A Study on Post-Mortem Assessment of Coronary Artery Disease and The Role of Clinical Cardiology Diagnostic Techniques in Further Development of Minimally Invasive Autopsy

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Abstract

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Background: There have been significant developments in recent years towards the use of imaging-based post-mortem examinations. Such new approach has to accurately diagnose coronary artery disease and should aim to overcome various limitations of conventional methods in determining whether the disease identified is a likely cause of death.

Aim: This study aims to determine the accuracy of current post-mortem computed tomographic angiography (PMCTA) techniques in diagnosing coronary stenosis and systematically investigates the effect and implication of repressurisation of post-mortem coronary arteries and the use of clinical cardiology diagnostic tools in this context.

Methods: PMCTA was systematically compared against histology to determine its accuracy in diagnosing the severity of coronary stenosis. A systematic study was performed to demonstrate the effect of repressurisation of post-mortem coronary arteries using coronary pressure wire and optical coherence tomography (OCT). Finally, a proof of concept work was undertaken in the application computational fluid dynamic (CFD) techniques to PMCTA.

Results: The accuracy of PMCTA to identify coronary stenosis was modest (sensitivity 30%, specificity 96%, PPV 65% and NPV 84%) compared to histology of collapsed post-mortem vessels. Repressurisation significantly increased vessel area in post-mortem porcine (3.3±0.5 mm^2 vs. 8.4±1.5 mm^2, N=6) and cadaveric coronary arteries (4.26±3.47 mm^2 vs. 5.74±5.38 mm^2, P<0.001). However, compliance was significantly different among normal and atherosclerotic segments (35.94±20.44 vs. 20.30±15.30 $mmHg^{-1}$x1000, P=0.006), systematically effecting stenosis severity assessment. Finally, CFD techniques could be applied to post-mortem images to derive information on pressure and flow.

Conclusion: This study identified potential areas of systematic error in the current gold standard in assessing coronary stenosis severity at autopsy. It also showed that PMCTA is a promising tool and, with addition of CFD techniques, can provide information on both anatomical and functional significance of a stenosis with the potential to become a new and improved gold standard.

This thesis is dedicated to my parents:

Mrs. Mohsena Begum

&

Late Mr. Kazi Shahidul Huq

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Academic output resulting from this thesis

Publications

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Robinson, C. *et al.* (2015) 'Measuring pressure during coronary artery angiography in ex-vivo hearts', *Journal of Forensic Radiology and Imaging*. Elsevier, pp. 1–5. doi: 10.1016/j.jofri.2015.11.005.

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Poster

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ABBEREVIATIONS

Apo B	Apolipoprotein B
CAD	Coronary artery disease
CFD	Computational fluid dynamic
CTCA	Computed Tomographic Coronary Angiography
CI	Confidence interval
CSA	Cross sectional area
CT	Computed tomography
DNA	Deoxyribonucleic acid
FFR	Fractional flow reserve
HTA	Human tissue act
IHD	Ischemic heart disease
IVUS	Intravascular ultrasound
LAD	Left anterior descending
LCx	Left circumflex
LDL	Low density lipoprotein
MRI	Magnetic resonance imaging
MPPMCTA	Multi-phase post-mortem computed tomographic angiography
NCEPOD	National confidential enquiry into patient outcome and death
OCT	Optical coherence tomography
PMCT	Post-Mortem computed tomography
PMCTA	Post-mortem computed tomographic angiography
PPV	Positive predictive value
NPV	Negative predictive value
SCCT	Society of cardiovascular computed tomography
St. Dev	Standard deviation
TcFA	Thin cap fibroatheroma

RCA	Right coronary artery
VCAM	Vascular cell adhesion molecule
WSS	Wall sheer stress

1 Chapter 1: Introduction

Coronary artery disease affects a very large population worldwide. According to the WHO, this is the commonest cause of adult death globally, resulting in 31% of all global deaths in 2016[1]. In the UK, despite significant resource deployment for the prevention, diagnosis and treatment of coronary artery disease, this still remains the second most common cause of death after dementia and the commonest cause of death in men [2,3]. Many of these deaths are unexpected and as a result a large proportion of those individuals undergo post-mortem examination to identify the cause of their death. Therefore, it is important that the post-mortem examination process is robust and up to date for accurate identification of coronary artery disease as the cause of death. With this view, we discuss autopsy as the current gold standard for post-mortem diagnosis, inadequacies of these current methods and how new technology might be useful in improving post-mortem assessment. We investigate the role of applying clinical diagnostic tools in the post-mortem setting with a view to achieving a better and more accurate post-mortem diagnosis.

