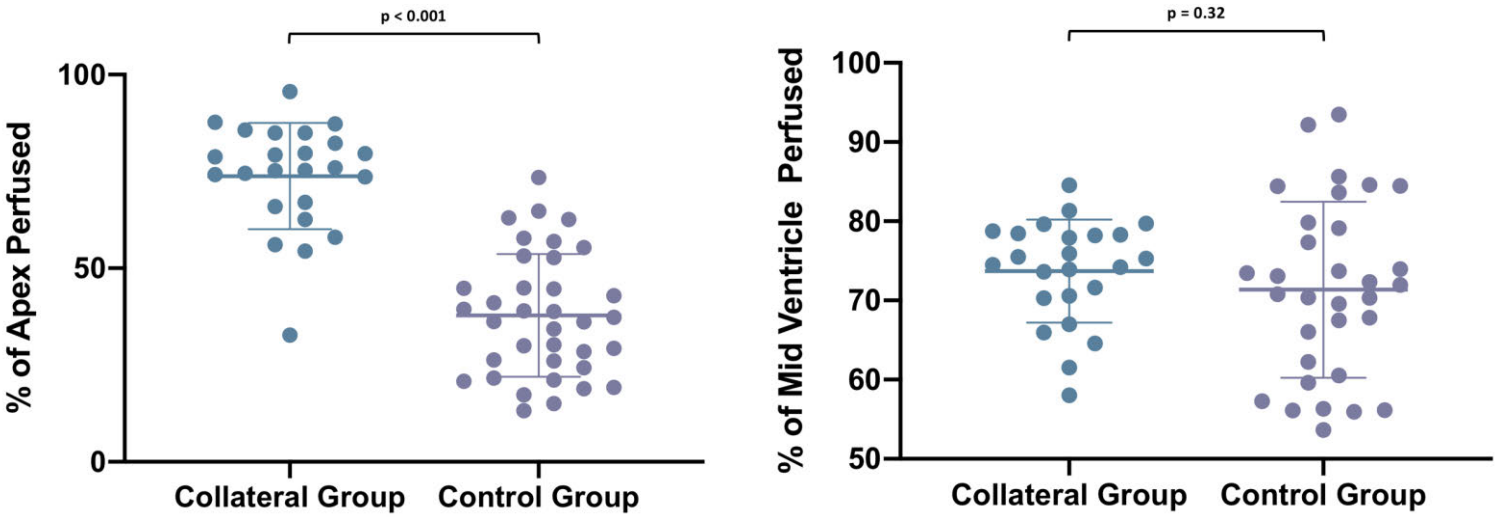
Rats in the collateral group had a significantly higher proportion of total myocardial perfusion compared to the control group (77.1% ± 9.8% vs 49.1% ± 11.9, p<0.001) (Figure 12.4). Rats in the collateral group had a significantly higher proportion of myocardial perfusion at the apex as compared to the control group (73.8% ± 13.7% vs 37.8% ± 15.8%, p<0.0001), although there was no difference in the proportion of mid cavity myocardial perfusion between groups (73.7% ± 6.5% vs 71.4% ± 11.1%, p=0.33) (Figure 12.5).

Figure 12.4: Percent of myocardial perfusion in rats in the collateral group compared with rats in the control group.



[Left] Graph illustrating significantly greater myocardial perfusion in the collateral group compared with the control group (77.1% vs 49.1%, p<0.001). [Top right]: Following injection of Evans blue dye in the rat, note blue colouring of the paw, foot and tail. [Bottom right]: Representative slices showing heart slices following Evans blue injection. The blue stained myocardium corresponds with perfused myocardium.

Figure 12.5: Percent of myocardial perfusion at the apex and mid ventricle in rats in the collateral group compared with rats in the control group.



12.4.2 ELISA Protein Concentrations

There was no difference in tissue concentration of VEGF in rats in the collateral group as compared to the control group (708.8pg/mL ± 495.5pg/mL vs 1053pg/mL ± 233.0pg/ml, p=0.13). There was similarly no difference in tissue concentration of IL-6 or IL-1ß between groups (4310pg/mL ± 2462pg/mL vs 6559pg/mL ± 1787pg/ml, p = 0.11 and 7291pg/mL ± 3596pg/mL vs 8688pg/mL ± 1792pg/ml, p = 0.39 respectively). There was also no difference in tissue concentration of TGF-ß in rats in the collateral group as compared to the control group (852.9pg/mL ± 513.1pg/mL vs 1224pg/mL ± 309.2pg/ml, p = 0.15) (Figure 12.6). There was a higher tissue concentration of GM-CSF in rats in the collateral group as compared to the control group (3752pg/mL ± 2477pg/mL vs 206pg/mL ± 155.5pg/ml, p<0.01) (Figure 12.7).

Figure 12.6: Tissue Protein Concentrations of VEGF, IL6, IL-1ß and TGF-ß as detected by ELISA

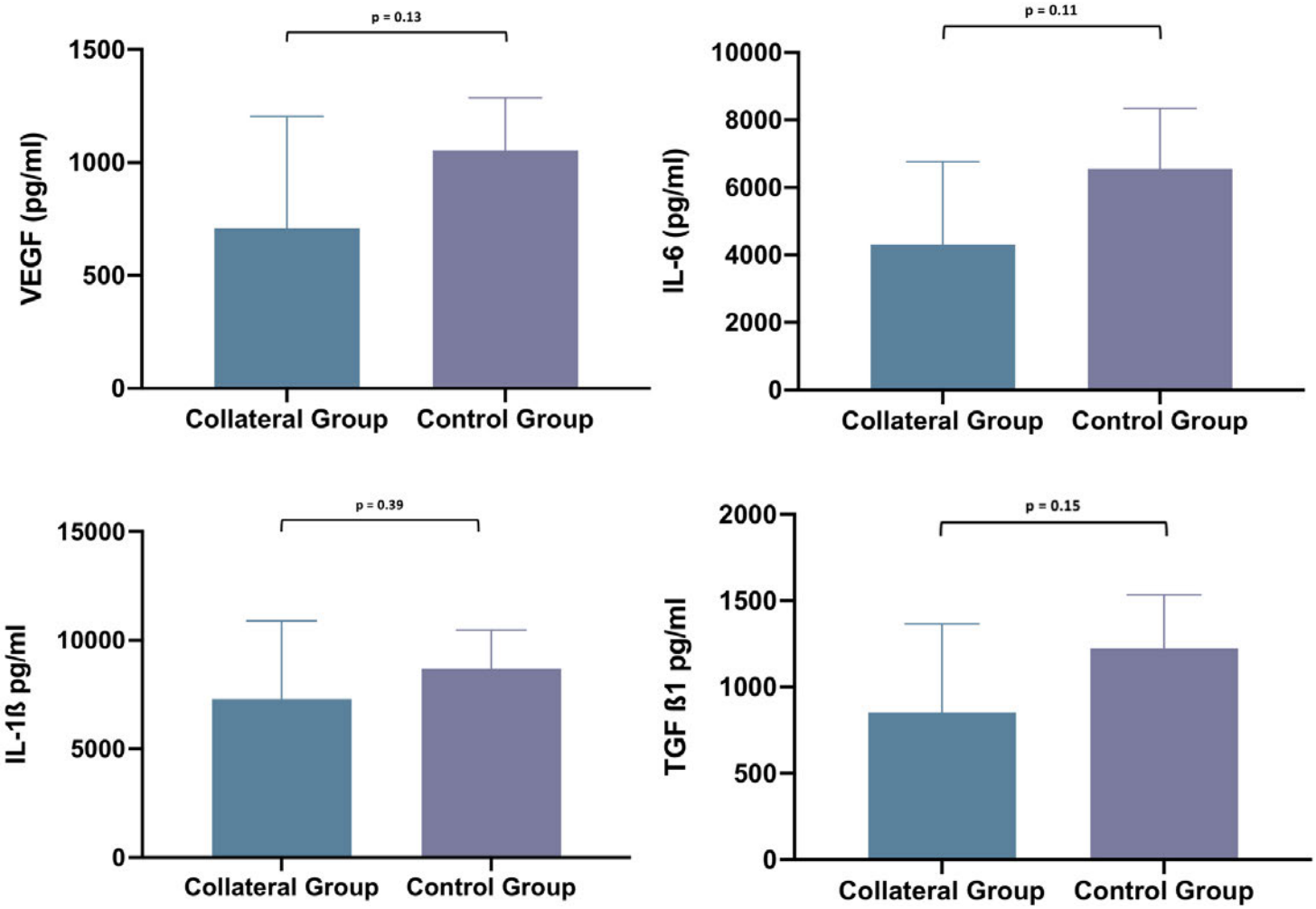
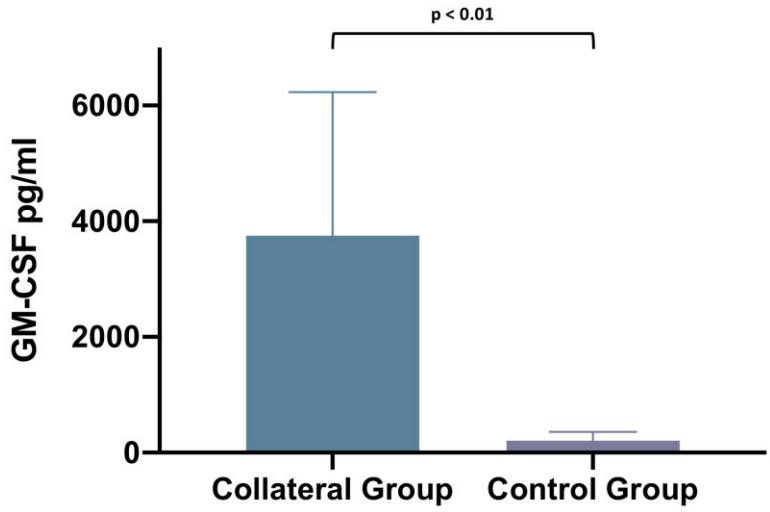


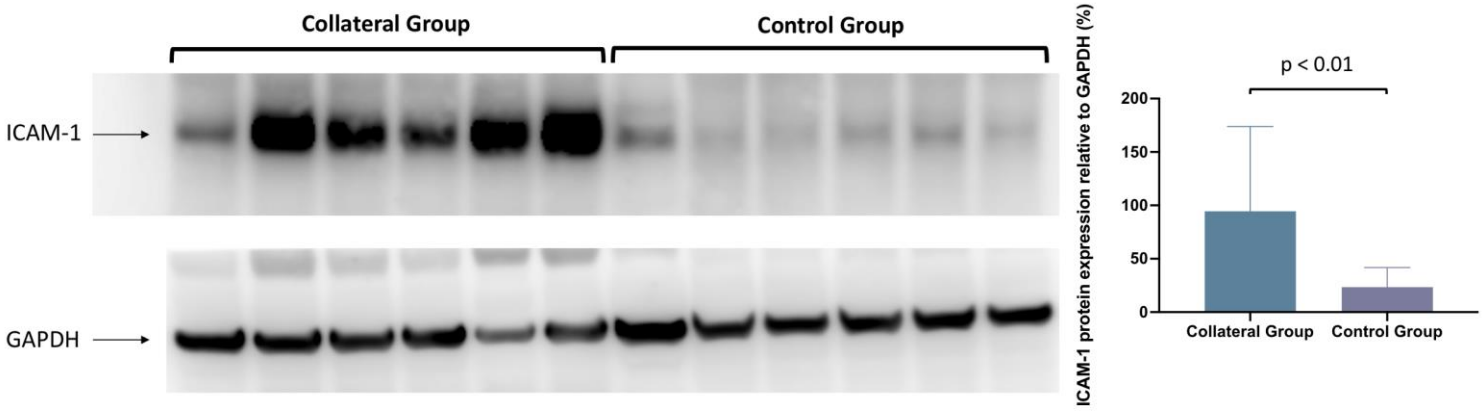
Figure 12.7: Tissue Protein Concentrations of GM-CSF as detected by ELISA



12.4.3 Western Blot

There was greater tissue protein expression of ICAM-1 relative to GAPDH in rats in the collateral group as compared to the control group (94.6% ± 79.3% vs 23.5% ± 18.3%, p<0.01) (Figure 12.8).

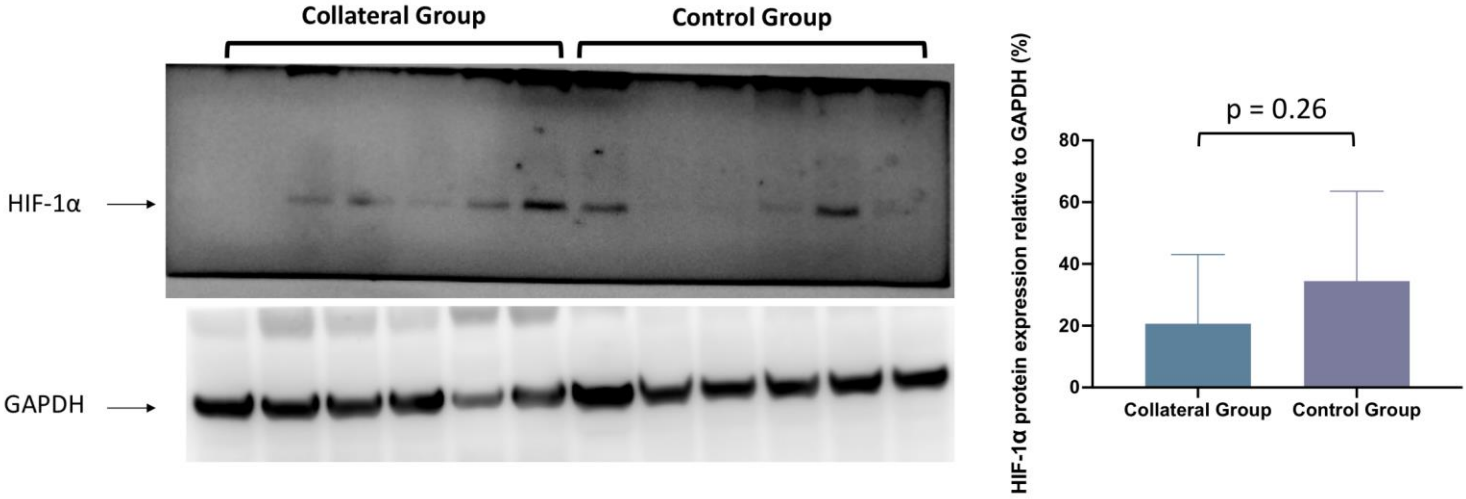
Figure 12.8: Western Blot Protein Concentration of ICAM-1 relative to GAPDH in collateral vs control group



Representative immunoblot from lysates from rat heart in collateral and control groups. Results are expressed as mean ± SD of ICAM-1 relative to GAPDH, n=22.

There was no difference in tissue protein expression of HIF-1α relative to GAPDH (20.6% ± 22.4% vs 34.4% ± 29.1%, p=0.26) (Figure 12.9).

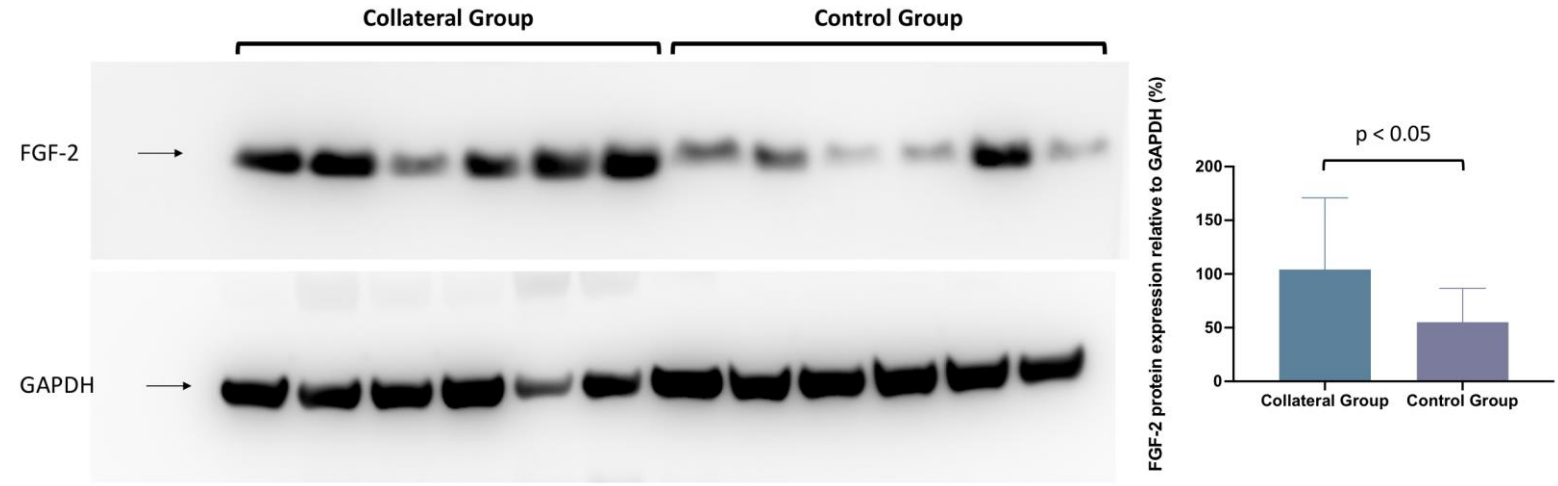
Figure 12.9: Western Blot Protein Concentration of HIF-1α relative to GAPDH in collateral vs control group



Representative immunoblot from lysates from rat heart in collateral and control groups. Results are expressed as mean ± SD of HIF-1α relative to GAPDH, n=22.

There was greater protein expression of FGF-2 relative to GAPDH in rats in the collateral group as compared to the control group (104.2% ± 66.7% vs 55.2% ± 31.5%, p<0.05) (Figure 12.10).

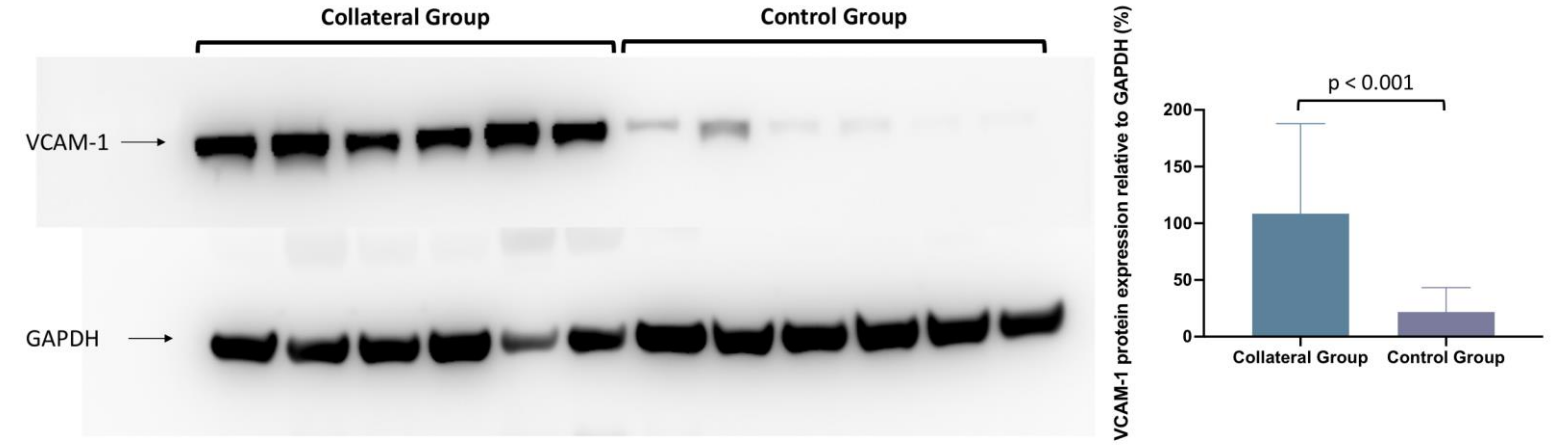
Figure 12.10: Western Blot Protein Concentration of FGF-2 relative to GAPDH in collateral vs control group



Representative immunoblot from lysates from rat heart in collateral and control groups. Results are expressed as mean ± SD of FGF-2 relative to GAPDH, n=22.

There was greater tissue protein expression of VCAM-1 relative to GAPDH in rats in the collateral group compared to rats in the control group (108.5% ± 79.4% vs 21.7% ± 21.5%, p<0.001) (Figure 12.11).