1.1 History of autopsy

The word 'autopsy' has both Greek and Latin roots and literally translates as 'seeing with one's own eyes'. Autopsy or necropsy refers to the post-mortem examination of the body to determine the cause and nature of death and the extent of disease[4].

Dissection of the human body has been performed for a variety of religious and cultural beliefs from as early as Egyptian era. Galen and subsequently many other physicians performed dissection of the human corpse to improve their understanding of human anatomy. During the medieval period, some physicians started performing autopsy to correlate clinical illness with anomalies found within the body organs [5].

In the 18th century, Giovanni Morgagni systematically studied 640 cases and linked clinical signs and symptoms with detailed post-mortem findings of those patients. He described all these findings in his book called “De sedibus et causis morborum per anatomen indignatis” (About the seats and causes of diseases investigated by anatomical investigations). This, arguably, was the beginning of modern autopsy as we know it now. Subsequently, other prominent pathologists such as Carl von Rokitansky and Virchow improved the process with the addition of histological examination. Those methods became widely used and established with further development using contemporary tools and techniques. However, the fundamental approach has remained unchanged [5–7].

1.2 Importance of autopsy

1.2.1 Role in advancement of medical knowledge, teaching and education

The knowledge derived from post-mortem examination of the deceased played a significant role in the development of modern medicine. Xavier Bichat, one of the key founders of the discipline of histopathology, strongly emphasized the interrelations between anatomy, physiology and necropsy without which, he considered, there could not be any good anatomists, physiologists or physicians. In many cases, physicians looking after the patients during life were either actively involved or performed the autopsy themselves, which provided in-depth understanding of many disease processes and improved subsequent treatment approaches. Consequently, there was a steady increase in the autopsy rates in hospitals across the globe reaching as high as 50% by the latter half of the 20th Century in most parts of the western world and other developed nations like Japan[5,6,8–10].

Also, its role in providing emotional comfort to the relatives, educating the clinical team involved in the deceased’s care and thus leading to improvements in health care are all vital to this date [8,10–13].

1.2.2 As a medico-legal tool

Autopsy played a vital role in the medico-legal and judicial system from the very early part of the 19th century. It was, and continues to be, routinely used for accurate identification of the deceased in unexpected deaths, finding out the time and cause of death, as well as the manner of death where foul play is suspected. The addition of toxicology and DNA fingerprinting has made it an essential tool for contemporary criminal investigations [14,15].

1.2.3 Providing data for health policy making

One of the other major roles of autopsy has been to provide accurate mortality data which in turn has a significant impact on national and international health policies and resource allocations for research and public health. Prevention and treatment of conditions like cardiovascular disease and many infectious diseases has improved significantly over the past few decades which would not have been possible without the information and knowledge derived from many autopsies performed in mid to late 20th century [16,17].

1.3 Autopsy in contemporary practice

Currently, there are two major routes to autopsy; the hospital autopsy and the medico-legal autopsy. Both types of autopsies are performed at varying rates and under different legal frameworks in different countries.

1.3.1 Hospital autopsy

The hospital or clinico-pathological autopsy is performed when a patient dies in hospital and the physicians and/or relatives want to have a better understanding of the disease process and in cases of uncertainty, the actual cause of death. These autopsies are performed with relative's consent in most countries. They provide important answers to the relatives and medical community such as missed or obscured diagnoses, beneficial or harmful effect of any treatment provided etc. and

over the last century, they have played a key role in the advancement of modern medicine as discussed above (*Section1.2*).

1.3.1.1 Current practice of hospital autopsy: A declining trend

There has been a dramatic decline in hospital autopsy rates over the last few decades. A Dutch study on hospital deaths and autopsies over 35 years (1977 -2011) showed a decline in autopsy rate from 31.4% to 7.7%. In Nova Scotia, Canada, clinical autopsy rate dropped from 30% to 15% in 1999 over a 13year period. A study of autopsies at a university hospital in Japan showed the rate declining from above 40% in 1983 to below 10% in 2012. In USA, the rate was as high as 40% which dropped to below 10% in the last decade. Importantly, in the UK, this decline has been even more dramatic. A study of hospital autopsy rates in 2013 performed in all the NHS trusts across the UK showed a mean rate of only 0.69% compared to previous rates of 30-40% in 1970 [18–21].