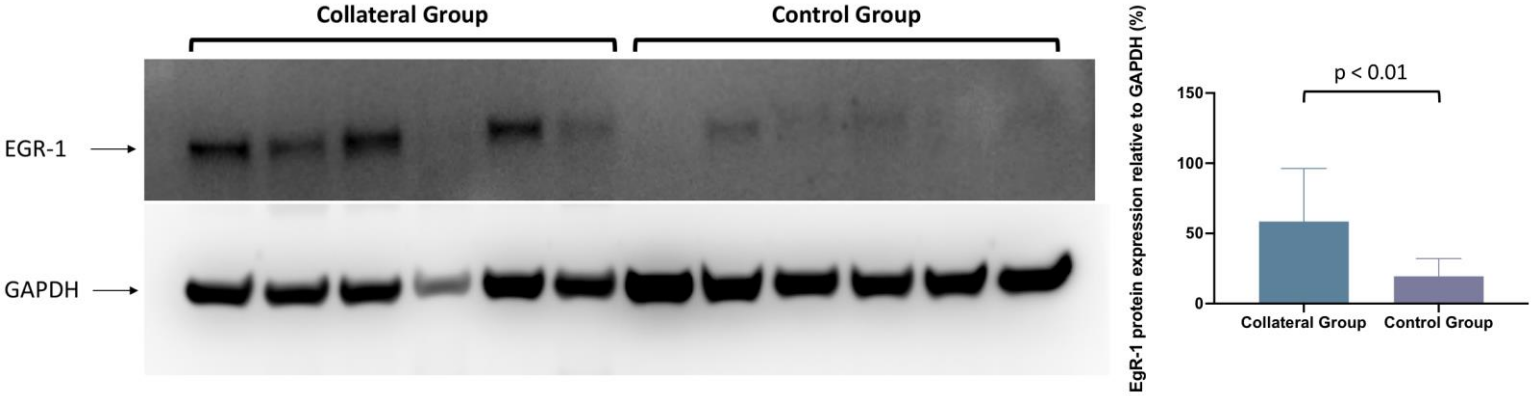
Figure 12.11: Western Blot Protein Concentration of VCAM-1 relative to GAPDH in collateral vs control group



Representative immunoblot from lysates from rat heart in collateral and control groups. Results are expressed as mean ± SD of VCAM-1 relative to GAPDH, n=22.

There was also greater tissue protein expression of EgR-1 in rats in the collateral group compared with the control group (58.5% ± 13.8% vs 19.5% ± 12.7%, p<0.01) (Figure 12.12).

Figure 12.12: Western Blot Protein Concentration of EgR-1 relative to GAPDH in collateral vs control group

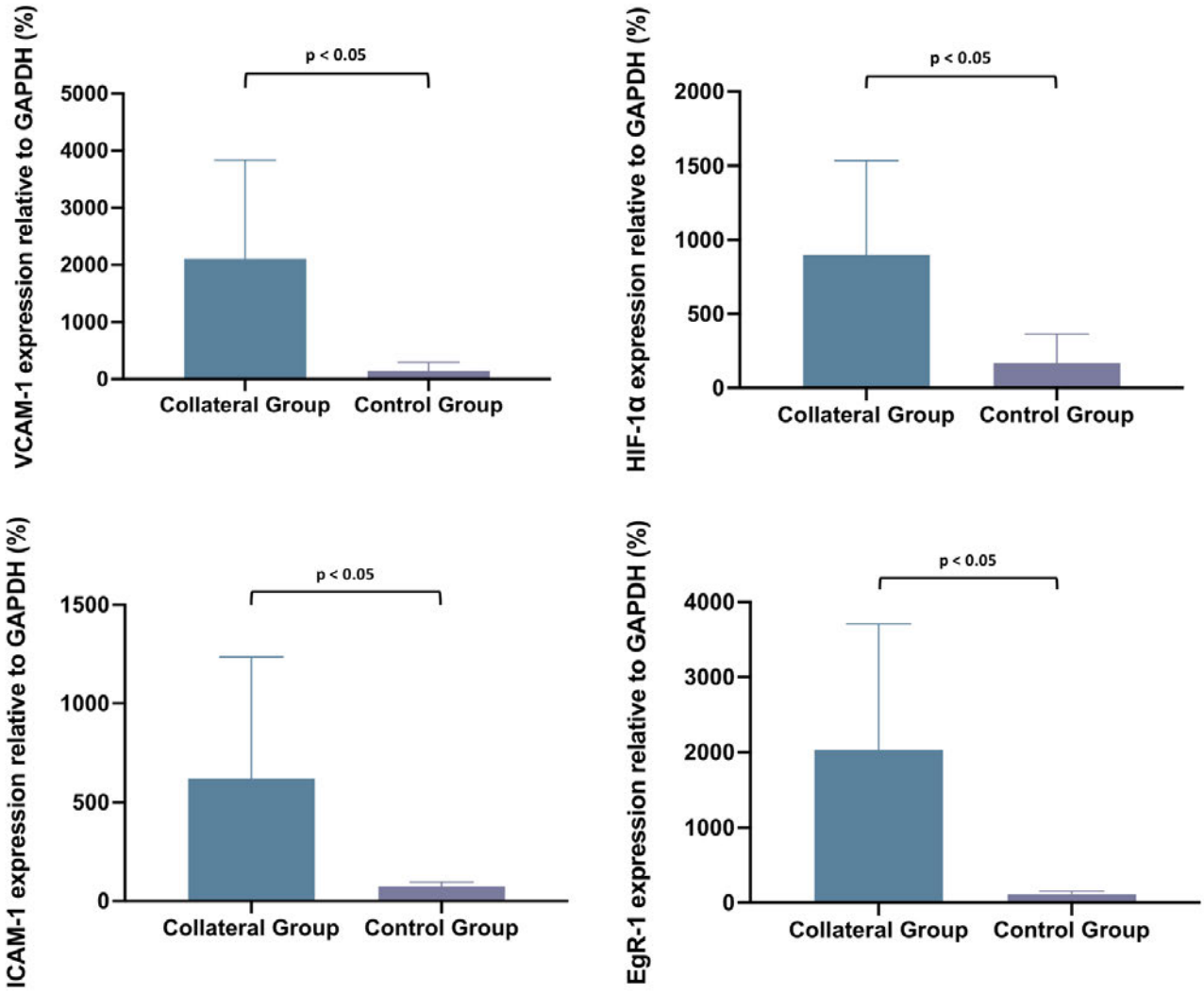


Representative immunoblot from lysates from rat heart in collateral and control groups. Results are expressed as mean ± SD of EgR-1 relative to GAPDH, n=22.

12.4.4 Quantitative Real Time Polymerase Chain Reaction (qPCR)

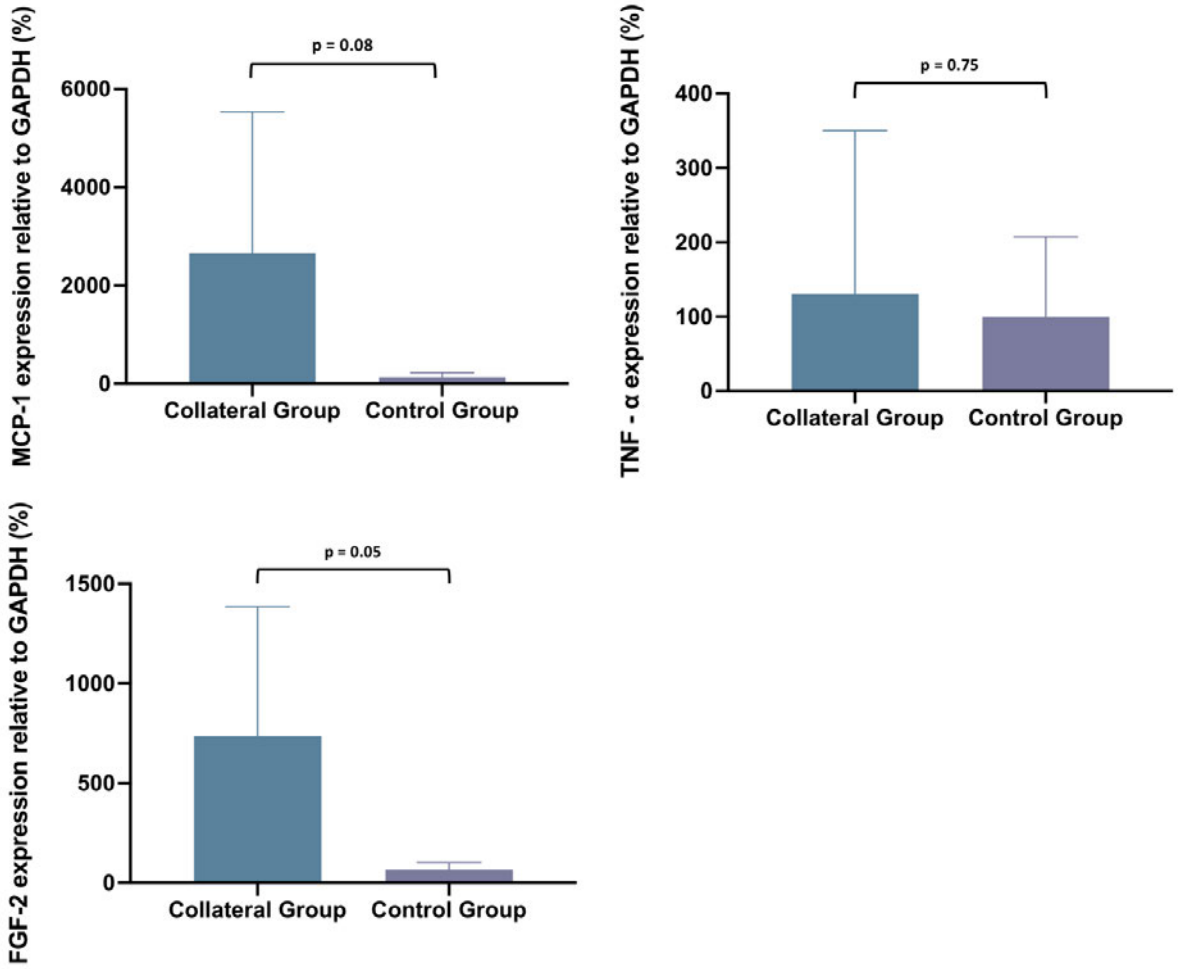
There was greater tissue expression of *VCAM-1* and *HIF-1*α mRNA relative to *GAPDH* mRNA in rats in the collateral group compared with the control group (2109% ± 1725% vs 146.6% ± 145.2%, p<0.05 and 898.4% ± 636.8% vs 167.8% ± 196.2%, p<0.05 respectively). There was also a greater tissue expression of *ICAM-1* and *EgR-1* mRNA relative to *GAPDH* *m*RNA in rats in the collateral group compared with the control group (621% ± 614.9% vs 75.1% ± 20.6%, p<0.05 and 2035% ± 1673% vs 110.1% ± 42.0%, p<0.05 respectively) (Figure 12.13).

Figure 12.13: qPCR concentrations of mRNA tissue expression of *VCAM-1*, HIF1- *α, ICAM-1* and *EgR-1* relative to *GAPDH* in collateral and control group rats



There was no difference in the tissue expression of *MCP-1 m*RNA or *TNF-α* mRNA relative to *GAPDH* mRNA in rats in the collateral group as compared to the control group (2661% ± 2874% vs 131.6% ± 94.9%, p=0.08 and 130.8% ± 219.4% vs 100.0% ± 107.5%, p=0.75 respectively). There was also no difference in the tissue expression of *FGF-2* mRNA relative to *GAPDH* mRNA in rats in the collateral group as compared to the control group (735.5% ± 650.3% vs 67.5% ± 34.9%, p=0.05) (Figure 12.14).

Figure 12.14: qPCR concentrations of mRNA tissue expression of MCP-1, TNF-α and FGF-2 relative to GAPDH in collateral and control group rats



12.5 Discussion

12.5.1 Collateral Perfusion following Repetitive Occlusive Ischaemia

In a rat model, a 10 day protocol of repetitive occlusive ischaemia results in an increase in perfusion of myocardium subtended by the occluded vessel by 50%, with an absolute increase in perfusion of 28%. As perfusion could only be occurring in a retrograde manner, due to the occluded vessel, this must be as a result of an increase in collateral perfusion to the territory. This increase in collateral perfusion is isolated to the apex, with perfusion in the mid cavity, similar to that of the control group. In patients with a CTO of the LAD,

analogous to the situation in this study, 65.4% of collaterals supply the distal LAD, which would perfuse the apex and distal anterior wall, suggesting greater perfusion from collaterals in this territory (23), as seen in the present study. Furthermore, previous studies have suggested a longitudinal, base-to-apex myocardial perfusion gradient, with less myocardial blood flow to the apex in the setting of coronary artery disease (24) and in normal coronary arteries in patients with cardiovascular risk factors (25). This suggests that the apex has greater ischaemic potential as compared to the mid cavity and therefore more likely to be hypoxic, as well as lower driving perfusion pressure distally, allowing greater contralateral pressure and flow in the pre-existing collateral connections, both of which are drivers of collateral recruitment and maturation.

12.5.2 Chemokine alterations following repetitive occlusive ischaemia

Following a 10 day protocol of repetitive occlusive ischaemia, there was a significant increase in the tissue mRNA and protein expression of ICAM-1 as determined by qPCR and Western Blot respectively. ICAM-1 is expressed on endothelial cells (26) and is involved in the adhesion of inflammatory cells such as monocytes, neutrophils and lymphocytes to the endothelium, to prime the location to mature into functional collateral vessels (27,28). While animal studies have previously implicated ICAM-1 in the development of collaterals in the setting of peripheral vascular disease (4), this study has identified its association with the early phase of coronary collateral recruitment and function. As discussed in Section V Chapter 10, in human participants with a CTO, serum ICAM-1 concentrations were significantly higher than in patients without, and following collateral regression, there was an increase in circulating levels suggesting a role of pre-conditioning and collateral recruitment.

Following a 10 day protocol of repetitive occlusive ischaemia, there was no change in the tissue mRNA expression of MCP-1. MCP-1 serves to attract and recruit monocytes to the

endothelium of the vasculature (6), and has been implicated in arteriogenesis in animal models (29). However, MCP-1 has also been suggested as a marker of inflammation (30), and has been noted to be elevated following cardiac surgery (31), which may account for similar levels in the sham group. These findings suggest that the role of MCP-1 in arteriogenesis may reflect downstream effects on inflammatory cells rather than direct effects on vascular tissue.

VCAM-1 tissue mRNA and protein expression were significantly elevated following a 10 day protocol of repetitive occlusive ischaemia. VCAM-1 is secreted by endothelial cells following adhesion of leukocytes (4), which facilitate binding of monocytes to the endothelium promoting further endothelial activation (32) and arteriogenesis (4). Inhibition of VCAM-1 has been associated with an inhibition of arteriogenesis (33). It is thus unsurprising that with intermittent coronary occlusion, a driver of collateral recruitment in the form of VCAM-1 is elevated.

12.5.3 Inflammatory cytokine alterations following repetitive occlusive ischaemia

As a consequence of the increase in chemoattractant proteins, and hence accumulation of inflammatory cells such as monocytes and other leukocytes, local inflammation is believed to promote collateral growth (7). There was no change in tissue mRNA expression of TNF-α following repetitive occlusive ischaemia. TNF-α, a pro-inflammatory cytokine which is secreted by monocytes (34) plays a role in a number of chronic inflammatory processes. Invasive human studies have suggested that there is an inverse relationship between TNF-α concentration and collateral maturity (35). However, as the control group had sham procedures performed, which would also activate an inflammatory process during recovery, it is possible that no difference was detected as a result of the comparator group. Similarly,

there were no differences in the tissue protein concentrations of IL6 or IL-1ß following the 10 day repetitive occlusive ischaemia protocol. Interleukins, which are expressed by a number of cells including monocytes and endothelial cells (36), are inflammatory cytokines which can activate downstream gene pathways to allow endothelial migration and tubular formation (37). While there was no correlation between inflammatory cytokines and increased myocardial perfusion following artery occlusion, this may represent either a high baseline reading in the control group (given the sham surgical procedure), or alternatively suggest that following initial recruitment of collaterals, inflammatory mediators may lessen as other growth factors promote maturation.

12.5.4 Growth Factor alterations following repetitive occlusive ischaemia

Following a protocol of repetitive occlusive ischaemia, there was no difference in concentration of tissue VEGF protein expression compared with control groups. VEGF is a potent mediator of angiogenesis, involved in monocyte chemotaxis (38), smooth muscle cell proliferation (39) and angiogenesis through activation of two main receptors (40). However, despite animal studies suggesting a role of VEGF in mediating collateral maturation (17,41), human studies have been negative for VEGF to induce clinically relevant arteriogenesis (42). VEGF is also upregulated in inflammation (43) and hence its role may be contributory rather than directive in the setting of arteriogenesis.

There was similarly no change in tissue protein concentration of TGF-β following repetitive occlusive ischaemia. Previous studies have been contradictory, with some finding a correlation between this growth factor and coronary collaterals (44) whilst others have not (45). Furthermore, it has been suggested that TGF-β is associated with the early recruitment of collaterals (46), and that over time the levels reduce to baseline. TGF-β has also been implicated in upregulating VEGF (47). It is thus possible that TGF-β is either not directly

implicated in arteriogenesis, or alternatively, the time point chosen for this study may not have identified the temporal changes in TGF-β concentration.

FGF-2 tissue protein expression was significantly higher following repetitive occlusive ischaemia, suggesting its correlation with collateral recruitment. FGF-2 has been implicated in coronary vasodilation (48,49), and may be an early factor which is activated following release from monocytes and endothelial cells. GM-CSF tissue protein expression was significantly elevated following a protocol of repetitive occlusive ischaemia. GM-CSF is predominantly expressed by myeloid cells (50), and is involved in increased survival of monocytes and macrophage (51,52). As monocyte adherence and activation is one of the initiating steps of collateral maturation, it is unsurprising that GM-CSF appears to be upregulated with collateral recruitment.

12.5.5 Regulatory Chemokine alterations following repetitive occlusive ischaemia

Following repetitive occlusive ischaemia, there was a significantly higher expression of tissue HIF-1α mRNA, although this was not seen with respect to tissue protein expression of HIF-1α. Previous studies have suggested that discrepancy between expression levels of mRNA and protein are in the region of 40% (53,54), attributable to other levels of regulation between transcript and protein production (55). HIF-1α is a transcriptional activator that functions as a master regulator of oxygen homeostasis, with its target genes encoding proteins that increase oxygen delivery and mediate adaptive responses to oxygen deprivation (56). Some clinical studies have correlated HIF-1α with coronary collaterals (57), although others found no correlation (58). Whilst it would appear that HIF-1α is upregulated in the setting of ischaemia, both experimentally as in the current study or pathologically as in the setting of a CTO, a situation where the myocardium is in a chronic state of ischaemia, it does

not appear to result in transcription upstream. This reflects a contributory effect of hypoxia on arteriogenesis, with an elevation in shear stress remaining the predominant driving force.

EgR-1 mRNA and protein tissue expression were significantly elevated following a protocol of intermittent occlusive ischaemia. EgR-1 is a transcription factor of the zinc-finger family (59) which is activated by the effects of fluid shear stress on the EgR-1 gene promoter (60). EgR-1 has been implicated in arteriogenesis through modulating levels of other growth factors, including TGF-β (61), and recruitment and proliferation of leukocytes (62). It thus appears that whilst EgR-1 is involved intimately with collateral recruitment and maturation, its role is that of a regulator, influencing effects on inflammatory cells as well as production of growth factors and other cytokines.

12.5.6 Limitations

The process involved in inducing collateral perfusion was repetitive occlusive ischaemia, which lacks the hallmark of CTO in humans, namely atherosclerosis. Nevertheless, by stimulating downstream hypoxia and a reduction (obliteration) in anterograde blood flow, thereby increasing fluid shear stress in the primitive collateral connections, this is analogous to the haemodynamic situation of a progressive CTO. Furthermore, the process of surgical implantation of a device onto the cardiac surface undoubtedly would increase local inflammation. However by including a sham arm which also underwent surgery, this aimed to obviate the effects of the surgery itself. Rats in the sham arm did not have routine anaesthesia administered, unlike rats in the surgical arm who had this done for their ROI protocol, which may theoretically introduce a confounder for these results. However it is unlikely that the anaesthetic would induce collateral development. Finally, the increase in perfusion in the myocardium supplied by the occluded artery must come from retrograde

filling via collaterals. However, whether this was a result of an increase in the number of collaterals, or alternatively due to an increase in the size of the existing collaterals was not directly visualised. Although previous studies have validated this method of inducing collaterals, nevertheless, future studies including immunohistochemical staining to identify the structure of the collaterals should be conducted to identify the mechanism of increased collateral dependent perfusion.

12.6 Conclusion

In a rat model, repetitive occlusive ischaemia to the left anterior descending artery results in an increase in myocardial perfusion in the territory subtended by the epicardial artery as a result of increased collateral blood flow at the apex. This increase in regional myocardial perfusion through collateral perfusion is associated with an increase in chemoattractant proteins ICAM-1 and VCAM-1, growth factors GM-CSF and FGF-2 along with the regulator protein EgR-1. Inflammatory cytokines did not appear to be associated with an increase in collateral perfusion. Further investigation of the mechanistic effect on collateral size and number as well as further assessment of causative processes should be performed.