1.3.1.2 Challenges and misperceptions contributing to such decline

Several factors have influenced the medical community, the public and the policy makers leading to this marked decline in hospital autopsy some of which are discussed below:

- Clinicians understanding of the benefit of hospital autopsy process:

Studies involving various groups including medical students, nursing staff and clinicians have shown that there is a significant lack of training and understanding of the process and purpose of the hospital autopsy [22–25]. Most consider it as a means of merely identifying the cause of death with no other benefit. Also clinicians have increasing confidence in new diagnostic modalities and don't consider autopsy as a valuable means for research and education anymore [6,8].

However, findings from research looking into the accuracy of clinical diagnosis compared to post-mortem examination is rather the contrary. Goldman et el. performed a detailed review of clinico-pathological correlation of 300 autopsies in

their institute from three different decades between 1960- 1980, selecting 100 random cases from each decade. They classified the different types and extent of discrepancies between clinical diagnosis and autopsy findings as described in *Table 1.1* [26].

They identified approximately 10% of class I and 12% of class II discrepancies in each time period with no decline over time despite advancement in diagnostic techniques over that period.

Table 1-1: *Classification of discrepancies of autopsy findings* (adapted from Goldman et al [26])

Type of Discrepancy	Description
Class I	Autopsy revealed major missed diagnosis for which treatment might have resulted in prolongation of survival
Class II	Autopsy revealed major missed diagnosis the detection of which before death might not have changed management depending on the available therapies / technologies at that time
Class III	Missed minor diagnosis related to terminal disease process but not directly related to death
Class IV	Missed unrelated minor diagnosis that might eventually have affected prognosis

In another study, the authors reviewed a large number of post-mortem studies from 1919 to 1998 and found that rate of major discrepancies or alternative cause of death found during autopsy compared to clinical diagnosis was in the range of 3 to 68%. [27]. Even for patients in intensive care, where it is likely that they had undergone the most extensive investigations, the rates of such discrepancies were similar [28,29].

One might argue that in the last few decades there have been further developments in diagnostic techniques at an exponential rate and the figures presented above might not reflect contemporary practice. However, a retrospective analysis of autopsies performed in an expert pathology centre in 2007 and 2012/2013 identified and average of 23.5% major (class I and II) and 32.6% minor (class III and IV) discrepancies between clinical and post-mortem diagnosis [10]. Important diagnoses such as cancer, myocardial infarction and pulmonary embolism were missed even in the setting of intensive care.

- Consent

One of the other key constraints to hospital autopsy is the requirement for the consent of the relatives of the deceased. There is a general assumption in the clinical community that the likelihood of obtaining consent from a recently bereaved next of kin for an invasive post-mortem examination is relatively low. This is further compounded by lack of specific training in consenting for hospital autopsy which can be emotionally sensitive.

However, findings from several studies show the rate of successful consent from the next of kin for autopsy or post-mortem research ranges from 65% to as high as 96.6% across the globe [25,30,31]. A common theme from these studies was that proper explanation of the purpose and the process of autopsy by trained individuals has a much greater chance of achieving successful consent.

Also, even in countries such as Austria where consent is not a legal requirement for hospital autopsy, the rate of hospital autopsy still declined significantly from 34.2% in 1990 to 17.5% in 2009 [32]. This suggests that there is interplay of many societal factors influencing the declining rate of autopsy besides consent.

- Religious and cultural factors

Most religions do not specifically forbid autopsy. However, as shown in the *Table 1.2* below, there is a requirement for early burial or cremation by many religions. This has a significant impact on the ability of the clinical team obtaining consent for an autopsy as this would usually add further delay in the final disposal of the deceased's body. Even when consent is not required legally, there is lower probability of hospital autopsy among these groups suggesting that the religious beliefs of the patient and relatives have a strong influence on this matter [32].

- Resources

The estimated cost of a coronial autopsy is approximately £1000 [33]. Due to many competing interests and expensive new treatment and diagnostic tools, autopsy is likely to get a lower priority, unless the benefit can be established with good evidence.

However, while there might be an upfront cost to autopsy, this is also true for any research projects exploring unchartered territory and autopsy should be viewed in the same spirit. There is potentially a significant clinical, societal and financial return from well conducted autopsy, by improved public health, enhanced diagnostic skills of the clinicians learning from its findings and the improved quality of education and training.

1.3.2 Medico-legal autopsy

The second route to autopsy is medicolegal. This approach is a requirement of the judicial system in cases of deaths resulting from unnatural circumstances or where there is suspicion of foul play. They are also required in non-suspicious deaths where

the cause of death is unknown, and a death certificate cannot be issued. These requirements vary according to the jurisdiction where the person died and according to the local law[7,19].

1.3.2.1 Current state

There has been a marked and steady decline in hospital autopsy to the level of near extinction. However, the medico-legal autopsy rates have remained relatively high despite some declining trend. In the mid to late 1990s, the rate of medicolegal or coronial autopsy in England and Wales was around 62% of the referred cases. A study from Canada showed an increase in medico-legal autopsy from 40% in 1986 to 62% in 1999 for all the cases referred to the coroner [19,34].

However, there has been some reversal in the above trend of high percentage of coronial autopsy in the last decade. In the year 2016, in England and Wales, 36% of all the cases referred to the coroner underwent a post-mortem examination. This is nearly a quarter reduction compared to the proportion of cases referred to the coroner undergoing autopsy in 1995 [34]. But this still resulted in 86,545 autopsies carried out that year which is nearly 16% or one in six of all deaths. As most of these cases are still performed for non-suspicious but unexpected deaths, the legal prerogative is to find “a cause of death” on the balance of probability, rather than “the definitive cause of death” without any reasonable doubt. This makes this whole process much less reliable from a clinical and scientific point of view.

Table 1-2: *Religious attitudes towards autopsy, retention of tissue and organs, and disposal of the body* (adapted from Burton & Underwood [8])

	Autopsy	Tissue Section	Disposal of the body
Atheism	No prohibition	No prohibition	Burial or Cremation
Baha'I	No religious prohibition	No religious prohibition	Burial within 1 h journey of place of death
Buddhism	No religious prohibition	No religious prohibition	Cremation
Christianity	No religious prohibition	No religious prohibition	Burial or cremation
Christian Scientist	No religious prohibition but usually not acceptable	No religious prohibition but usually not acceptable	Burial or cremation
Church of Jesus Christ of Latter Day Saints	No religious prohibition	No religious prohibition	Burial
Hinduism	No religious prohibition	No religious prohibition	Cremation without unnecessary delay
Islam	Usually only if required by law	Returned to the body or, if released after funeral, buried	Burial, ideally within 24 h of death
Jainism	No religious prohibition	No religious prohibition	Cremation

Jehovah Witness	No religious prohibition but usually not acceptable	No religious prohibition but usually not acceptable	Burial or cremation
Judaism	Usually only if required by law	Returned to the body or, if released after funeral, buried	Burial, ideally within 24 h of death
Rastafarianism	Only if required by law	Only if required by law	Burial or cremation
Shintoism	No religious prohibition	No religious prohibition	Cremation is usual
Sikhism	No religious prohibition	No religious prohibition	Cremation without unnecessary delay
Taoism	No religious prohibition	No religious prohibition	Burial or cremation

1.3.2.2 Medico-legal autopsy in the UK: Quality assessment

Due to the high volume, there have been several reviews over the last two decades on the quality and purpose of coronial autopsy in England and Wales. In 2003, the Luce report on 'Death Certification in England and Wales', presented to Parliament by the Secretary of State, was very critical of such high numbers of invasive autopsies [35]. Subsequently a full review of the quality of autopsy reports was undertaken by the NCPEOD committee in 2006 [36]. Along with the primary objective of assessing the quality of autopsy reports, they set out to answer if there was justification for so many autopsies and whether these coronial autopsies had any additional benefits such as educational value, better understanding of disease process and answering questions for the family. They looked at 1877 autopsy reports from 121 coronial jurisdictions over a period of one week. The findings by an expert panel highlighted several major concerns regarding the quality of the coronial autopsy and its benefit.

They found –

- Only 52% reports were satisfactory and 26% were poor or unacceptable
- In about one fifth of the cases, the cause of death mentioned on the autopsy report was questionable
- Conditions like cardiomyopathy and epilepsy were not properly investigated by the pathologists despite established guidelines
- The rate of histology was only 19% and
- In nearly 40% of the cases, there were no clinico-pathological correlation detailed on the report.

Overall, this report was highly critical and recommended changes in the whole process.