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SECTION VII: ROLE OF CORONARY ARTERY BYPASS GRAFTING AND ALTERATIONS ON CORONARY BLOOD FLOW ON COLLATERAL RECRUITMENT

Chapter 13: Impact of previous Coronary Artery Bypass Grafting (CABG) of the Donor Vessel on Coronary Collateral Recruitment

13.1 Abstract

Background & Aims: A chronic total occlusion (CTO) is found commonly in patients with prior coronary artery bypass grafting (CABG). The success rates of revascularisation for a CTO in patients with previous CABG is lower and associated with more complications. The aim of this study was to determine the effect of prior CABG on collateral recruitment.

Methods: Patients with a CTO diagnosed on coronary angiography between July 2010 and December 2019 were included in this study. Patients were classified as either CTO supplied by a functional graft, CTO supplied by collaterals from a non-grafted donor vessel (non-grafted) or a CTO supplied by collaterals from a grafted donor vessel (grafted). The degree of collateral robustness was determined by the Rentrop classification and collateral connection (CC) grade. Demographic, angiographic and clinical outcomes were recorded.

Results: A total of 2,088 CTO lesions were identified, of which 878 (42.0%) were supplied by a functional graft, 994 (47.6%) CTOs were supplied by a non-grafted donor vessel and 216 (10.3%) CTOs were supplied by a grafted donor vessel. CTOs supplied by a grafted donor vessel had lower rates of robust collaterals (37.0% vs 83.0%, $p<0.0001$) with less mature collaterals as determined by the Rentrop grade ($p<0.0001$) and CC grade ($p<0.0001$) as compared to CTOs supplied by a non-grafted donor vessel.

Conclusion: In patients with a previous CABG, a donor vessel which is grafted results in poorer coronary collaterals with lower Rentrop grade and CC grade compared to an ungrafted donor vessel. This may be attributable to changes in coronary blood flow and shear stress, and may be a factor in the lower procedural success rates for CTO intervention in patients with prior CABG.

13.2 Introduction

A coronary chronic total occlusion (CTO) is appreciated angiographically as the presence of late filling of the occluded vessel by collaterals (1). The true incidence of a CTO varies widely in the published literature, with up to 90% in patients undergoing angiography with a history of prior coronary artery bypass grafting (CABG) (2), 15-30% in those without prior CABG (3) and 6.6% in those presenting with an acute coronary syndrome (4).

Bypass grafting results in significant alterations in coronary blood flow and consequently vascular shear stress in the native circulation distal to the graft (5). As collateral recruitment and maturation is exquisitely related to vascular shear stress (6), bypass grafting may affect the collateral circulation. However, what impact the presence of a bypass graft, and consequent alterations in coronary blood flow have on coronary collaterals has not previously been assessed.

The aim of this study was to determine the effect of previous CABG on the coronary collateral circulation as determined by invasive angiography. Furthermore, the aim was to determine the effect of graft type and location on collateral recruitment as well as demographic, clinical and angiographic differences in patients with a CTO.

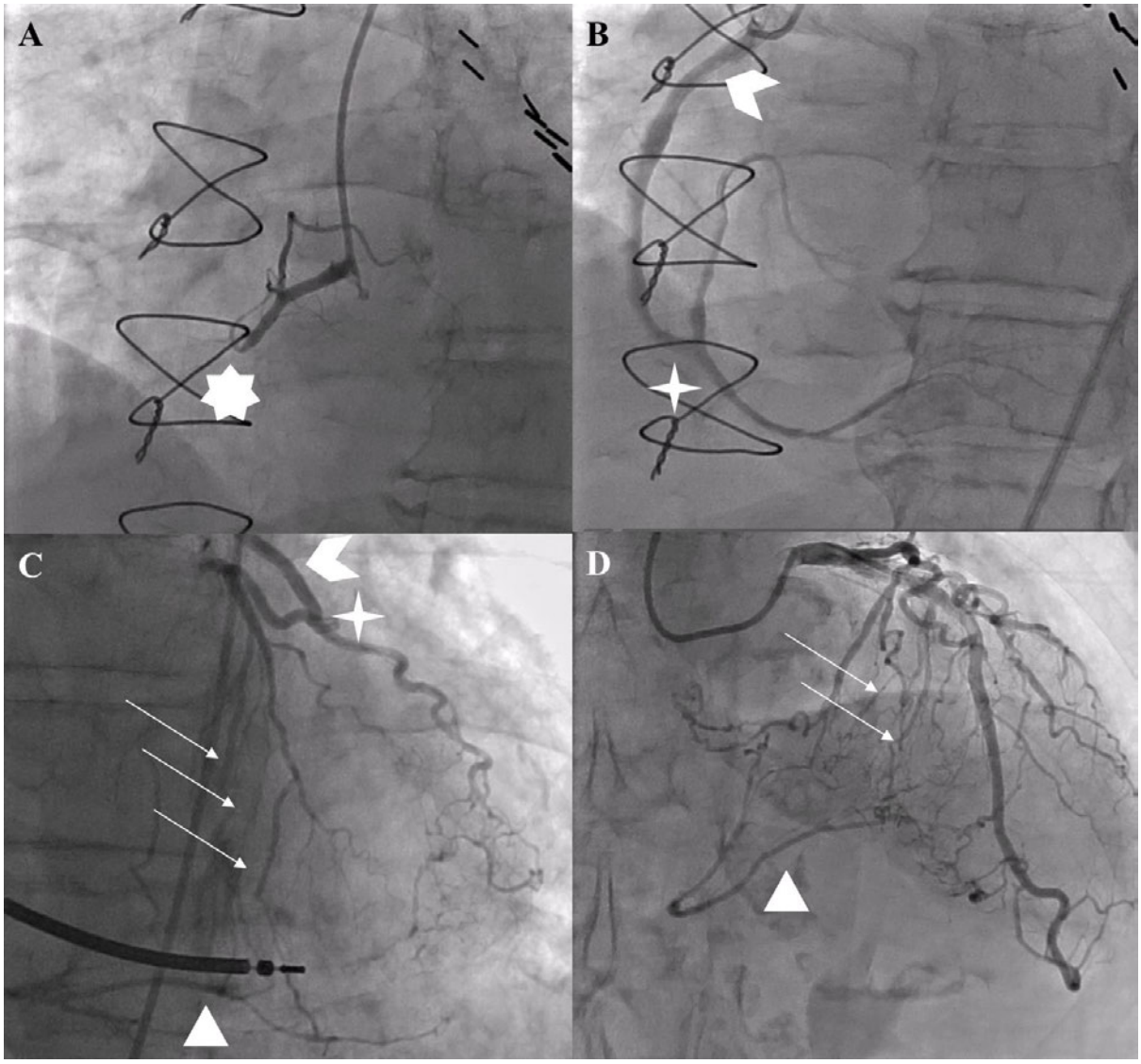
13.3 Methods

All patients undergoing clinically indicated coronary angiography at a tertiary centre from July 2010 to December 2019 were reviewed. Patients who had a reported CTO identified through a commercially available reporting system on a local server (McKesson, Irving TX, USA) were included. Patients presenting with ST elevation myocardial infarction (STEMI) or those with only ipsilateral collaterals such as bridging collaterals, were excluded. Procedural

characteristics, baseline medications, in-hospital course along with left ventricular function, and biochemical results were reviewed using electronic medical records. Mortality and last medical contact was determined through medical record linking systems. Left ventricular function was assessed by transthoracic echocardiography, or if not performed, by ventriculography at the time of angiography.

Patients were divided into one of 3 groups based on the perfusion to the CTO (Figure 13.1): CTO supplied by a functional graft, CTO supplied by collaterals from a non-grafted donor vessel or a CTO supplied by collaterals from a grafted donor vessel. In those patients with a CTO supplied by a non-grafted or grafted vessel, the presence and degree of collaterals was graded according to the Rentrop classification (7), where robust collaterals were defined as Rentrop grade 2 or 3(8-11). The Collateral Connection (CC) grade was also assessed (12). The donor vessel was defined as the epicardial coronary artery from which collaterals arose. In cases where 2 vessels provided collaterals, the vessel from which the predominant collaterals arose was defined as the donor vessel. Stenosis in the donor vessel and graft was calculated using quantitative coronary angiography (QCA) (McKesson, Irving Tx, USA). In the setting of a grafted donor, this was assessed between the graft anastomosis and collaterals, thereby determining the degree of stenosis (and hence alteration to blood flow) impacting on the collaterals. Bypass grafts were recorded as left internal mammary artery, right internal mammary artery, saphenous vein graft or radial arterial graft.

Figure 13.1: Differing perfusion of a chronic total occlusion of the right coronary artery



A) Diagnostic angiography identifying a CTO of the right coronary artery (RCA) [7-point star]. B) a patent SVG graft (chevron) anastomosed to the distal RCA (4-point star) perfusing the CTO in both anterograde and retrograde manner. C) A patent SVG graft (chevron) anastomosed to a diagonal artery (4-point star) perfusing the LAD in a retrograde manner which subsequently perfuses through septal collaterals (thin arrows) the occluded RCA (triangle). D) Septal collaterals (thin arrows) from an ungrafted LAD supplying the occluded RCA (triangle).

Indication for angiography was defined as either emergent (unstable angina, non ST elevation myocardial infarction, ventricular arrhythmia or cardiac arrest not fulfilling criteria for STEMI) or non-emergent (stable angina or angina equivalent symptoms). Left ventricular impairment was defined as left ventricular ejection fraction (LVEF) ≤ 50% while valvular

heart disease was defined as moderate or severe mitral or aortic valve disease. Medications were based on regular medications at the time of angiography. Project approval by the local human ethics committee was obtained prior to data analysis.

13.3.1 Statistical Analysis

Continuous variables were presented as means ± standard deviation in those with normally distributed data or medians and interquartile ranges in those with non-normally distributed data. Categorical data was presented as percentages. Comparisons between groups were performed using Pearson's chi square test for all categorical variables. Continuous variables were firstly assessed by the Shapiro-Wilk test to ascertain normality of distribution, after which a student t-test was used for data that was normally distributed or Mann-Whitney U test for non-normally distributed continuous data. All tests were 2-sided, and $p < 0.05$ was considered statistically significant. Analyses were performed using SPSS (version 24, IBM, New York, New York).

13.4 Results

A total of 2,088 CTO lesions were included in the analysis, of which 1,129 (54.1%) had previous CABG. 878 (77.1%) patients had a functional graft perfusing the CTO while 216 (19.1%) had a grafted donor vessel and 35 (3.1%) had a CTO supplied by either an ungrafted vessel or vessel with an occluded graft. Consequently, 994 (47.6%) CTOs were supplied by an ungrafted vessel, 216 (10.3%) CTOs supplied by a grafted vessel and 878 (42.0%) supplied by a functional graft (figure 13.2).

Figure 13.2: Flow diagram of CTO lesions and their classifications

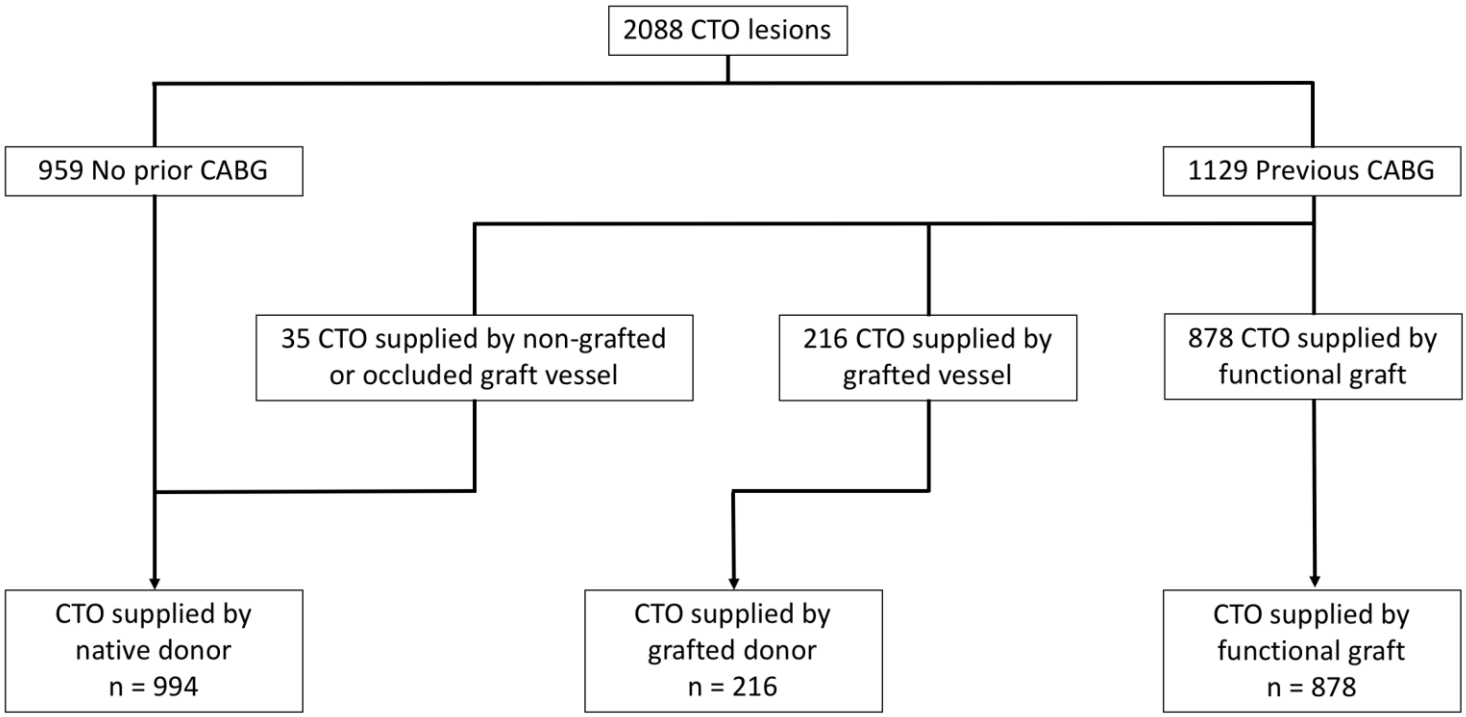


Table 13.1 shows the baseline characteristics of CTO lesions classified on their perfusion. Patients with a CTO supplied by a grafted donor were less likely to have a LAD CTO (7.9% vs 23.6% vs 49.8%, p<0.0001) and a lower left ventricular ejection fraction (45% vs 55% vs 50%, p<0.0001) compared with those with a CTO supplied by an ungrafted donor or functional graft respectively. Patients with a CTO supplied by a functional graft were older (76.3yrs vs 75.5yrs vs 71.8yrs, p<0.0001) compared to those with a CTO supplied by a grafted donor or ungrafted donor respectively.

Table 13.1: Demographic and angiographic differences in CTO vessel supplied by bypassed vessel, non-bypassed vessel and functional graft

	CTO supplied by ungrafted vessel n=994	CTO supplied by grafted vessel n=216	CTO supplied by functional graft n=878	p-value
Age *(yrs)*	**71.8 (62.1 – 80.1)**	**75.5 (66.7 – 82.5)**	**76.3 (69.0 – 76.3)**	**<0.0001**
Female Sex *(n)*	188 (18.9%)	31 (14.4%)	158 (18.0%)	0.29
BMI *(kg/m^2)*	27.1 (24.2 – 30.6)	27.0 (23.9 – 29.6)	27.2 (24.5 – 30.3)	0.60
Number of CTOs *(n)*				**<0.0001**
1	**801 (80.6%)**	**20 (9.3%)**	**157 (17.9%)**	
2	**176 (17.7%)**	**106 (49.1%)**	**403 (45.9%)**	
3	**17 (1.7%)**	**90 (41.7%)**	**318 (36.2%)**	
Previous CABG *(n)*	**36 (3.6%)**	**216 (100%)**	**878 (100%)**	**<0.0001**
CTO Vessel *(n)*				**<0.0001**
LAD	**235 (23.6%)**	**17 (7.9%)**	**437 (49.8%)**	
LCx	**209 (21.0%)**	**36 (16.7%)**	**217 (24.7%)**	
RCA	**550 (55.3%)**	**163 (75.5%)**	**224 (25.5%)**	
LAD CTO *(n)*	**235 (23.6%)**	**17 (7.9%)**	**437 (49.8%)**	**<0.0001**
Emergent Indication for angiogram *(n)*	518 (52.1%)	129 (59.7%)	458 (52.2%)	0.11
Valvular Heart disease *(n)*	114/856 (13.3%)	27 (12.5%)	147/878 (16.7%)	0.08
LV impairment *(n)*	**441 (46.5%)**	**113 (66.5%)**	**328 (52.6%)**	**<0.0001**
LVEF (%)	**55 (40 – 60)**	**45 (35 – 55)**	**50 (40 – 60)**	**<0.0001**

**BMI = body mass index; CABG = coronary artery bypass graft; CTO = chronic total occlusion; kg = kilogram; LAD = left anterior descending artery; LCx = left circumflex artery; LV = left ventricular; LVEF = left ventricular ejection fraction; m = metre; n = number; RCA = right coronary artery; yrs = years;*

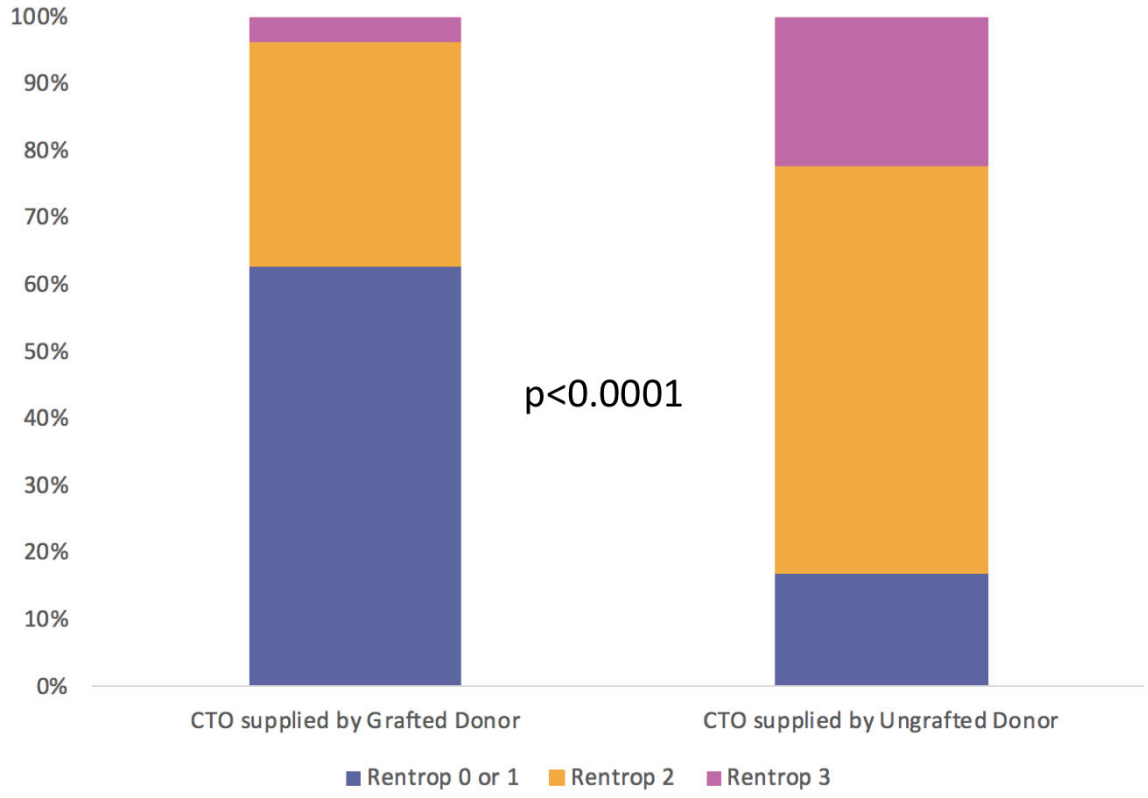
Table 13.2 shows the demographic and angiographic differences between the CTO supplied by a grafted donor compared with the CTO supplied by an ungrafted donor. Those with a grafted donor were older (74.0yrs vs 70.5yrs, p<0.0001) and more likely to have multiple CTOs (90.8% vs 27.4%, p<0.0001). Those with a grafted donor vessel had a lower degree of stenosis in the donor vessel, proximal to the collaterals (37.1% vs 50.8%, p<0.0001). CTOs supplied by a grafted vessel had lower rates of robust collaterals (37.0% vs 83.0%, p<0.0001) with less mature collaterals as determined by the Rentrop grade (p<0.0001) and CC grade (p<0.0001) (Figure 13.3).