1.4 Coronary artery disease: The pathophysiology, its evaluation at autopsy and weakness in the current approach

As coronary artery disease is globally one of the leading causes of death, it is essential that the post-mortem examination process in place is robust from a scientific point of view to identify coronary artery disease where present and ascertain its' direct or indirect role in causing death as accurately as possible.

Therefore, it is important to briefly discuss the coronary artery disease process, the various modes by which it may cause death and how it is assessed currently during post-mortem examination.

1.4.1 Coronary atherosclerosis

Coronary artery disease manifests through progressive coronary atherosclerosis, which is a complex process. Several hypotheses exist regarding the initiation and progression of atherosclerosis. While no single process has been identified as having a principle role, factors such as endothelial dysfunction, endothelial injury resulting from sheer stress, increased endothelial permeability to lipid molecules such as low density lipoprotein (LDL) and apolipoprotein B (Apo B) and endothelial retention of the lipid molecule have been identified as playing key roles in initiating atherogenesis. The process is further accelerated by expression of adhesion molecules such as vascular cell adhesion molecule-1 (VCAM-1) by the early atheroma which recruits leucocytes into the lesions and leads to further inflammatory change and lesion progression. The result is a gradual change in the arterial wall with initial stages of fatty streak to lipid rich plaque or atheroma [37].

This atheroma can gradually progress to cause significant stenosis leading to symptoms of stable angina. However, in many instances there is rupture of plaque exposing the highly thrombogenic necrotic core leading to platelet activation and thrombus formation. Depending on the extent and location of thrombus formation, this can result in acute coronary syndrome or sudden death [37–42].

Figure 1.1 shows the schematic of various components of two types of atherosclerotic plaque and *Figure 1.2* shows histology and a schematic of different types of coronary plaques in cases of sudden cardiac death.

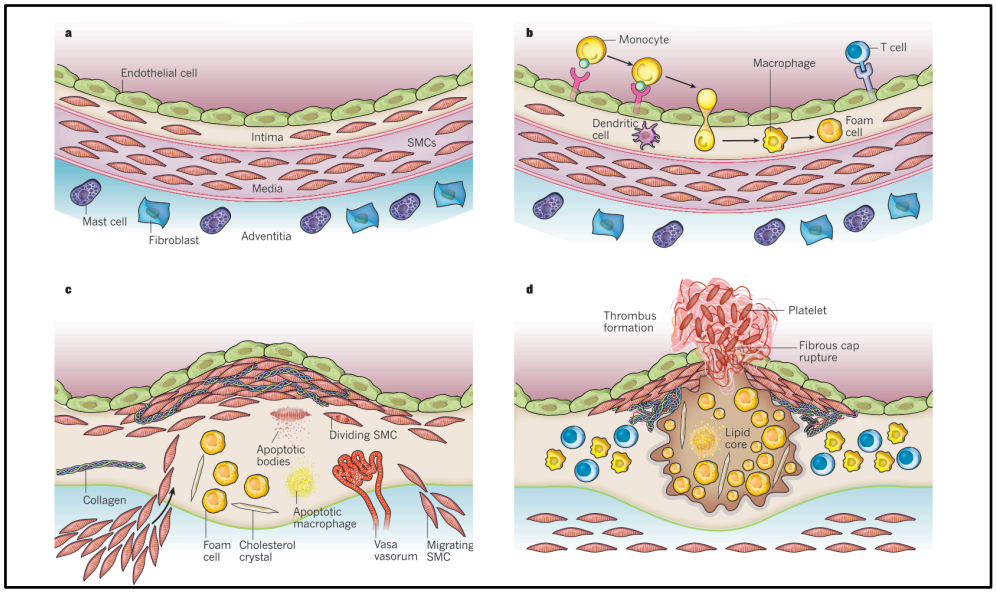

Figure 1-1: *Schematic of atherosclerotic plaque and its various components* (adapted with permission from Libby et al.[41]). a) Normal vascular wall with no atherosclerosis b) Inflammation and activation of monocytes and macrophages c) development of a stable atherosclerotic plaque with thick fibrous cap d) acute rupture of a thin cap fibro-atheroma leading to thrombosis

The progression of atherosclerotic lesions is not necessarily a linear phenomenon. Virmani and colleagues have demonstrated the heterogeneity of plaque characteristics and progression associated with sudden cardiac death as shown in *Figure 1.3* below.