Table 13.2: Demographic and angiographic differences in CTO vessel supplied by bypassed vessel compared with non-bypassed vessel

	CTO supplied by grafted donor vessel n = 216	CTO supplied by ungrafted donor vessel n = 994	p-value
Age *(yrs)*	**74.0 ± 11.1**	**70.5 ± 12.3**	**<0.0001**
Female Sex *(n)*	31 (14.4%)	188 (18.9%)	0.11
BMI *(kg/m^2)*	27.6 ± 5.1	27.8 ± 5.4	0.53
Previous AMI *(n)*	**97 (48%)**	**320 (33.4%)**	**<0.0001**
Prior CABG *(n)*	**216 (100%)**	**36 (3.6%)**	**<0.0001**
Emergent Indication for angio *(n)*	**129 (59.7%)**	**518 (52.1%)**	**0.04**
Medications *(n)*			
Aspirin	**157 (78.1%)**	**806 (85.1%)**	**<0.05**
P2Y12 Inhibitor	119 (59.5%)	560 (59.1%)	0.92
Beta Blockers	134 (67.3%)	664 (70.1%)	0.44
ACE-I/ARB	**99 (49.5%)**	**584 (61.7%)**	**<0.001**
Nitrate	**86 (43.0%)**	**197 (20.8%)**	**<0.0001**
Statin	**182 (91.9%)**	**791 (83.5%)**	**<0.01**
Number of CTOs *(n)*			**<0.0001**
1	**20 (9.3%)**	**801 (80.5%)**	
2	**106 (49.1%)**	**176 (17.7%)**	
3	**90 (41.7%)**	**17 (9.7%)**	
CTO Vessel *(n)*			**<0.0001**
LAD	**17 (7.9%)**	**235 (23.6%)**	
LCx	**36 (16.7%)**	**209 (21.0%)**	
RCA	**163 (75.5%)**	**550 (55.3%)**	
LAD CTO *(n)*	**17 (7.9%)**	**235 (23.6%)**	**<0.0001**
CTO Vessel grafted *(n)*	**153 (70.8%)**	**22 (2.2%)**	**<0.0001**
CTO of stented vessel *(n)*	9 (4.2%)	56 (5.6%)	0.39
Donor Vessel *(n)*			**<0.0001**
LAD	**158 (73.1%)**	**525 (52.8%)**	
LCx	**38 (17.6%)**	**258 (26.0%)**	
RCA	**20 (9.3%)**	**211 (21.2%)**	
Stenosis in donor vessel (%)	**37.1 ± 23.6**	**50.8 ± 27.1**	**<0.0001**
Rentrop Grade			**<0.0001**
0 or 1	**136 (63%)**	**169 (17.0%)**	
2	**72 (33.3%)**	**606 (61.0%)**	
3	**8 (3.7%)**	**219 (22.0%)**	
Robust Collaterals *(n)*	**80 (37.0%)**	**825 (83.0%)**	**<0.0001**
CC grade			**<0.0001**
0	**42 (19.4%)**	**61 (6.1%)**	
1	**108 (50.0%)**	**236 (23.7%)**	
2	**66 (30.6%)**	**697 (70.1%)**	
Valvular heart disease *(n)*	27 (12.5%)	114 (13.3%)$^+$	0.75
LV impairment *(n)*	**129 (63.2%)**	**442 (46.4%)**	**<0.0001**
LVEF (%)	**45 (35 – 55)**	**55 (40 – 60)**	**<0.0001**

ACE-I = angiotensin converting enzyme inhibitor; AMI = acute myocardial infarction; ARB = angiotensin II receptor blocker; BMI = body mass index; CABG = coronary artery bypass graft; CC = collateral connection; CTO = chronic total occlusion; kg = kilogram; LAD = left anterior descending artery; LCx = left circumflex artery; LV = left ventricular; LVEF = left ventricular ejection fraction; m = metre; n = number; RCA = right coronary artery; yrs = years;

Figure 13.3: Degree of coronary collaterals perfusing a CTO from a grafted and ungrafted donor vessel



In CTOs supplied by a grafted donor, neither the graft type nor the site of anastomoses relative to the collaterals were associated with robustness of coronary collaterals. Similarly, there was no difference in the degree of stenosis in the donor vessel graft or donor vessel and robustness of coronary collaterals (Table 13.3)

Table 13.3: Baseline and Angiographic differences in patients with a CTO supplied by a grafted donor with robust or poor coronary collaterals.

	Robust Collaterals n=80	Poor Collaterals n=136	p-value
CTO Vessel *(n)*			0.47
LAD	4 (5.0%)	13 (9.6%)	
LCx	13 (16.3%)	23 (16.9%)	
RCA	63 (78.8%)	100 (73.5%)	
LAD CTO *(n)*	4 (5.0%)	13 (9.6%)	0.23
CTO of stented vessel *(n)*	2 (2.5%)	7 (5.1%)	0.36
Donor Vessel *(n)*			0.74
LAD	57 (71.3%)	101 (74.3%)	
LCx	14 (17.5%)	24 (17.6%)	
RCA	9 (11.3%)	11 (8.1%)	
Stenosis in Donor vessel graft *(%)*	10 (0 – 20)	5 (0 – 20)	0.92
Stenosis in donor vessel proximal to collaterals (%)	40 (20 – 50)	30 (22.5 – 50)	0.99
Graft Type *(n)*			0.38
LIMA/RIMA	52 (65.0%)	92 (67.6%)	
SVG/Radial	28 (35.0%)	44 (32.4%)	
Graft anastomoses relative to collaterals *(n)*			0.75
Proximal	53 (66.3%)	93 (68.4%)	
Distal	27 (33.8%)	43 (31.6%)	
Valvular Heart Disease *(n)*	13 (16.3%)	14 (10.3%)	0.20
LV impairment	54 (71.1%)	75 (58.6%)	0.07

CTO = chronic total occlusion; LAD = left anterior descending artery; LIMA = left internal mammary artery; LCx = left circumflex artery; LV = left ventricular; RCA = right coronary artery; RIMA = right internal mammary artery; SVG = saphenous vein graft

13.5 Discussion

CTOs supplied by a grafted donor vessel have significantly poorer and less mature collaterals than those with an ungrafted donor, irrespective of location of the graft relative to collaterals, or type of graft. Previous studies have suggested that in patients undergoing CTO PCI, the presence of a prior CABG is associated with lower procedural success rates, higher risk of in-hospital mortality and higher complication rates (13,14). Histopathological studies (15) have correlated bypass grafts with more extensive calcification and severe negative remodelling (16). Whilst the degree of calcification is associated with lower CTO PCI success (17), as is the presence of poorer coronary collaterals (18). This is attributable not only to the ability to

utilise collaterals as a retrograde option for intervention, but also reflecting better distal opacification and hence vessel visualisation. The finding of poorer collaterals may be a factor in the lower success rates of CTO PCI in patients with a prior CABG.

Extensive calcification is identified in both segments proximal and distal to the CTO, providing a histological explanation for the clinical finding of more rapid progression of atherosclerosis in grafted coronary arteries (19,20). It has been postulated that blood stasis and lower shear stress resulting from competitive flow between the native vessel and the bypass graft may be the underlying mechanism of greater calcification (19,21). These alterations in flow and shear stress are also associated with poor collateral maturation.

Coronary blood flow, particularly in the left coronary system is predominantly (>60%) diastolic due to the effect of systolic myocardial compressive forces reducing coronary driving pressure and maximally increasing coronary vascular resistance (22). However in certain situations, such as a non-dominant RCA, owing to the thin walled right ventricle and low systolic intra-cavitatory pressure, there is lower vascular resistance and greater systolic flow. Similarly, in the setting of a dyskinetic or hypokinetic segment, graft and coronary systolic blood flow can significantly increase, thereby affecting endothelial shear stress and the ability to recruit collaterals (22). Furthermore, canine studies suggest that, in the acute setting, diastolic flow through a LIMA anastomosed to the LAD is significantly lower than in the native coronary setting (23). Over time however, flow through the LIMA has a large diastolic component, characteristic of native coronary artery flow (24-26), with modulation from predominantly systolic flow proximally, to predominantly diastolic in the distal segment to match coronary vascular resistance (27). SVGs, however, act as passive conduits with diastolic flow throughout their length (27). Diastolic flow velocity in a LIMA graft is greater

and more sustained than in an SVG (28) and as a result wall shear stress is higher (27). The radial artery as a conduit is more susceptible to spasm (29), which may reflect its relative muscular structure and endothelial and smooth muscle cell response to platelet activation (29). These dynamic and varied perturbations to flow and shear stress likely impact on the ability to recruit and mature coronary collaterals.

Computational fluid dynamic modelling suggests that wall shear stress is significantly lower in bypass grafts compared with native coronary flow, with resultant downstream reduction in shear stress in the native circulation (30). In a doppler wire study (31), wall shear stress was greater in the LIMA as compared with the SVG, suggesting acceleration of atherosclerosis in the low flow state of a grafted coronary artery. This unfavourable low flow and decreased shear stress may also result in poorer collateral recruitment (6). Although the current study did not detect any difference in robustness of collaterals in patients with an SVG graft compared with a LIMA graft, this requires further assessment with larger numbers. Similarly, the effect of the graft location relative to collaterals did not affect robustness of collaterals. In the setting of an occluded donor vessel, all flow will be in a single direction originating from the graft. However, in the setting of persisting native flow in the donor vessel, there may be areas of competitive flow, which is associated with unfavourable wall shear stress, endothelial dysfunction and possible impairment of collateral recruitment (32).

In a previous study (33) of 217 patients, a prior CABG was associated with improved collaterals, with a significantly lower rate of non-interventional collaterals compared with those patients who had not undergone prior CABG. Furthermore, they found no difference in patients with an occluded graft compared to those without prior grafts with respect to degree of collaterals. Despite more robust collaterals, the presence of a previous CABG was

associated with a significantly lower rate of successful CTO PCI, although no differences in complication rates. Whilst the authors of that study did not quantify how they classified 'non-interventional collaterals', in the present study patients with a grafted donor vessel had lower CCS scores along with Rentrop grade. These differences may reflect a predilection to utilising an occluded graft itself for intervention to a native vessel CTO, thereby classifying a vessel as having 'non-interventional collaterals'. Instead, the Rentrop classification which relates to contrast opacification of the occluded vessel removes any inherent bias in consideration of a collateral for intervention.

13.5.1 Limitations

This is a single centre retrospective study which has limitations with respect to possible underlying bias, however the relatively large numbers allow hypothesis generating analysis of the data. The semi-quantitative method of collateral grading may have been influenced by degree of catheter engagement and duration of cine acquisition. However, robust collaterals are generally seen relatively early with a previous study suggesting that collaterals opacify the epicardial vessel in 20-30 frames (34). In the setting of a cine acquisition of 15 frames per second, which is standard in most catheter laboratories, this does not require prolonged injections and acquisition compared to usual care. However further assessment particularly to assess the impact of alterations in wall shear stress and flow dynamics is required to determine the impact of collateral maturation in patients with previous CABG and an ungrafted CTO. Finally, as angiograms prior to CABG were not reviewed, it is possible that some collaterals were pre-existing to the CABG. However, as collaterals are dynamic, with rapid regression and recruitment, the influence of flow alterations following grafting are more relevant.

13.6 Conclusion

In patients with a previous CABG, a donor vessel which is supplied by a bypass graft results in poorer coronary collaterals and less interventional coronary collaterals. This likely reflects alterations in coronary blood flow through the graft and native circulation with resultant modifications in endothelial shear stress. This may be a factor in the lower procedural success rates for CTO intervention in patients with prior CABG. Further research into the effect of alterations in coronary flow dynamics on collateral recruitment and maturation in the setting of CABG should be considered.

13.7 References

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SECTION VIII: ROLE OF OBSTRUCTIVE SLEEP APNOEA ON CORONARY COLLATERAL RECRUITMENT

Chapter 14: The Prognostic Impact of Obstructive Sleep Apnoea on patients presenting with ST Elevation Myocardial Infarction – The role of the Coronary Collateral Circulation

This chapter includes material presented at TCT Connect 2020; published as: Allahwala UK, Cistulli P, Ciofani JL, Dissanayake H, Ward M, Weaver J, Bhindi R. Influence of Obstructive Sleep Apnea on outcomes in patients with ST elevation myocardial infarction (STEMI) – the role of the coronary collateral circulation. *Journal of the American College of Cardiology*. 76(17): Suppl B 2020

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TCT CONNECT-43

Abstract Withdrawn

TCT CONNECT-44

Influence of Obstructive Sleep Apnea on Outcomes in Patients With ST-Segment Elevation Myocardial Infarction (STEMI): The Role of the Coronary Collateral Circulation

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BACKGROUND Obstructive sleep apnea (OSA) occurs frequently in patients with coronary artery disease, with associated intermittent hypoxia a possible stimulus for coronary collateral recruitment through ischemic pre-conditioning. It is uncertain whether OSA affects recruitment of coronary collaterals and prognosis of patients presenting with ST-segment elevation myocardial infarction (STEMI).

METHODS Patients with a STEMI undergoing percutaneous coronary intervention (PCI) from July 2010 to December 2019 were reviewed. Electronic medical records were accessed to determine documented patient history of OSA. Patients with robust collaterals were defined as Rentrop grade 2 or 3.

RESULTS In total, 1,863 patients were included, of which 143 (7.7%) patients had documented evidence of OSA in their health record. Patients with OSA had a higher body mass index (BMI) (30.2 kg/m^2 vs. 27 kg/m^2; $p < 0.0001$), greater rate of hypertension (61.1% vs. 45.1%; $p < 0.0001$), hypercholesterolemia (47.4% vs. 38.4%; $p < 0.05$), and diabetes mellitus (22.6% vs. 15.9%; $p < 0.05$). Patients with OSA were more likely to have robust coronary collaterals (35.7% vs. 20.2%; $p < 0.0001$). Patients with documented OSA had a lower rate of left ventricular (LV) impairment (50.7% vs. 63.1%; $p < 0.01$), a higher LV ejection fraction (50.3% vs. 46.7%; $p < 0.0001$), and a lower peak troponin-I level (26,452 ng/l vs. 39,469 ng/l; $p < 0.01$). There were no differences in rates of in-hospital or longer-term mortality in patients with OSA compared with those without.

CONCLUSION Patients with documented OSA presenting with STEMI appear to have more robust coronary collaterals observed on angiography, which likely mediates lower myocardial necrosis. Broader implications of this finding on treatment require further investigation.

CATEGORIES **CORONARY:** Acute Coronary Syndromes

TCT CONNECT-45

The Influence of Frailty and Multimorbidity on PCI Utilization Among Older Adults With Acute Myocardial Infarction

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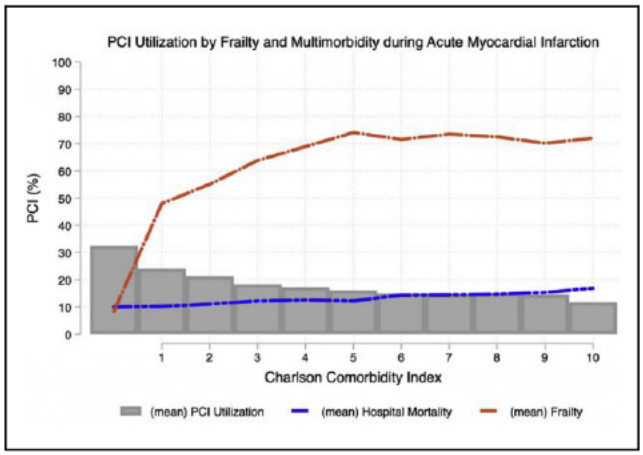
BACKGROUND The objective of this study was to examine the influence of frailty and multimorbidity on percutaneous coronary intervention (PCI) utilization rate among adults age 75 years or older during acute myocardial infarction (AMI) admission.

METHODS The Premier health care database was used to identify older adults with primary diagnoses of AMI. Individuals were classified as frail or not using the validated claims-based frailty index (CFI). Patients' characteristics were described and receipt of PCI stratified by frailty and Charlson comorbidity index.

RESULTS From 2000 to 2016, 501,581 encounters were identified for patients age ≥75 years admitted with AMI. The median age was 82 years (interquartile range: 77 to 88 years), 52% were women, 75% were Caucasian, and 4% were Hispanic. The prevalence of frailty was 18%, and the mean (SD) Charlson comorbidity index was 0.6 ± 1.4. With increasing comorbidities, the prevalence of frailty increases substantially (Figure). There is 58% (95% CI: 57% to 59%) lower adjusted odds of receiving PCI compared with the nonfrail group. Similarly, with a 1-U increase in Charlson comorbidity index, there is 9% (95% CI: 8% to 9%) lower adjusted odds of receiving PCI during AMI admission. Among older adults who received PCI, frailty increased adjusted risk of hospital mortality (adjusted OR: 1.51; 95% CI: 1.41 to 1.62) after adjusting for Charlson comorbidity index.



CONCLUSION In the United States, frailty and multimorbidity are common among older patients during index AMI hospitalization and the risk increases with age. From a public health perspective, research efforts should focus on screening for frailty and multimorbidity to improve patient selection and minimize complications after PCI.

CATEGORIES **CORONARY:** Acute Coronary Syndromes

TCT CONNECT-46

Trends and In-Hospital Outcomes of Percutaneous Coronary Intervention in Non–ST-Segment Elevation Myocardial Infarction and Prior Coronary Artery Bypass Graft

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BACKGROUND The data on percutaneous coronary intervention (PCI) in patients presenting with non-ST-segment elevation myocardial infarction (NSTEMI) and a prior coronary artery bypass graft (CABG) is sparse. This study sought to compare the trends and in-hospital outcomes of PCI with medical management in patients hospitalized for NSTEMI with prior CABG without cardiogenic shock.

METHODS International Classification of Diseases-Ninth and -Tenth Revision codes from the National Inpatient Sample (NIS) database between 2012 and 2017 were utilized to identify patients hospitalized for NSTEMI with a prior coronary artery bypass graft (CABG). Patients undergoing PCI were compared with those who did not after propensity score matching. The outcomes of interest were in-hospital complications and mortality.

RESULTS In total, 2,89,735 hospitalizations were identified: 71.4% did not have PCI and 28.6% got PCI. There was a steady increase in the number of PCI procedures from 24.2% in 2012 to 32.2% in 2017 (p trend <0.01). However, the trend for in-hospital mortality has steadily increased from 0.7% to 1.1% without reaching significance in the PCI group (p trend = 0.20) (Figure 1A). After propensity score matching, 82,770 hospitalizations were identified each for PCI and no-PCI groups. The risk of in-hospital mortality in the PCI group was lower (1% vs. 2.6%; $p < 0.001$). In-hospital complications and procedures were lower in the PCI compared with no-PCI group (Figure 1b). The duration and the cost of hospitalization was higher in the PCI group ($p < 0.01$).

14.1 Abstract

Background: Obstructive sleep apnoea (OSA) occurs frequently in patients with coronary artery disease, with associated intermittent hypoxia a possible stimulus for coronary collateral recruitment through ischaemic preconditioning. The aim of this study was to determine whether OSA effects recruitment of coronary collaterals and prognosis of patients presenting with ST elevation myocardial infarction (STEMI).

Methods: Patients with a STEMI undergoing percutaneous coronary intervention (PCI) from July 2010 to December 2019 were reviewed. Electronic medical records were accessed to determine documented patient history of OSA. Patients with robust collaterals were defined as Rentrop grade 2 or 3.

Results: 1,863 patients were included, of which 143 (7.7%) patients had documented evidence of OSA in their health record. Patients with OSA had a higher body mass index (BMI) (30.2kg/m2 vs 27 kg/m2, p <0.0001) and greater rates of hypertension (61.1% vs 45.1%, p<0.0001), hypercholesterolaemia (47.4% vs 38.4%, p<0.05) and diabetes mellitus (22.6% vs 15.9%, p<0.05). Patients with OSA were more likely to have robust coronary collaterals (35.7% vs 20.2%, p<0.0001), lower rate of left ventricular (LV) impairment (50.7% vs 63.1%, p<0.01), a higher LV ejection fraction (50.3% vs 46.7%, p<0.0001) and a lower peak troponin-I (26,452ng/L vs 39,469ng/L, p<0.01). There were no differences in rates of mortality in patients with OSA compared to those without.

Conclusions: Patients with documented OSA presenting with STEMI appear to have more robust coronary collaterals observed angiographically which likely mediates lower myocardial necrosis. Broader implications of this finding on treatment require investigation.

14.2 Introduction

Obstructive Sleep Apnoea (OSA) is a chronic, sleep-related breathing disorder characterised by periodic obstruction of the pharyngeal airway during sleep, resulting in repetitive apnoeas (1), affecting 34% of men and 17% of women. The incidence of OSA is rising, with a 30% increase in diagnoses noted over the last 20 years (2). OSA has been associated with a number of differing cardiovascular diseases, including systemic hypertension (3), pulmonary hypertension (4), heart failure (5) and atrial fibrillation (6). The key perturbations associated with OSA include intermittent hypoxia, frequent arousals from sleep, and exaggerated intrathoracic pressure swings, all of which have acute and chronic effects on haemodynamics and cardiovascular function.

The reported prevalence of OSA in patients with coronary artery disease (CAD) ranges between 38%-65%, significantly higher than in the general population (7). This association has been linked to an increase in sympathetic nervous system activity, oxidative stress, endothelial dysfunction, and poorly controlled hypertension (8). However, the effect of OSA on outcomes in patients presenting with ST elevation myocardial infarction (STEMI) is uncertain. In particular, given the episodic apnoeas and hypoxia associated with OSA, it is possible a degree of ischaemic preconditioning may be present, which, in experimental settings has been associated with a reduction in infarct size (9). This has been proposed as an explanation for the age decline mortality risk in OSA (10). Clinical studies lend support to this notion, by demonstrating a lower troponin rise in patients with OSA, suggesting that OSA has a protective effect in the context of myocardial infarction (11). The mechanism by which preconditioning may result in improved outcomes includes improved coronary collateral recruitment (12) and neurohormonal and cellular adaptation mechanisms (13).

The aim of this study was to determine the prevalence of acute coronary collaterals in patients with OSA presenting with STEMI undergoing primary percutaneous coronary intervention (pPCI) as well as to determine the impact of OSA on prognosis.

14.3 Methods

14.3.1 Patient Selection

All patients presenting to a tertiary centre with a diagnosis of STEMI, undergoing pPCI or rescue PCI following failed thrombolysis, from July 2010 until December 2019 were reviewed. Patients were included if they had a final diagnosis of STEMI using previously accepted definitions (14). All patients had pre-treatment with aspirin, unless they were unable to tolerate per oral medications, with a P2Y12 inhibitor administered either before or immediately following pPCI. The procedure was performed either through the femoral or radial artery based on operator discretion. Therapeutic intravenous heparin was administered at the beginning of the case, whilst use of vasodilators such as intracoronary nitrates prior to intervention was not routinely given, due to the emergent nature of the procedure. The use of either intracoronary or intravenous glycoprotein IIb/IIIa inhibitors was left to the discretion of the operating physician.

Patients who had had a prior coronary artery bypass graft (CABG) were excluded from the analysis to allow characterisation of native collaterals. Similarly patients in whom angiography of the contralateral vessel was not performed prior to pPCI were excluded. To ascertain whether patients had co-existent OSA, electronic medical records and records from the hospital's sleep investigation laboratory were reviewed to identify a documented history of OSA. Procedural characteristics, in-hospital course along with left ventricular function, and biochemical results were also reviewed. Left ventricular function was assessed by

transthoracic echocardiogram, or if not performed, ventriculography at the time of the procedure. Electronic medical records were also reviewed to determine date of death or last date of medical contact for survival analysis.

14.3.2 Angiographic Assessment

Analysis of coronary angiography was performed to determine the presence and maturity of collaterals, graded according to the Rentrop classification (15) and the Collateral Connection grade (CC) (16). Rentrop grade 0 or 1 were defined as poor collateral recruitment and Rentrop grade 2 or 3 were defined as robust collaterals (17). A chronic total occlusion (CTO) was defined based on accepted criteria (18). Pre-hospital arrest and ventricular arrhythmia during the case was defined as sustained ventricular arrhythmia with loss of cardiac output necessitating cardio-pulmonary resuscitation and/or defibrillation. Ischaemic time was the time between symptom onset and first angiographic image. Left ventricular impairment was defined as a left ventricular ejection fraction (LVEF) of ≤50%, assessed by transthoracic echocardiogram or ventriculography. Serum troponin levels were collected as per clinical indication, and the peak troponin as defined as the highest concentration during index admission.

14.3.3 Statistical Analysis

Continuous variables were presented as means (± standard deviation) in the setting of normally distributed data or as medians and interquartile ranges, if the distribution of data was not normal. Categorical variables were reported as percentages. Comparisons between groups were performed using Pearson's chi square test for all categorical variables and a student's T-Test for all continuous variables normally distributed or the Mann-Whitney U test for continuous data not distributed normally. Cumulative event rates were calculated

according to the Kaplan–Meier method and compared using the log-rank test. All tests were 2-sided, and a $p < 0.05$ was considered statistically significant. All analyses were performed using SPSS (version 24, IBM, New York, New York).

14.4 Results

14.4.1 Patient Demographics & Baseline Characteristics

A total of 2,012 patients presenting with STEMI were identified over the study period, with 102 excluded due to previous CABG, 21 due to no image acquisition of the contralateral artery prior to pPCI, 14 due to no culprit vessel identified and 12 which were facilitated PCIs. Of the remaining 1,863 patients, 143 (7.7%) had a documented history of OSA within their medical record, whilst 1,720 (92.3%) had no documented history of OSA. The mean age in the population was 64.9 years, with 77.1% males.

Baseline and angiographic differences are summarised in table 14.1. Patients with OSA had a higher body mass index (BMI) (30.2kg/m^2 vs 27 kg/m^2, $p < 0.0001$), greater rate of hypertension (61.1% vs 45.1%, $p<0.0001$), hypercholesterolaemia (47.4% vs 38.4%, $p<0.05$) and diabetes mellitus (22.6% vs 15.9%, $p<0.05$). At angiography, patients with OSA were more likely to have had a previous stent (17.7% vs 9.4%, $p<0.01$), and more likely to have robust coronary collaterals (35.7% vs 20.2%, $p<0.0001$) and higher grade collateral recruitment based on both Rentrop classification ($p<0.0001$) and the CC grade ($p<0.0001$). The right coronary artery (RCA) was more likely to be culprit vessel in those patients with OSA compared to those without (47.5% vs 37.3%, $p<0.05$). Those patients with OSA had a longer length of stented segment (33.0mm vs 29.8mm, $p<0.05$) (Table 14.2).

Table 14.1: Baseline Demographics and Angiography Findings

	OSA n= 143	**No Hx of OSA n= 1720**	**p-value**
Age *(yrs)*	63.3 (± 12.5)	65.0 (± 13.8)	0.11
Male *(n)*	117 (81.8%)	1319 (76.7%)	0.16
BMI *(kg/m²)*	**30.2 (± 6.3)**	**27.0 (± 4.6)**	**<0.0001**
Cardiovascular risk factors			
Hypertension *(n)*	**85 (61.1%)**	**722 (45.1%)**	**<0.0001**
Hypercholesterolaemia *(n)*	**65 (47.4%)**	**612 (38.4%)**	**<0.05**
Smoking *(n)*			0.43
Never	51 (45.5%	553 (39.7%)	
Previous	31 (27.7%)	400 (28.7%)	
Current	30 (26.8%)	440 (31.6%)	
Diabetes Mellitus *(n)*	**31 (22.6%)**	**247 (15.9%)**	**<0.05**
Family History of premature CAD *(n)*	39 (31.2%)	419 (28.9%)	0.59
Pre Hospital Cardiac Arrest *(n)*	15 (10.5%)	203 (11.8%)	0.64
Thrombolysis *(n)*	15 (10.6%)	207 (12.2%)	0.56
Prior Stent *(n)*	**25 (17.7%)**	**161 (9.4%)**	**<0.01**
Culprit Vessel *(n)*			**<0.05**
RCA	**68 (47.5%)**	**641 (37.3%)**	
LCx	**20 (14.0%)**	**249 (14.5%)**	
LAD	**55 (38.5%)**	**830 (48.2%)**	
TIMI Flow>1 at angiography *(n)*	48 (33.6%)	563 (32.7%)	0.84
Heart Rate *(bpm)*	83.1 (± 19.7)	79.6 (± 19.9)	0.90
SBP *(mmHg)*	124.6 (± 27.2)	122.5 (± 28.3)	0.36
Rentrop *(n)*			**<0.0001**
0	**73 (51.0%)**	**1136 (66.0%)**	
1	**19 (13.3%)**	**236 (13.7%)**	
2	**40 (28.0%)**	**308 (17.9%)**	
3	**11 (7.7%)**	**40 (2.3%)**	
Robust Collaterals *(n)*	**51 (35.7%)**	**348 (20.2%)**	**<0.0001**
CC Grade *(n)*			**<0.0001**
0	**93 (65.0%)**	**1328 (77.2%)**	
1	**19 (13.3%)**	**234 (13.6%)**	
2	**31 (21.7%)**	**158 (9.2%)**	
Stenosis in Donor Vessel *(%)*	44.6 (± 24.0)	42.5 (± 25.4)	0.26
CTO in remote vessel *(n)*	13 (9.1%)	103 (6.0%)	0.14
Ischaemic Time *(hours)*	8.3 (± 11.8)	8.0 (± 10.7)	0.55

BMI = body mass index; CAD = coronary artery disease; CC = collateral connection; CTO = chronic total occlusion; kg = kilograms; LAD = left anterior descending artery; LCx = left circumflex artery; m = metre; n = number; RCA = right coronary artery; SBP = systolic blood pressure; TIMI = thrombolysis in myocardial infarction; Yrs = Years;

Table 14.2: Percutaneous Coronary Intervention Characteristics

	OSA n= 143	**No Hx of OSA n= 1720**	**p-value**
pPCI performed *(n)*	136 (95.1%)	1578 (91.7%)	0.08
No. of stents *(n)*	1.2 (± 0.6)	1.1 (± 0.6)	0.16
Length of stents *(mm)*	**33.0 (± 17.3)**	**29.8 (± 16.0)**	**<0.05**
TIMI Flow Post PCI <3 *(n)*	8 (5.6%)	180 (10.4%)	0.06
GPIIbIIIa Inhibitor *(n)*	75 (53.6%)	927 (54.9%)	0.76
Inotrope use during intervention *(n)*	19 (13.4%)	250 (14.5%)	0.70
Ventricular Arrhythmia *(n)*	6 (4.2%)	118 (6.9%)	0.22
IABP/ECMO *(n)*	2 (1.7%)	42 (2.7%)	0.52
Non-IRA stented *(n)*	2 (2.0%)	97 (7.1%)	0.05

ECMO = extracorporeal membrane oxygenation; GPIIbIIIA = glycoprotein IIb/IIIa; IABP = intra-aortic balloon pump; IRA = infarct related artery; mm = millimetres; No. = number; PCI = percutaneous coronary intervention; pPCI = primary percutaneous coronary intervention; TIMI = thrombolysis in myocardial infarction;

14.4.2 Clinical Outcomes & Survival

With respect to in-hospital outcomes (Table 14.3), patients with a history of OSA had a lower rate of LV impairment (50.7% vs 63.1%, p<0.01) and a higher left ventricular ejection fraction (LVEF) (50.3% vs 46.7%, p<0.0001). Patients with OSA also had a lower peak troponin I level (26,452ng/L vs 39,469ng/L, p<0.01) however there were no differences in rates of in-hospital mortality or ICU admissions.

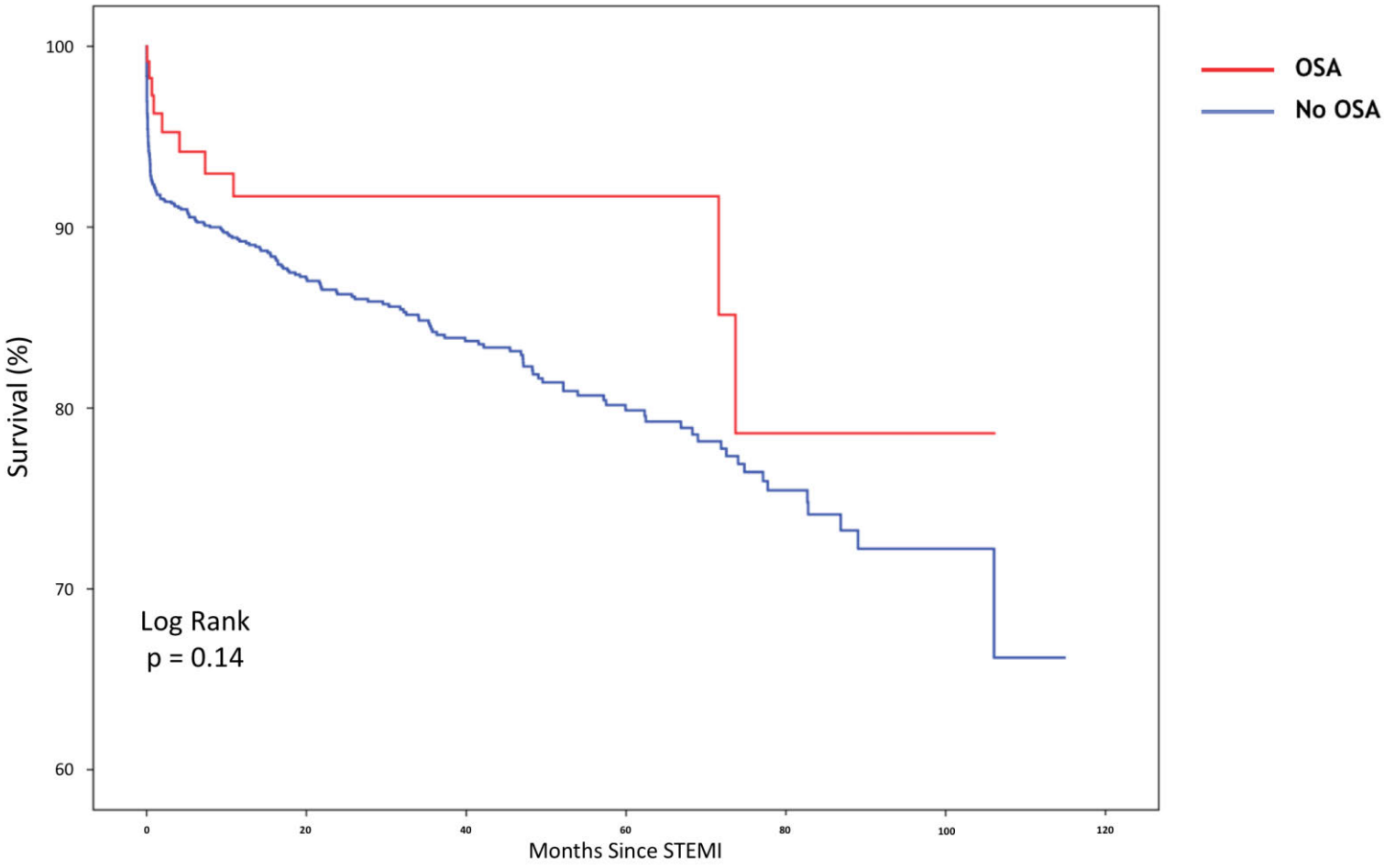
Table 14.3: In-Hospital Outcomes

	OSA n=143	**No Hx of OSA n= 1720**	**p-value**
LV impairment *(n)*	**70 (50.7%)**	**1044 (63.1%)**	**<0.01**
LVEF *(%)*	**50.3 (± 8.7)**	**46.7 (± 10.3)**	**<0.0001**
Troponin *(ng/L)*	**26,452.5 (11,291.2 – 94,793.7)**	**39,469 (8,747.7 – 53,434)**	**<0.01**
Mortality *(n)*	5 (3.5%)	124 (7.2%)	0.09
ICU admission *(n)*	23 (16.3%)	269 (15.9%)	0.89

ICU = intensive care unit; ng = nanograms; L = litre; LV = left ventricular; LVEF = left ventricular ejection fraction;

Kaplan-Meier survival analysis showed no difference in long term mortality between patients with OSA and those without, with a mean survival of 83 months vs 89.3 months (p=0.14) [Figure 14.1].

Figure 14.1: Kaplan Meier Curve showing survival in patients with and without OSA.



14.5 Discussion

A history of OSA in patients presenting with STEMI undergoing pPCI is associated with increased robustness of coronary collaterals. This results in a reduction in myocardial necrosis as evaluated by serum biomarker release and improved left ventricular function. The prevalence of OSA in the current population of patients with STEMI was 7.7%, which is significantly higher than in another study of such patients, which utilised in-patient medical records to identify a prevalence of 1.3% (19). However, the rates were lower than in other

forms of acute coronary syndrome (ACS) patients (20,21). This disparity may be attributable to differing rates of OSA between different forms of ACS, or perhaps more likely, due to differing rates based on inclusion criteria, between self-reporting, previously documented in medical records and polysomnographic confirmed diagnoses.

There is limited but conflicting data regarding the effects of OSA on coronary collaterals. The results of the current study are similar to a study of 71 patients admitted following STEMI who underwent polysomnography, finding those patients with higher apnoea–hypopnea index had more robust collaterals (22). However in another study of 86 patients presenting with acute myocardial infarction, there was no correlation between degree of collaterals and sleep apnoea hypopnoea syndrome (23). Both these studies were relatively small with the latter including patients of Chinese ethnicity only with a mean BMI of 25.6 kg/m^2 and a mean age of 52.4 years, which may reflect a differing specific population to those seen in other demographics. Furthermore, in the setting of a CTO (24), a previous study found that patients with OSA had greater rates of robust collaterals compared to those who did not (25). One proposed mechanism for the association between OSA and collaterals is that sleep disordered breathing may activate adaptive mechanisms that improve endothelial function (26).

Patients with OSA had a higher BMI (27) and were more likely to have a diagnosis of hypertension (28) and diabetes mellitus (29) than those patients who did not, an association which has been extensively described in the literature. This is unsurprising given the epidemiological relationship between OSA and metabolic syndrome as well as the growing literature suggesting a causal relationship mediated through inflammation, hypoxia and generation of reactive oxygen species (ROS).

Patients with OSA have significant nocturnal haemodynamic alterations, often in extremes, with changes in heart rate, systemic arterial blood pressure and cardiac function (30,31). Bradycardia and ventricular asystole are commonly associated with OSA (32) and are believed to be mediated through hypoxia induced autonomic nervous system activation. A lower resting heart rate has been associated with reduced cardiovascular mortality (33,34), including following acute coronary syndrome (35). Although a lower mortality rate was not identified in the present study, the beneficial effects on LV function may stem from this finding. Furthermore, bradycardia has been associated with better coronary collateral recruitment (36), which may be a result of increased coronary and capillary blood flow during diastole and resultant increased shear stress and tensile force on the immature collateral vessels. Given the significant relationship between patients with OSA and well developed collaterals in this study, this may be mediated through episodic bradycardia.

The role between hypoxia and coronary collaterals remains controversial, with hypoxia being the predominant mediator of angiogenesis, it is less well understood for its role in arteriogenesis, the maturation of pre-existing arteriolar connections into collaterals. Intermittent hypoxia can lead to oxidative stress by reducing reductive molecules during hypoxia and increasing ROS during reoxygenation, termed an ischaemia-reperfusion injury (37). In the setting of coronary revascularisation (and reperfusion), an influx of ROS results in cellular destruction, leading to stunning and necrosis of tissue mediated by the effects of ROS on cell death by lipid peroxidation, interruption of survival signalling pathways by protein modification, and DNA damage (38). Whilst ROS generation is believed to accelerate the development of cardiovascular disease (39), there is increasing evidence that their presence is necessary for coronary collateral growth with a so called “redox window” (40) whereby some degree of ROS is required for collateral growth, although a state of oxidative

stress is detrimental. Indeed, ischaemic pre-conditioning, whereby brief, intermittent ischaemia to an organ, renders it tolerant to subsequent ischaemia and reperfusion, is mediated, in part through promotion of collaterals (41). The strong finding of increased collaterals in patients with OSA may alternatively be mediated through intermittent hypoxia.

Previously studies have shown that the presence of robust collaterals is associated with improvement in left ventricular function (17). This protective effect of coronary collaterals on degree of myocardial damage is also manifested with a reduction in peak serum troponin, which is a marker of left ventricular function and expected recovery following revascularisation (42). In a smaller study of 127 patients admitted with an ACS, patients with OSA also had a significantly lower troponin than in patients without OSA although no comment on degree of collaterals was made (11).

14.5.1 Limitations

This is the largest study to date assessing the impact of OSA on outcomes in patients with STEMI, however being a retrospective single centre study has inherent limitations. Whilst formally documented apnoea-hypopnoea index to confirm the diagnosis of OSA was not performed, self-reported medical history from patients has been shown to be reliable (43). Nevertheless as not all patients were screened for OSA, it is probable that a number of patients with undiagnosed OSA were included in the study, and hence the observed prevalence of 7.7% is likely to be an underestimate. This makes the finding of greater collaterals in OSA patients conservative. Furthermore, the severity of OSA as well as whether patients were being treated with positive pressure therapy was not recorded. Given the known challenges of positive airway pressure in the real world many patients may be receiving suboptimal therapy. These confounders may have significant implications for

effect, and in particular the protective effect of collaterals may be lost with more severe or untreated OSA. These factors underline the importance of further prospective research into the cardiovascular implications of OSA following STEMI.

14.6 Conclusions

Patients with a history of OSA who present with STEMI appear to have more robust coronary collaterals diagnosed on angiography which likely facilitate in mediating lower myocardial necrosis as determined by higher left ventricular function and lower serum biomarker release. The mechanism of collateral recruitment is likely through hypoxia mediated endothelial activation, inflammation and reactive oxygen species formation. Further studies are required to determine the pathophysiological basis of this finding and to determine potential therapeutic and management implications for patients with OSA.

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Chapter 15: Obstructive Sleep Apnoea (OSA) in patients with a coronary chronic total occlusion (CTO) is associated with robust collaterals and reduced mortality

15.1 Abstract

Background: A chronic total occlusion (CTO) is frequently identified in patients undergoing coronary angiography. The prognostic implications of intermittent hypoxia from Obstructive Sleep Apnoea (OSA) on patients with a CTO, and effects on collateral recruitment, are unknown. The aim of this study was to determine the prevalence, vascular effects and prognostic implications of the presence of OSA in patients with a CTO.

Methods: Patients with an angiographically confirmed CTO between July 2010 and December 2019 were reviewed. Electronic medical records were accessed to determine documented history of OSA, demographics and clinical course. Patients with robust collateral recruitment were defined as Rentrop grade 2 or 3.

Results: 948 patients were included in the study, of which 127 (13.4%) had a documented history of OSA. OSA patients were younger (67.0 years vs 70.6 years, $p<0.01$), had a higher BMI (29.6kg/m^2 vs 26.7 kg/m^2, $p<0.0001$), higher rates of hypertension (91.3% vs 83.2%, $p<0.05$), higher rates of smokers (63.3% vs 49.0%, $p<0.01$) and more use of beta blockers (79% vs 68.5%, $p<0.05$) and statins (92.7% vs 82.1%, $p<0.01$). A documented history of OSA was independently associated with robust collaterals (OR: 3.0 95%CI: 1.5 – 5.8, $p<0.01$) and lower mortality (HR: 0.3 95% CI: 0.1 – 0.7, $p<0.01$) with a mean survival of 10.8 years, as compared to 8.1 years (log rank $p<0.0001$).

Conclusions: In patients with a CTO, documented OSA is independently associated with more robust coronary collaterals and lower mortality. The possible cardioprotective implications of intermittent hypoxia in OSA, as well as treatment effect requires further investigation.

15.2 Introduction

A coronary chronic total occlusion (CTO) (1) is identified in almost 7% of patients presenting with an acute coronary syndrome (2) and 18-52% of patients with stable coronary artery disease (CAD) (3-6). The precise aetiology and predictors of collateral recruitment and maturation, vital for the development of a CTO, remain uncertain (7,8). However, one of the possible mechanisms by which this may occur is through ischaemic preconditioning (9), whereby brief, intermittent ischaemia, renders tissue, either remote to, or at the site of, tolerant to subsequent ischaemia (10).

Obstructive sleep apnoea (OSA) is a chronic, sleep-related breathing disorder characterised by periodic obstruction of the pharyngeal airway during sleep, resulting in repetitive apnoeas (11). The prevalence of OSA in patients with CAD is 38-65% (12). The hallmarks of OSA, namely intermittent hypoxia, frequent arousals from sleep, and exaggerated intrathoracic pressure swings have acute and chronic effects on haemodynamics and cardiovascular function. Thus it is plausible, that the concurrent presence of OSA may be associated with development of, and in turn prognosis in, patients with a CTO. The aim of this study was to determine the prevalence of OSA in patients with an angiographically diagnosed CTO, as well as determine the prognostic implications of the presence or absence of OSA on outcomes in patients with a CTO. The secondary aim of the study was to determine the predictors of coronary collaterals in this population.

15.3 Methods

15.3.1 Patient Selection

All patients undergoing diagnostic coronary angiography at a tertiary centre from July 2010 to December 2019 were reviewed. Patients were identified through a commercially available reporting system on a local server (McKesson, Irving TX, USA). Patients who had had a prior coronary artery bypass graft (CABG) were excluded from the analysis to allow characterisation of native collaterals alone. Patients presenting with ST elevation myocardial infarction (STEMI), whereby acute recruitment of robust collaterals is associated with improved prognosis (13), were also excluded to focus on patients with chronically developed collaterals. To ascertain whether patients had a co-existent diagnosis of OSA, electronic medical records and records from the hospital's sleep investigation laboratory, were reviewed to identify a documented history of OSA. Procedural characteristics, in-hospital course along with left ventricular function, and biochemical results were also reviewed.

15.3.2 Angiographic & Collateral Assessment

The presence and degree of collaterals was graded according to the Rentrop (14), and Collateral Connection (CC) (15) grades. The donor vessel was defined as the epicardial vessel which provided the predominant collaterals to the occluded vessel. Emergent indication for coronary angiography was defined as acute coronary syndrome, including unstable angina and non ST elevation myocardial infarction or ventricular arrhythmia or cardiac arrest not fulfilling criteria for STEMI. Left ventricular impairment was defined as left ventricular ejection fraction (LVEF) ≤ 50% as determined by echocardiography, or if unavailable, by ventriculography during angiography. Valvular heart disease was defined as moderate or severe mitral or aortic valve disease as determined on echocardiography. CTO percutaneous coronary intervention (CTO PCI) technical success was defined as <30%

residual diameter stenosis within the treated segment and restoration of Thrombolysis in Myocardial Infarction (TIMI) grade 3 antegrade flow. Management of the CTO was based upon both intention to treat and 'as treated'. Project approval by the local human ethics committee was obtained prior to data analysis.

15.3.3 Statistical Analysis

Categorical variables were reported as percentages, whilst continuous variables were presented as means (± standard deviation) or as medians and interquartile ranges, depending on distribution of data. Comparisons between groups were performed using Pearson's chi square test for all categorical variables. Continuous variables were firstly assessed by the Shapiro-Wilk test to ascertain normality of distribution, after which, a student's T-Test was used for normally distributed data, while the Mann-Whitney U test was used for continuous data not distributed normally. Multivariate logistic regression analyses were performed to determine variables associated with the recruitment of robust collaterals, which was built by forward linear regression. Variables included in the model were those which had a correlation on univariate analysis with robust collateral recruitment, with entry and exit criteria of variables included in the model set at $p<0.01$. Cox regression analysis was performed to determine the independent predictors of mortality with entry set at $p<0.05$ and removal at $p<0.1$ from the model. All tests were 2-sided, and a $p < 0.05$ was considered statistically significant. Analyses were performed using SPSS (version 24, IBM, New York, New York).

15.4 Results

15.4.1 Patient Demographics & Baseline Characteristics

A total of 948 patients with a CTO were identified over the study period, of which 127 (13.4%) had a documented history of OSA within their medical records, while 821 (86.6%)

patients had no documented history of OSA. The mean age was 70.2 years (± 12.3) with 773 (81.5%) males. The indication for angiography was stable angina in 458 (48.3%) with the remaining 490 patients having emergent indications. The CTO vessel was the left anterior descending artery (LAD) in 224 (23.6%) patients, the left circumflex artery (LCx) in 197 (20.8%) patients and the right coronary artery (RCA) in 527 (55.6%) patients. Two hundred and twenty patients (23.2%) had an attempt at CTO percutaneous coronary intervention (PCI), of which 185 (84.1%) achieved technical success. 291 patients (30.7%) underwent coronary artery bypass grafting, whilst 472 (48.8%) underwent medical management to the CTO, including PCI to a non-CTO lesion.

Baseline and angiographic differences between patients with documented OSA and those without OSA are summarised in table 15.1. Patients with OSA were younger (67.0 years vs 70.6 years, $p<0.01$), had a higher BMI (29.6kg/m^2 vs 26.7kg/m^2, $p<0.0001$), higher rates of hypertension (91.3% vs 83.2%, $p<0.05$) and higher rates of smokers (63.3% vs 49.0%, $p<0.01$) compared to those without a history of OSA. With respect to medications at the time of angiography, patients with a documented history of OSA were more likely to be prescribed beta blockers (79% vs 68.5%, $p<0.05$) and statins (92.7% vs 82.1%, $p<0.01$).

Table 15.1 Baseline Characteristics in patients with and without a documented history of OSA

Variable	OSA n = 127	No OSA n = 821	p-value
Age *(yrs)*	**67 (± 14.1)**	**70.6 (± 12.0)**	**<0.01**
Male Sex	108 (85%)	665 (81.0%)	0.27
BMI *(kg/m^2)*	**29.6 (26.2 – 33.6)**	**26.7 (24.0 – 29.9)**	**<0.0001**
Risk Factors			
Hypertension	**116 (91.3%)**	**672 (83.2%)**	**<0.05**
Hypercholesterolaemia	111 (87.4%)	647 (80.7%)	0.07
Smoking History			
Never	**44 (36.7%)**	**385 (51.0%)**	**<0.01**
Ex-Smoker	**59 (49.2%)**	**260 (34.4%)**	
Current	**17 (14.2%)**	**110 (14.6%)**	
Diabetes Mellitus	49 (38.9%)	277 (35.0%)	0.39
Previous AMI	49 (39.5%)	256 (32.5%)	0.12
Medications			
Aspirin	109 (87.9%)	670 (86%)	0.57
P2Y12 Inhibitor	78 (62.9%)	458 (58.8%)	0.39
Beta Blockers	**98 (79.0%)**	**534 (68.5%)**	**<0.05**
ACE-I/ARB	76 (61.3%)	488 (62.6%)	0.77
Nitrates	30 (24.2%)	145 (18.6%)	0.14
Statin	**115 (92.7%)**	**640 (82.1%)**	**<0.01**
LVEF *(%)*	55 (40 – 55)	55 (40 – 60)	0.93
LV Impairment	59 (47.6%)	363 (46.1%)	0.76
Valvular Heart Disease	10 (9.3%)	96 (13.7%)	0.20
Emergent Indication for angiogram	58 (45.7%)	432 (52.6%)	0.14
Number of CTOs			0.49
1	103 (81.1%)	677 (82.5%)	
2	21 (16.5%)	135 (16.4%)	
3	3 (2.4%)	9 (1.1%)	
CTO Vessel			0.12
LAD	26 (20.5%)	198 (24.1%)	
LCx	20 (15.7%)	177 (21.6%)	
RCA	81 (63.8%)	446 (54.3%)	
CTO of stented vessel	11 (8.7%)	41 (5.0%)	0.09
Stenosis in Donor Vessel *(%)*	50 (30 - 72.5)	50 (30 - 75)	0.81
Rentrop			**<0.001**
0/1	**11 (8.7%)**	**154 (18.8%)**	
2	**75 (59%)**	**500 (60.9%)**	
3	**41 (32.3%)**	**167 (20.3%)**	
Collateral Connection Score (CCS)			0.46
0	6 (4.7%)	55 (6.7%)	
1	27 (21.3%)	201 (24.5%)	
2	94 (74.0%)	565 (68.8%)	

**ACE-I = angiotensin converting enzyme inhibitor; ARB = angiotensin II receptor blocker AMI = acute myocardial infarction; BMI = body mass index; CTO = chronic total occlusion; LAD = left anterior descending artery; LCx = left circumflex artery; LV = left ventricular, LVEF = left ventricular ejection fraction; OSA = obstructive sleep apnoea; RCA = right coronary artery.*

15.4.2 Angiographic Data & Management

Patients with a documented history of OSA had a significantly higher rate of robust (Rentrop grade 2 or 3) collaterals (91.3% vs 81.2%, p<0.001) compared to those without a documented history of OSA. There were no differences in the CCS (p=0.46) or management strategies with respect to intention to revascularise the CTO (p=0.10), successful CTO revascularisation (p=0.23) or final management of the CTO (p=0.49) (Table 15.2).

Table 15.2: Management of patients with a CTO stratified by history of OSA.

	OSA n = 127	**No OSA n = 821**	**p-value**
CTO Revascularisation (ITT)	77 (60.6%)	434 (52.9%)	0.10
Successful CTO Revascularisation (PCI or CABG)	70 (55.1%)	406 (49.4%)	0.23
Management			0.66
CTO PCI	27 (21.2%)	158 (19.2%)	
CABG	43 (33.9%)	248 (30.2%)	
Medical Management	36 (28.3%)	246 (30.0%)	
PCI to Non Donor Vessel	9 (7.1%)	90 (11.0%)	
PCI to Donor Vessel	12 (9.4%)	79 (9.6%)	
Final Management of CTO			0.49
CTO PCI	27 (21.3%)	158 (19.2%)	
CABG	43 (33.9%)	248 (30.2%)	
Medical Management	57 (44.9%)	415 (50.5%)	

**CABG = coronary artery bypass grafting; CTO = chronic total occlusion; ITT = intention to treat; PCI = percutaneous coronary intervention;*

Multivariate analysis was performed to determine the independent predictors of robust collaterals, with variables which had a correlation on univariate analysis of p<0.10 included in the model, namely; a documented history of OSA, beta blocker therapy, a history of hypertension and BMI. The independent predictors or robust collaterals were a documented history of OSA (OR: 3.0 95%CI: 1.5 – 5.8, p<0.01), whilst a history of hypertension (OR: 0.4 95%CI: 0.2 – 0.7, p<0.01) and beta blockers (OR: 0.5 95%CI: 0.3 – 0.8, p<0.01) were associated with poorer collaterals (Table 15.3).

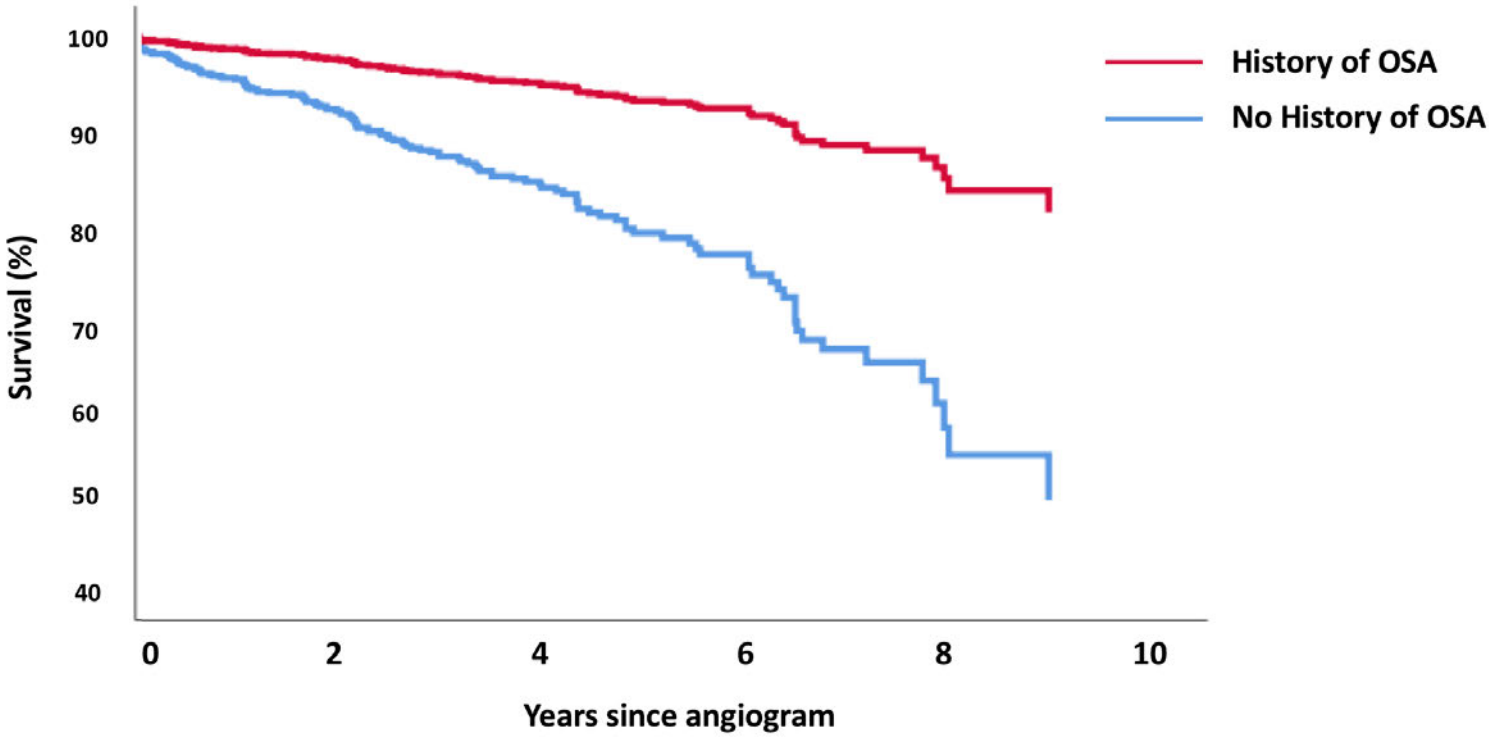
Table 15.3: Independent predictors of Robust Collaterals

Variable	Odds Ratio	95% CI	p-value
History of OSA	3.0	1.5 – 5.8	<0.01
Beta Blockers	0.5	0.3 – 0.8	<0.01
Hypertension	0.4	0.2 – 0.7	<0.01

15.4.3 Clinical Outcomes & Morality

Cox regression analysis was performed to determine the independent predictors of mortality. The variable included in the model were; age, history of OSA, male sex, multiple CTOs, Rentrop grade, history of diabetes mellitus, body mass index (BMI), indication for diagnostic angiography, CTO revascularisaton and left ventricular impairment. The independent predictors of mortality were older age (HR: 1.6 for every 10 years of age 95%CI: 1.4 – 1.9, p<0.0001), a history of diabetes mellitus (HR: 1.8, 95%CI: 1.2 – 2.5, p<0.01) and left ventricular impairment (HR: 2.3, 95% CI: 1.6 – 3.4, p<0.0001). Meanwhile, a documented history of OSA was associated with a significant reduction in mortality (HR: 0.3, 95% CI: 0.1 – 0.7, p<0.01), as was CTO revascularisaton (HR: 0.5, 95%CI: 0.4 – 0.8, p<0.01). Patients with a documented history of OSA had a mean survival of 10.8 years, as compared to 8.1 years for patients without a documented history of OSA (p<0.01) [Figure 15.1].

Figure 15.1: Survival in patients with and without documented OSA following diagnosis of a CTO on coronary angiography



15.5 Discussion

In patients with a CTO, a documented history of OSA was identified in 14.1% of patients, and was associated with robust coronary collaterals and lower mortality. The prevalence of OSA in patients with stable coronary disease is likely underreported, with a cross-sectional study of 772 patients finding OSA in 38.9% of these patients (16). Whilst this was higher than in the present study, this may reflect differing populations of all-comers with CAD as compared to only patients with a CTO as in the current study. Alternatively, this may be due to the methods of diagnosis of OSA, whereby the former study performed polysomnography in all patients, the current study utilized medical record linkage to determine the diagnosis of OSA.

There is increasing evidence that OSA is associated with development of CAD, as a result of increased oxidative stress, poorly controlled hypertension, increased sympathetic nervous system activity and endothelial dysfunction (17). Furthermore, in patients with stable CAD undergoing PCI, the presence of OSA is associated with poorer prognosis (18). However, in the setting of a CTO, a diagnosis of OSA was independently associated with robust coronary collaterals. These findings are similar to a previous small study of 34 patients (19) with a CTO, of whom 15 had polysmographically confirmed sleep apnoea (Apnoea-Hypopnoea index >10/hr), whereby patients with OSA were alo found to have more robust coronary collaterals. This cardioprotective mechanism may be driven by intermittent hypoxia, the hallmark of OSA, resulting in upregulation of transcription factors associated with collateral development, including vascular endothelial growth factor (VEGF) (20,21) and hypoxia inducible factor 1α (HIF-1α) (21). Meanwhile the complex interplay between OSA and generation of reactive oxygen species (ROS) (22), and the so-called “redox window”, whereby an equilibrium of oxidative and reductive factors are necessary for the maturation of collaterals is also likely implicated in this association (8).

Patients with a CTO and concurrent diagnosis of OSA were younger, had a higher BMI and were more likely to have a history of hypertension and prior or current smoking history. Despite the close relationship between OSA and development of systemic hypertension (23), patients with a documented history of hypertension are less likely to have robust collaterals (24), related to endothelial dysfunction (25). It is possible that the putative advantage of intermittent hypoxia may override any disadvantage of systemic hypertension on collateral maturation in patients with OSA. A documented history of OSA was associated with greater usage of beta blocker therapy, which may be attributed to higher rates of atrial fibrillation (26), although this was not assessed in the current study. A lower resting heart rate is

associated with more robust collaterals, possibly due to prolonged diastolic time and promotion of endothelial shear stress (27,28), although whether exogenous beta blockers have similar effects is controversial (29). Furthermore, patients with OSA were more likely to be taking statins, which has also been independently associated with more robust collaterals (30).

The presence of a documented history of OSA was independently associated with 70% lower risk mortality in patients with a CTO, irrespective of revascularisation strategy, age or left ventricular dysfunction. This protective effect of OSA with respect to developing collaterals, may explain the paradoxical finding of a survival advantage in older patients with OSA (31), who may have developed protective collaterals. It is possible that intermittent hypoxia and resultant ischaemic preconditioning is protective against future cardiovascular events. This is particularly relevant, as any potential advantage of OSA with respect to mortality cannot simply be explained by demographic variations. Whilst the presence of a CTO has been associated with poorer prognosis, including mortality, recent data from randomised trials have not shown an improvement in mortality with percutaneous revascularisation (32). Non-randomised trials have consistently shown that compared to either medical management, or failed CTO PCI, revascularisation is associated with a significant improvement in survival (33-35). Future studies assessing the implications of a concurrent diagnosis of OSA in patients with CTO and, indeed, stable coronary disease should be considered. Furthermore, the effect of treatment of OSA should also be prospectively studied to ascertain whether the apparent protective effect of OSA remains.

15.5.1 Limitations

This is the largest study of patients with a CTO to assess the prognostic implications of a concurrent diagnosis of OSA, however given this is a single centre retrospective study, there are inherent limitations. Most importantly, OSA diagnosis was based on medical records, which, although reliable (36), presumably under-estimates the prevalence of OSA. Furthermore, the impact of severity or treatment on patients with OSA was not determined. These confounders may have significant implications for effect, and in particular the protective effect of collaterals may be lost with more severe or untreated OSA. These factors underline the importance of further prospective research into the cardiovascular implications of OSA in patients with stable coronary disease and CTOs.

15.6 Conclusion

In patients with a CTO, a concurrent documented diagnosis of OSA is independently associated with more robust coronary collaterals, and subsequently independently associated with reduced mortality. The cellular processes and mechanism by which collaterals are recruited and matured require further investigations, and may include hypoxia mediated endothelial activation, reactive oxygen species formation and upregulation of transcription factors implicated in collateral recruitment. The apparent protective effect of OSA on mortality in patients with CTO also requires further investigation to identify the underlying mechanisms of an apparent “sleep apnoea collateral paradox”. Further studies should be performed to ascertain the pathophysiological basis of these findings and to determine therapeutic and management implications for patients with OSA.

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Chapter 16: The Severity of Obstructive Sleep Apnoea (OSA) Impacts on Coronary Collateral Recruitment during Coronary Occlusion

16.1 Abstract

Background & Aims: Obstructive sleep apnoea (OSA) and its resultant hypoxia and downstream tissue ischaemia along with effects on coronary blood flow, may affect the ability to recruit coronary collaterals. The aim of this study was to determine whether the severity as determined by plethysmography, of OSA affects collateral recruitment and maturation in patients with total coronary occlusions.

Methods: All patients with total coronary artery occlusion, either in the setting of an ST elevation myocardial infarction (STEMI) or chronic total occlusion (CTO) were reviewed. Subsequently, records from the sleep investigation laboratory were reviewed to identify those patients who had undergone diagnostic polysomnography. Robust coronary collaterals were defined as those with Rentrop grade 2 or 3 angiographic collaterals.

Results: Sixty four patients with a total coronary occlusion had diagnostic polysomnography performed, of whom 60 patients had OSA. Thirty two patients (53.3%) has poor collaterals while 28 (46.7%) had robust collaterals. Twenty four (40%) patients had mild OSA, 10 (16.7%) were diagnosed as moderate OSA and 26 (43.3%) patients had severe OSA.

Patients with robust collaterals were more likely to be males (96.4% vs 74.3%, $p<0.05$), more likely to have a history of hypercholesterolaemia (88.9% vs 51.6%, $p<0.01$) and be currently taking a statin (70.4% vs 32.3%, $p<0.01$). Patients with robust collaterals had a lower apnoea-hypopnoea index (AHI) (13.6 vs 45.5, $p<0.05$), a higher MinS_aO_2 (85.4% vs 79.8%, $p<0.05$), less time S_aO_2 <90% (0mins vs 30.4mins, $p<0.05$) and lower oxygen desaturation index (6.9 vs 26.8, $p<0.05$).

Conclusion: The presence of more severe OSA is associated with poorer coronary collateral recruitment in patients with total coronary artery occlusion. This process likely stems from direct effects of hypoxia and ischaemia as well as alterations in coronary blood flow and endothelial function. The effect of treatment of OSA on subsequent ability to recruit collaterals and other cardioprotective mechanisms requires further research.

16.2 Introduction

The coronary collateral circulation is a rich anastomotic network of primitive vessels, which have the ability to augment in size, connecting the territory supplied by one epicardial coronary artery with that supplied by another. Although ubiquitous in all species to varying degrees (1), the processes by which collaterals mature and function, remain uncertain (2). The proposed mechanisms by which coronary collaterals are recruited include hypoxia and resultant downstream tissue ischaemia, as well as changes in haemodynamic load and vascular shear stress. Obstructive sleep apnoea (OSA) is a chronic, sleep-related breathing disorder characterised by periodic airway obstruction during sleep, resulting in repetitive apnoeas (3). The key clinical manifestations of OSA include intermittent hypoxia, frequent arousals from sleep, and exaggerated alterations in intrathoracic pressure, which have effects on haemodynamics and cardiovascular function. OSA has been associated with a variety of cardiovascular diseases, including systemic and pulmonary hypertension (4,5), coronary artery disease, heart failure (6), and atrial fibrillation (7). Whether the severity of OSA is associated with the presence, recruitment and function of coronary collaterals, however, has not been investigated previously.

The aim of this study was to determine whether the presence of polysomnographically defined OSA was associated with visible coronary collaterals in patients presenting with acute coronary syndromes, as well as those with a CTO. Furthermore the aim of the study was to characterise polysomnographic metrics which correlate with the degree of coronary collateral recruitment.

16.3 Methods

16.3.1 Patient Selection

All patients diagnosed with a total coronary artery occlusion on angiography presenting to a tertiary centre between 2010 to 2019 were reviewed. This included patients with a STEMI as well as those with a CTO (8). Patients were identified through a commercially available angiography reporting system on a local server (McKesson, Irving TX, USA). Patients with a previous history of coronary artery bypass graft (CABG) or those without an angiographic acquisition of the contralateral artery prior to percutaneous coronary intervention (PCI) were excluded. This was to allow accurate quantification of native collaterals arising from the contralateral vessel. Subsequently, records from the hospital's sleep investigation laboratory were reviewed to identify those patients, who had undergone diagnostic polysomnography.

16.3.2 Polysomnography

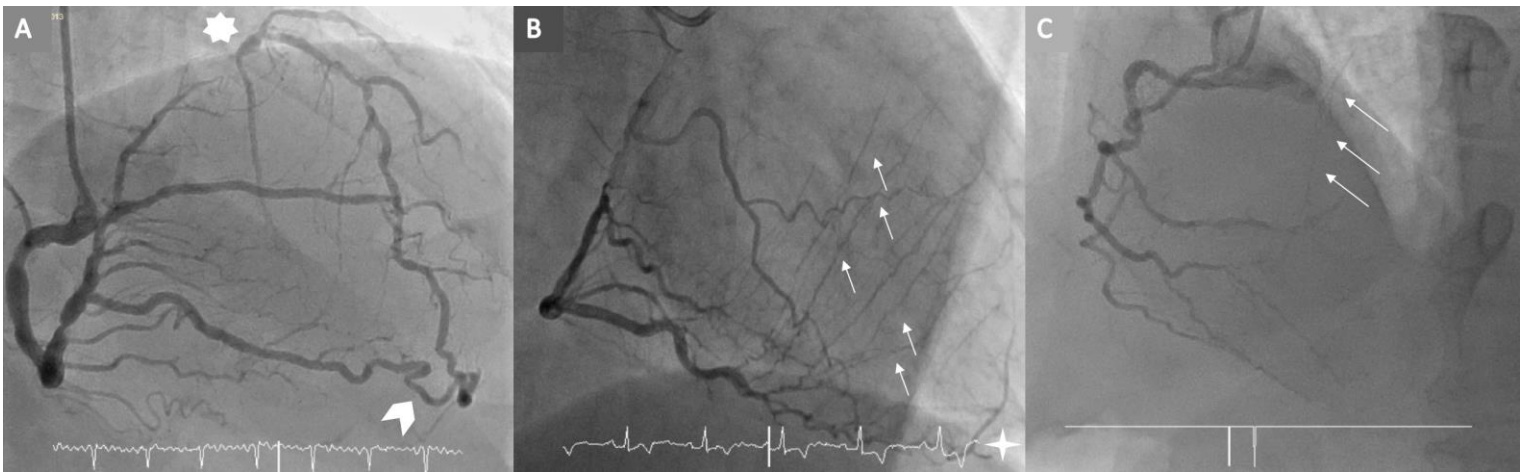
Standard nocturnal polysomnography was performed for a clinical indication, with electroencephalogram (EEG), electrooculogram, and submental electromyogram (EMG) electrodes applied for sleep stage determination as has been previously described (9). Respiratory variables included chest wall and abdominal movement, diaphragm EMG, nasal airflow and pressure, and oxygen saturation by pulse oximetry. Calculated respiratory variables were apnoea-hypopnea index - AHI (number of apnoeas and hypopneas per hour of sleep), longest apnoea, mean apnoea-hypopnoea duration, lowest oxyhaemoglobin saturation ($MinS_aO_2$), oxygen desaturation index - ODI (calculated as the number of times the SaO_2 dropped by ≥4% per hour), and time spent below oxygen saturation of 90%. *Apnoea* was defined as cessation of airflow for at least 10 seconds with oxygen desaturation of more than 3% and/or associated with arousal. *Hypopnoea* was defined as a reduction in amplitude of

airflow or thoracoabdominal wall movement of greater than 50% of the baseline measurement for more than 10 seconds with an accompanying oxygen desaturation of at least 3% (no time limit), and/or associated with arousal. These events were considered obstructive if they occurred in association with continued diaphragm EMG activity and thoracoabdominal wall movement. All variables were recorded continuously on a 20-channel computerized sleep monitoring system (Compumedics, Vic, Australia). Sleep recordings were scored in 30 second epochs by an experienced polysomnographer. An AHI <5 was considered normal, while OSA was characterised as mild if the AHI was 5-15/hr, moderate if the AHI was 15-30/hr, and severe if the AHI>30/hr.

16.3.3 Angiographic Assessment

Analysis of coronary angiography was performed to determine the presence and maturity of collaterals, graded according to the Rentrop classification (10) [figure 16.1]. Rentrop grade 0 or 1 collaterals were considered poor, while Rentrop grade 2 or 3 were considered robust. Left ventricular impairment was defined as a left ventricular ejection fraction (LVEF) of ≤50%, assessed by transthoracic echocardiogram or if unavailable, ventriculography.

Figure 16.1: Rentrop collateral grading of an occluded left anterior descending artery.



A. Rentrop grade 3 – Contrast injection of the right coronary artery (RCA) results in contrast opacification of the left anterior descending artery (LAD) via a large epicardial collateral (chevron) resulting in complete filling of the occluded LAD and its branches to the ostium (star). B. Rentrop grade 2 – Contrast injection of the RCA resulting in contrast opacification of septal collaterals (arrows) resulting in partial filling of the occluded LAD (4 point star). C. Rentrop grade 1- Contrast injection of the RCA results in filling of the side branch of the LAD, the septal artery (arrows) but no opacification of the occluded LAD.

16.3.4 Statistical Analysis

Categorical variables were reported as percentages, whilst continuous variables were presented as means (± standard deviation) for normally distributed data or as medians and interquartile ranges for those in whom data was not normally distributed. Comparisons between groups were performed using Fisher's exact test for all categorical variables. Continuous variables were firstly assessed by the Shapiro-Wilk test to ascertain normality of distribution, after which, a student's T-Test was used for normally distributed data, while the Mann-Whitney U test was used for continuous data not distributed normally. All tests were 2-sided, and a $p < 0.05$ was considered statistically significant. Analyses were performed using SPSS (version 24, IBM, New York, New York).

16.4 Results

16.4.1 Patient Demographics & Baseline Characteristics

Between 2010 to 2018, 64 patients with an occluded coronary artery on angiography had diagnostic polysomnography performed, of which 60 patients had a confirmed diagnosis of OSA. Polysomnography was performed a median 2.4 years (IQR: 0.7 – 5.6 years) prior to date of angiography. Twenty patients (33.3%) had a diagnosis of CTO, while 40 patients (66.6%) had a STEMI. Of the included population, 32 (53.3%) has poor collaterals while 28 (46.7%) had robust collaterals. The mean age was 60.7 (± 13.9) with 52 (86.7%) males. Twenty four (40%) patients had mild OSA, 10 (16.7%) were diagnosed as moderate OSA and 26 (43.3%) patients had severe OSA.

Patients with robust collaterals were more likely to be males (96.4% vs 74.3%, $p<0.05$), have a lower body mass index (BMI) (30.0 kg/m^2 vs 27.8 kg/m^2, $p<0.05$), more likely to have a history of hypercholesterolaemia (88.9% vs 51.6%, $p<0.01$) and be currently taking a statin (70.4% vs 32.3%, $p<0.01$) or beta blocker (53.6% vs 25.8%, $p<0.05$), but less likely to be taking a P2Y12 inhibitor (64.3% vs 96.8%, $p<0.01$). Patients with robust collaterals were more likely to present with a CTO (60.7% vs 9.4%, $p<0.0001$) than those with poor collaterals. There was no difference in age (62.8 vs 59.2, $p=0.68$), left ventricular function (50% vs 55%, $p=0.41$) or occluded vessel ($p=0.58$) (Table 16.1).

Table 16.1: Baseline characteristics in patients with poor and robust collaterals

	Poor Collaterals n = 32	Robust Collaterals n = 28	p-value
Age *(yrs)*	62.8 +/- 11.7	59.2 +/- 16.5	0.34
Male Gender *(n)*	**25 (78.1%)**	**27 (96.4%)**	**<0.05**
Body Mass Index (kg/m^2)	**30 (27.5 - 35)**	**27.8 (23.6 – 30.5)**	**<0.05**
Cardiovascular Risk Factors			
Hypertension	22 (68.7%)	24 (85.7%)	0.12
Hypercholesterolaemia	**16 (51.6%)**	**24 (88.9%)**	**<0.01**
Smoking History			0.56
Never	15 (55.6%)	15 (60%)	
Ex-Smoker	8 (29.6%)	8 (32%)	
Current	4 (14.8%)	2 (8%)	
Diabetes Mellitus	10 (32.2%)	6 (23.1%)	0.44
Medications			
Aspirin	32 (100%)	28 (100%)	N/A
P2Y12 Inhibitor	**30 (96.8%)**	**18 (64.3%)**	**<0.01**
Beta Blockers	**8 (25.8%)**	**15 (53.6%)**	**<0.05**
ACE-I/ARB	12 (38.7%)	16 (59.3%)	0.12
Nitrate	1 (3.2%)	2 (7.4%)	0.47
Statin	**10 (32.3%)**	**19 (70.4%)**	**<0.01**
Stenosis in donor vessel	**50 (35-70)**	**40 (30-70)**	**<0.0001**
LVEF (%)	50 (44.4 – 60)	55 (40-60)	0.41
LV Impairment *(n)*	16 (42.9%)	11 (39.3%)	0.18
Occluded Vessel *(n)*			0.58
LAD	15 (46.9%)	10 (35.7%)	
LCx	3 (9.4%)	2 (7.1%)	
RCA	14 (43.7%)	16 (57.1%)	
Presentation *(n)*			**<0.0001**
CTO	**3 (9.4%)**	**17 (60.7%)**	
STEMI	**29 (90.6%)**	**11 (39.3%)**	

** ACE-I = angiotensin converting enzyme inhibitor; ARB = angiotensin receptor blocker; CTO = chronic total occlusion; OSA = obstructive sleep apnoea; STEMI = ST elevation myocardial infarction;*

16.4.2 Polysomnographic Data

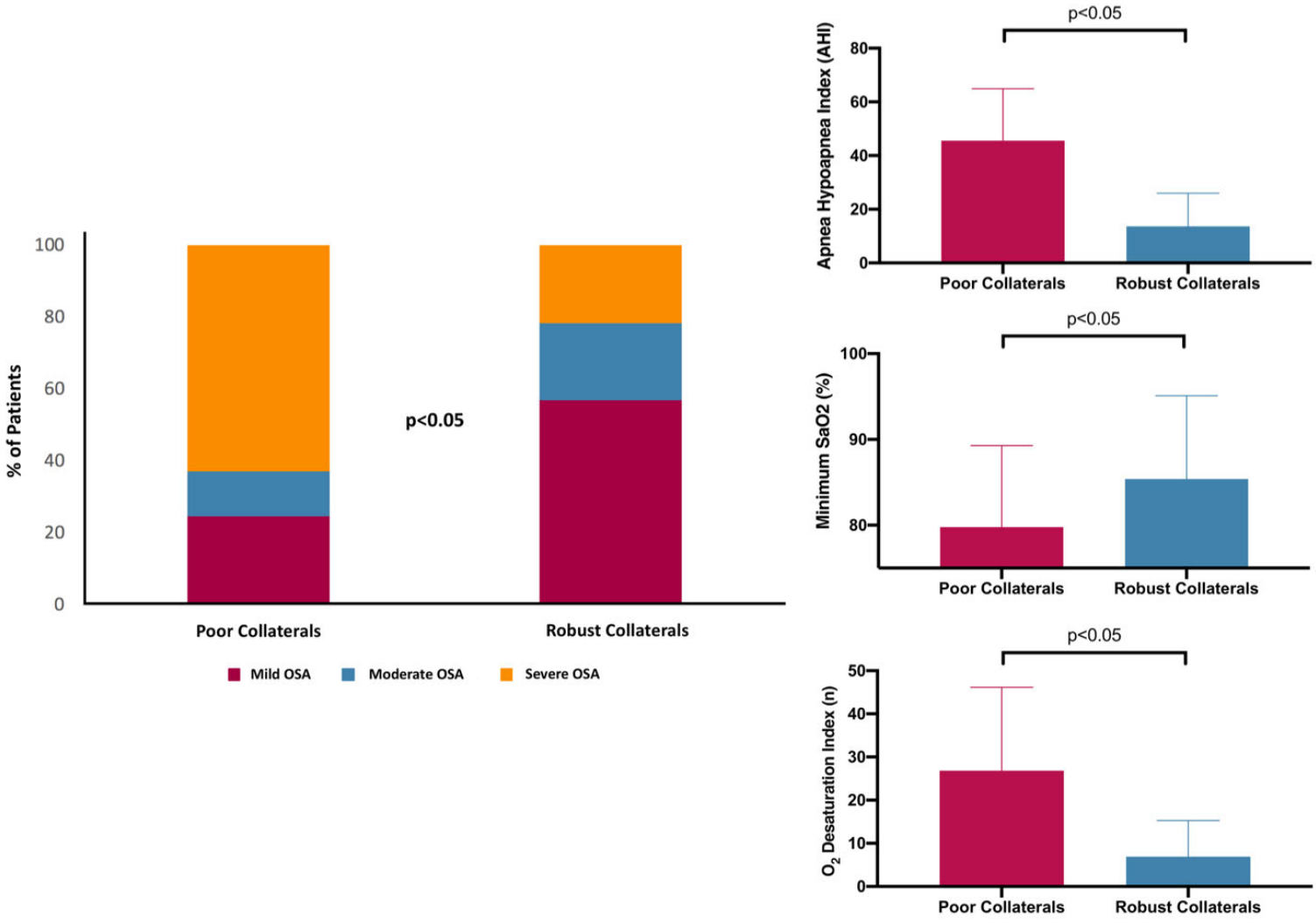
Patients with robust collaterals were more likely to have mild OSA (57.1% vs 25%, p<0.01) [Figure 16.2] and had a lower AHI (13.6 vs 45.5, p<0.05). Similarly, patients with robust collaterals had a higher MinS_aO_2 compared with those with poor collaterals (85.4% vs 79.8%, p<0.05), less time S_aO_2 was <90% (0mins vs 30.4mins, p<0.05) and lower ODI (6.9 vs 26.8, p<0.05) (Table 16.2).

Table 16.2: Polysomnographic characteristics in patients with poor and robust collaterals

	Poor Collaterals n = 32	Robust Collaterals n = 28	p-value
OSA Severity *(n)*			**<0.01**
Mild	**8 (25%)**	**16 (57.1%)**	
Moderate	**4 (12.5%)**	**6 (21.4%)**	
Severe	**20 (62.5%)**	**6 (21.4%)**	
Moderate or Severe OSA *(n)*	**24 (75%)**	**12 (42.9%)**	**<0.05**
Apnoea Hypopnoea Index (AHI) *(/hr)*	**45.5 (17.2 – 63.6)**	**13.6 (8.9 – 25.5)**	**<0.05**
Longest Apnoea *(sec)*	**43.4 +/- 18.5**	**40.6 +/- 31.6**	**0.69**
Mean apnoea-hypopnoea duration *(sec)*	26.9 (22.9 – 29.6)	28.3 (24.1 – 32.2)	0.19
Minimum S_aO_2 (%)	**79.8 +/- 9.5**	**85.4 +/- 9.7**	**<0.05**
O_2 Desaturation Index *(n)*	**26.8 (6.8 – 42.8)**	**6.9 (3.8 – 11.9)**	**<0.05**
S_aO_2<90 *(mins)*	**30.4 (3.3 – 88.1)**	**0 (0 – 0.9)**	**<0.01**
Epworth Sleepiness Score *(n)*	6.9 +/- 4.3	7.3 +/- 4.4	0.81
Excessive Daytime Sleepiness^	3 (13.6%)	3 (20%)	0.61

OSA = obstructive sleep apnoea; S_aO_2 = oxygen saturation by pulse oximetry; ^ Defined as Epworth Sleepiness Scale >10

Figure 16.2: OSA severity in patients with poor and robust coronary collaterals



Left panel: Distribution of OSA severity in patients with poor and robust collaterals. Top right panel: Apnoea-hypopnoea index in patients with poor and robust collaterals. Right centre panel: MinSaO2 in patients with poor and robust collaterals. Bottom right panel: ODI in patients with poor and robust collaterals.

Sub-group analysis was performed in patients who presented with a CTO (Table 16.3) and those who presented with a STEMI (Table 16.4). In patients presenting with a CTO, 17 (85%) has robust collaterals. Those with robust collaterals were more likely to have mild OSA (58.8% vs 0%, p<0.05).

Table 16.3: Baseline characteristics and polysomnography metrics in patients with a CTO.

	Poor Collaterals n = 3	Robust Collaterals n = 17	p-value
Age *(yrs)*	60.3 ± 1.7	58.3 ±19.4	0.68
Male Gender *(n)*	3 (100%)	16 (94.1%)	0.67
Body Mass Index (kg/m^2)	**37.0 (36.8 – 39.5)**	**27.2 (23.1 – 29.0)**	**<0.01**
Cardiovascular Risk Factors			
Hypertension	3 (100%)	17 (100%)	N/A
Hypercholesterolaemia	**2 (66%)**	**17 (100%)**	**<0.05**
Smoking History			**<0.05**
Never	**0 (0%)**	**9 (52.9%)**	
Ex-Smoker	**2 (66%)**	**7 (43.7%)**	
Current	**1 (33%)**	**0 (0%)**	
Diabetes Mellitus	2 (66%)	5 (71.4%)	0.52
Medications			
Aspirin	3 (100%)	16 (100%)	N/A
P2Y12 Inhibitor	2 (66%)	7 (43.8%)	0.47
Beta Blockers	3 (100%)	12 (75%)	0.33
ACE-I	2 (66%)	10 (62.5%)	0.89
Nitrate	1 (33%)	2 (12.5%)	0.36
Statin	3 (100%)	15 (93.8%)	0.66
Stenosis in donor vessel	70 (45 – 70)	50 (30 – 70)	0.91
LVEF (%)	50 (35 – 55)	55 (40 – 60)	0.50
LV Impairment *(n)*	2 (66%)	7 (41.2%)	0.41
Occluded Vessel *(n)*			0.47
LAD	0 (0%)	4 (23.5%)	
LCx	0 (0%)	2 (11.8%)	
RCA	3 (100%)	11 (64.7%)	
OSA Severity *(n)*			0.07
Mild	0 (0%)	10 (58.8%)	
Moderate	1 (33%)	3 (17.6%)	
Severe	2 (66%)	4 (23.5%)	
Moderate or Severe OSA *(n)*	**3 (100%)**	**7 (41.2%)**	**<0.05**
Apnoea Hypopnoea Index (AHI) *(/hr)*	44.7 ± 23.2	25.1 ± 22.8	0.28
Longest Apnoea *(sec)*	42.7 ± 4.0	38.6 ±17.3	0.41
Mean apnoea-hypopnoea duration *(sec)*	29 (26.5 – 29)	26.2 (23 – 32)	0.87
Minimum S_aO_2 (%)	81.7 ± 6.6	85.6 ± 7.0	0.42
O_2 Desaturation Index *(n)*	38.1 ± 23.6	18.2 ± 21.9	0.28
S_aO_2<90 *(mins)*	3.1 (3.1 – 3.1)	0.4 (0 – 2.15)	0.95
Epworth Sleepiness Score *(n)*	5 (3.5 – 6.5)	6 (5 – 8)	0.80
Excessive Daytime Sleepiness^	2 (100%)	9 (100%)	N/A

** ACE-I = angiotensin converting enzyme inhibitor; ARB = angiotensin receptor blocker; OSA = obstructive sleep apnoea; S_aO_2 = oxygen saturation by pulse oximetry; STEMI = ST elevation myocardial infarction;* ^ Defined as Epworth Sleepiness Scale >10

In those patients presenting with a STEMI, 11 (27.5%) had robust collaterals. Those patients with robust collaterals were more likely have mild OSA severity (54.5% vs 27.6%, $p<0.05$) and a lower AHI (20 vs 40.4, $p<0.05$).

Table 16.4: Baseline characteristics and polysomnography metrics in patients with STEMI.

	Poor Collaterals n = 29	Robust Collaterals n = 11	p-value
Age *(yrs)*	63.0 ± 12.2	60.5 ± 11.4	0.54
Male Gender *(n)*	22 (75.8%)	11 (100%)	0.07
Body Mass Index (kg/m^2)	30.7 ±5.8	28 ± 4.2	0.13
Cardiovascular Risk Factors			
Hypertension	19/29	7/11	0.91
Hypercholesterolaemia	14 (50%)	7 (70%)	0.27
Smoking History			0.60
Never	15 (62.5%)	6 (66.7%)	
Ex-Smoker	6 (25%)	1 (11.1%)	
Current	3 (12.55	2 (22.2%)	
Diabetes Mellitus	8 (28.6%)	1 (10%)	0.24
Medications			
Aspirin	28 (100%)	11 (100%)	N/A
P2Y12 Inhibitor	28 (100%)	11 (100%)	N/A
Beta Blockers	5 (17.9%)	3 (27.3%)	0.51
ACE-I	10 (35.7%)	6 (54.5%)	0.28
Nitrate	0 (0%)	0 (0%)	N/A
Statin	**22 (75.9%)**	**11 (100%)**	**<0.05**
Stenosis in donor vessel	**51.4** ± 21.2	**37** ± 22.1	0.20
LVEF (%)	49.8 ± 9.4	50.3 ± 11.5	0.91
LV Impairment *(n)*	14 (56%)	4 (36.4%)	0.28
Occluded Vessel *(n)*			0.53
LAD	15 (51.7%)	6 (54.5%)	
LCx	3 (10.3%)	0 (0%)	
RCA	11 (37.9%)	5 (45.5%)	
OSA Severity *(n)*			**<0.05**
Mild	**8 (27.6%)**	**6 (54.5%)**	
Moderate	**3 (10.3%)**	**3 (27.3%)**	
Severe	**18 (27.6%)**	**2 (18.2%)**	
Moderate or Severe OSA *(n)*	21 (72.4%)	5 (45.5%)	0.15
Apnoea Hypopnoea Index (AHI) *(/hr)*	**40.4 ± 24.8**	**20.0 ± 20.3**	**<0.05**
Longest Apnoea *(sec)*	43.5 ±19.5	44.0 ± 48.4	0.97
Mean apnoea-hypopnoea duration *(sec)*	**26.4** ± 7.0	**29.7** ± 6.4	0.17
Minimum S_aO_2 (%)	79.6 ± 9.8	85.1 ±13.3	0.23
O_2 Desaturation Index *(n)*	28.2 ± 22.4	12.1 ± 22.3	0.08
S_aO_2<90 *(mins)*	30.4 (2.6 – 88.1)	0 (0 – 0)	0.07
Epworth Sleepiness Score *(n)*	7.1 ± 4.4	9.3 ± 6.0	0.43
Excessive Daytime Sleepiness^	3 (15%)	3 (50%)	0.07

** ACE-I = angiotensin converting enzyme inhibitor; ARB = angiotensin receptor blocker; OSA = obstructive sleep apnoea; S_aO_2 = oxygen saturation by pulse oximetry; STEMI = ST elevation myocardial infarction;* ^ Defined as Epworth Sleepiness Scale >10

16.5 Discussion

In patients with total coronary artery occlusion, mild OSA appears to be associated with more robust coronary collaterals, whilst severe OSA appear to be associated with poor coronary collaterals. As shown in Chapter 14 and 15, patients with a documented history of OSA have more robust collaterals compared with patients without a history of OSA, and may be associated with prognostic improvement. However, the present study appears to illustrate a "J-curve" or graduated effect, with milder forms associated with more robust collaterals compared with those without OSA. However any putative advantage is lost with increasing severity of OSA, which correlates with poorer collaterals than those with milder forms.

This finding is likely associated with alterations in degree of tissue hypoxia. Although hypoxia plays a role in arteriogenesis, it is not the predominant factor (12), and an excess may be detrimental to collateral maturation (2). This is shown by the present study, whereby patients with robust collaterals had a higher minS_aO_2, lower ODI and less time spent with oxygen saturation <90%, compared with those with poor collaterals, suggesting severe hypoxia is in fact detrimental to collateral recruitment. Furthermore, it is probable that the chronicity of OSA also plays a factor, whereby there may be adaptive (or protective) changes initially, but over time, as the severity of untreated OSA progresses, these adaptive mechanisms may become overwhelmed.

In patients presenting with a CTO or a STEMI, severe OSA associated with poorer collateral recruitment compared with milder forms. While the time course over which collaterals are recruited differ between these populations, the underlying processes and mechanisms by which they occur are similar (13-17).

In a prior study of patients presenting with a STEMI (18), those with severe OSA had more visible collaterals than those with mild or moderate severity OSA. Furthermore, similar to the current study, a potential "J-shape" effect was described, whereby patients with increasing Rentrop grade had higher AHI scores. However those with Rentrop grade 3 collaterals had a lower mean AHI than those with grade 1 or 2. Similarly in a study of 34 patients with a CTO, patients with an AHI≥10 had greater rates of robust collaterals compared to those who had an AHI <10 (19), although the study did not stratify whether more severe forms of OSA were associated with poorer collateral recruitment.

An alternative potential mechanism by which OSA may affect collaterals is through alterations in coronary haemodynamics. Animal models have suggested that in the setting of OSA, there is a significant reduction in the ability to augment coronary blood flow in the setting of increased myocardial oxygen demand, particularly following apnoeas (20). As an elevation in coronary blood flow occurs in the setting of increased shear stress to induce collateral maturation, it is possible this effect is blunted following apnoeas and significant hypoxia. Endothelial dysfunction has been demonstrated in otherwise healthy patients with OSA with resultant impaired myocardial perfusion (21). The resultant microvascular dysfunction may suppress collateral recruitment (22).

16.5.1 Limitations

This study provides detailed polysomnographically derived data, correlating specific metrics of OSA severity with collateral formation, however there are limitations. Firstly, this is a retrospective single centre study, which has inherent limitations due to a range of potential biases. This data is hypothesis generating, and has plausible biological and mechanistic explanations, underlining the importance of prospective studies to further delineate the effect

of OSA on collateral recruitment. By including both patients with STEMI (whereby collateral recruitment is rapid) and those with a CTO (where this process is more gradual), potential bias may be introduced. More severe OSA was associated with poorer collaterals in both groups, suggesting this is a ubiquitous effect on the ability to recruit collaterals rather than time course specific. Furthermore, an assessment of the effect of treatment of OSA on collateral recruitment could not be determined. It is possible that effective treatment with positive pressure ventilation or other device therapy may have an important impact on collaterals.

16.6 Conclusion

The presence of more severe OSA and resultant worse intermittent hypoxia is associated with poorer coronary collateral recruitment in patients with coronary artery occlusion, both in the setting of STEMI and CTO. This process likely stems from direct effects of hypoxia and ischaemia as well as alterations in coronary blood flow as a result of acute thoracic pressure alterations with OSA. The effect of OSA treatment on subsequent ability to recruit collaterals and other cardioprotective mechanisms requires further research.

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SECTION IX: SUMMARY AND CONCLUSIONS

Chapter 17: Summary, Conclusions & Future Directions

17.1 Summary

The prevalence of robust coronary collaterals perfusing the territory subtended by an occluded artery during a STEMI is approximately 25%. The independent predictors of robust collaterals are the RCA as the culprit vessel, presence of a CTO in a remote vessel, longer ischaemic time and a younger age. These factors, reflect the importance of elevated fluid shear stress (1,2) as the initial driver of collateral recruitment augmented by hypoxia (3). The ability to rapidly recruit these collaterals is reproducible with future coronary occlusion recruiting a similar degree of collaterals. This suggests an underlying innate biological process which is activated in the setting of a sudden obliteration of coronary blood flow. Contrastingly in the setting of a CTO, the presence of robust collaterals is associated with female sex, while those with a history of significant renal impairment or prior CABG are more likely to have poorer collaterals.

Robust collaterals during a STEMI are associated with a lower in-hospital and longer term mortality, driven by an improvement in left ventricular function. The degree of collateral recruitment does not however reduce risk of repeat AMI or repeat revascularisation. This suggests that whilst collaterals reduce the adverse sequalae of STEMI, they are not preventative and do not preclude repeat plaque rupture or atherosclerotic progression. Contrary to these findings, in patients with a CTO, robust collaterals are not associated with a reduction in mortality or risk of AMI, but do improve the rate of successful PCI. While the presence of collaterals may reduce the acute effects of myocardial necrosis associated with coronary occlusion, over time, due to the exhausted ability to autoregulate blood flow and maximally vasodilate, this prognostic benefit is lost.

Autoregulation (4,5) occurs in the setting of a CTO, where there is maximal vasodilatation of collaterals to reduce microvascular resistance and thereby increase myocardial regional blood flow. This has significant implications on flow and pressure changes in the donor vessel supplying the collaterals to the CTO and thereby impacts on management decision making. The fractional flow reserve (FFR), which is the ratio of coronary pressure distal to a stenosis relative to pressure proximal to the stenosis in the setting of maximal hyperaemia, has been shown to be superior to angiography in guiding revascularisation (6,7). However, the FFR relies on assumptions related to blood flow and territory of myocardium subtended by a vessel.

In the setting of a CTO, there is a significant underestimation of the FFR of the donor vessel, which increases by an average of 0.03 following revascularisation of the occlusion. This change is driven by a reduction in coronary blood flow through the donor vessel, following CTO revascularisation and return of anterograde blood flow. While this absolute change is relatively small, this results in reclassification of a donor vessel from ischaemic to non-ischaemic in over 40% of patients with an initial ischemic FFR, significantly impacting on treatment for these patients. This change is apparent irrespective of which vessel is the donor vessel, although more pronounced in an LAD CTO, reflecting the greater change in myocardial blood flow. Similarly the degree of stenosis of the donor vessel does not affect the absolute or relative change in FFR, suggesting the territory of myocardium supplied by the vessel is the most important determinant.

The change in FFR was seen in patients with diabetes mellitus and those without, patients who commonly have more microvascular and endothelial dysfunction. Indeed, 75% of patients had an abnormal coronary flow reserve (CFR) of the donor vessel, which reflects

both epicardial stenosis and microvascular function. As only 26.9% of patients had an ischaemic FFR, the vast majority of these patients have significant microvascular dysfunction. CFR is independently associated with poorer prognosis even in the setting of a normal FFR (8), which may explain why randomised control trials of PCI in CTO (9-11), which treats only the epicardial stenosis, has failed to show a consistent prognostic benefit.

The effect of blood flow as an integral predictor of coronary collateral function was noted in patients with a prior history of CABG. Patients in whom the donor vessel was grafted had poorer collaterals. In the setting of a grafted vessel, arterial conduits have an initial significant systolic component of blood flow, which over time become more diastolic to reflect normal coronary blood flow (12-14). Venous grafts act as predominant passive conduits, however may affect shear stress in the native circulation (15). This is associated with a more rapid progression of atherosclerotic and calcific process (16,17), and likely also contributes to the poor collaterals seen arising from a grafted donor vessel. This lower prevalence of robust collaterals may be a contributing factor to the lower success rates of CTO PCI in patients with a prior CABG (18,19).

These alterations in coronary blood flow and pressure are manifested with biochemical changes through alterations in endothelial, biochemical and haematological biomarkers. Plasma fibroblast growth factor 2 (FGF-2), monocyte chemoattractant protein 1 (MCP-1) and intracellular adhesion molecule 1 (ICAM-1) are upregulated in the setting of a CTO. These chemokines are involved in monocyte and other inflammatory cell mobilisation and upregulation (20-27), further reflected by a lower peripheral lymphocyte count, higher monocyte count and higher neutrophil to lymphocyte ratio. Plasma MCP-1 and ICAM-1 are both upregulated 6 months following collateral regression, suggesting they may be involved

in ischaemic preconditioning and the initiating phase of subsequent potential to recruit collaterals.

Similarly, in a rat model, the use of intermittent repetitive occlusive ischaemia results in an increase in myocardial perfusion subtended by the epicardial artery as a result of collateral blood flow. This increase in collateral function is associated with an increase in tissue expression of FGF-2 and ICAM-1, as shown in human studies, as well as an increase expression of vascular cell adhesion molecule 1 (VCAM-1), granulocyte-macrophage colony stimulating factor (GM-CSF) and early growth response 1 (EgR-1). Although there was an increase in these chemoattractant proteins, growth factors and regulatory proteins, inflammatory cytokines were not upregulated. Whilst the accumulation, adhesion and activation of inflammatory cells and subsequent release of chemokines appears to be well established (25,26,28), it did not appear that there was a significant increase in these inflammatory cytokines. It is possible that the sham procedure itself in the comparator group induced a significant inflammatory response (29) thereby attenuating any difference between the groups. Alternatively it may reflect the timing of the samples being assessed, with inflammation more prominent early on in the maturation of collaterals. However, this evidence, which is in keeping with the findings of human studies, highlights the role of chemoattractant proteins, inflammatory cells and growth factors, which should be further assessed to determine causative or associative effects.

Along with the effects of fluid shear stress and endothelial activation resulting in release of these chemoattractant proteins, growth factors and transcription factors, there has long been controversy with respect to the effect of hypoxia as a driver of arteriogenesis. While some studies suggesting no role (30-33), others have suggested a strong role in augmenting the

effect of elevated fluid shear stress (34-36). Obstructive Sleep Apnoea (OSA), which is characterised by intermittent hypoxia along with exaggerated intrathoracic pressure swings was documented in 7.7% of patients presenting with STEMI and 13.4% of patients with a CTO. A documented history of OSA was associated with more robust collaterals, which resulted in significantly improved left ventricular function and lower peak troponin in STEMI, and independently associated with improved mortality in those with a CTO. This suggests that OSA confers a cardioprotective effect through possible ischaemic preconditioning and robust collaterals. However, this putative advantage is lost in patients with more severe OSA, potentially indicating a "J-curve" benefit with mild disease. Whether this augmented effect of OSA is driven by predominant hypoxia, or alternatively through alterations on afterload and preload remains to be further investigated.

17.2 Conclusions

Coronary collaterals are found frequently during angiography and associated with improved prognosis in STEMI and favourable percutaneous revascularisation in CTO. The drivers of collaterals are alterations in endothelial shear stress manifested as changes in coronary blood flow, with mild forms of hypoxia augmenting this effect. A number of chemoattractant proteins, growth factors and transcription factors are upregulated in the setting of altered coronary blood flow which likely play a role in collateral recruitment. The subsequent presence of functional collaterals intimately impacts on pressure and flow throughout the coronary circulation affecting clinically meaningful parameters of ischaemia assessment.

17.3 Future Directions

Future studies should continue to focus on the mechanisms and impact of collaterals in patients in both acute and chronic settings. Specifically, aims should include to determine whether the identified growth factors and mediators along with microvascular function may be prognostic markers and future therapeutic targets. Although significant associations between growth factors, chemokines and signalling pathways has been presented in this body of work, a causative link is yet to be established. These hypotheses should be further developed through initial animal studies being exposed to exogenous factors as described in these studies to determine their mechanistic effects. Subsequent studies in humans may also be required, in particular the effects of commonly used cardiovascular medications which appear to have an impact on the ability to recruit collaterals, including beta blocker, statins and aldosterone antagonists. This may particularly be beneficial in the setting of a CTO, which to date remains controversial with respect to the most appropriate management strategy.

Furthermore, the effect of metabolic disease processes such as diabetes mellitus should be investigated to determine whether its effect on endothelial function impacts on coronary collateral recruitment and function. Although diabetes is a well recognised factor associated with poorer prognosis with respect to cardiovascular disease, the specific effects on collateral recruitment and maturation remains uncertain. Future studies should look at diabetic animal models to determine whether the ability to recruit collaterals in the setting of ischaemic stimuli is augmented or attenuated. Subsequent clinical linking studies should continue to investigate the effect of diabetes on invasive measures of microvascular function to assist in diagnosis as well as develop tailored treatment strategies.

Finally, guidance of multivessel revascularisation based on alternative ischaemic thresholds for FFR assessment of donor vessels should be considered. Data from this body of work suggests that in patients with a CTO where the donor vessel is in the ischaemic “grey-zone” this vessel may not require revascularisation, which has dramatic implications for management strategies. This should be confirmed with prospective randomised studies in patients managed on currently accepted physiological cutoffs, or through an adjusted threshold, where only patients with an FFR of <0.75 in the donor vessel are treated. This would of course require a collaborative approach with involvement of multiple centres and experts to ensure harmony in trial design and execution.

Whilst this body of work has highlighted the prognostic and haemodynamic impacts of collaterals, as well determined those factors associated with their recruitment, more research is required to continue meaningful clinical impacts, determine causality and result in potential future therapeutics and management strategies.

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