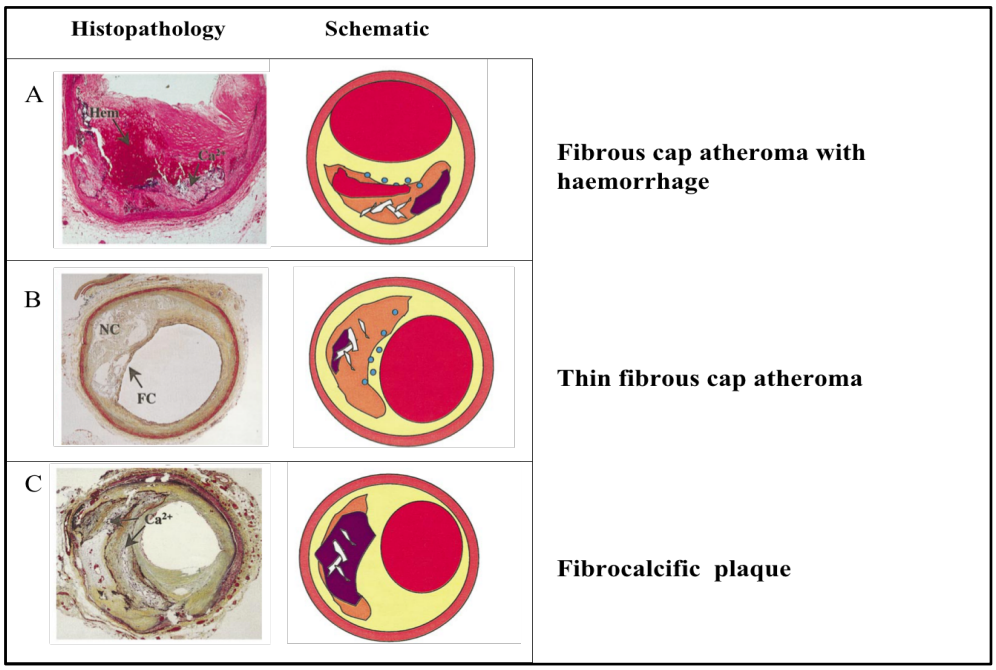

Figure 1-2: *Histology and schematic of various types of plaques associated with cases of sudden cardiac death* (reproduced with permission from Virmani et al. [43])

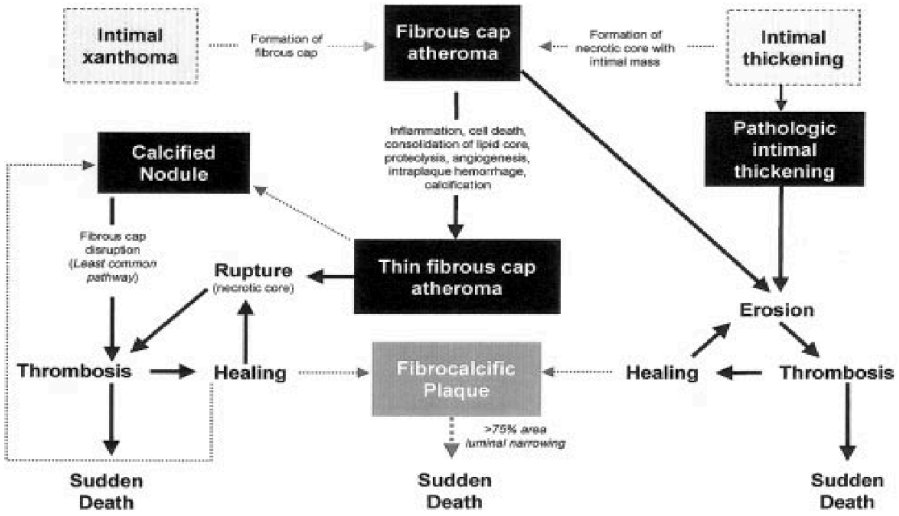

Figure 1-3: *Various pathways of progression of a coronary plaque leading to death.*(reproduced with permission from Virmani et al. [43])

1.4.2 Mode of death due coronary artery disease

Depending on the nature of progression of coronary artery disease in an individual, there are a number of broad pathways which can lead to death.

A person can develop an acute coronary thrombosis causing myocardial infarction. This subsequently leads to either acute ventricular arrhythmias causing sudden death or a large area of myocardial ischemia and necrosis, mechanical complications and pump failure leading to death in the immediate aftermath of the infarction. Or, they can develop an ischemic cardiomyopathy leading to pump failure or arrhythmic death in the future. Alternatively, depending on the severity and extent of stable coronary artery stenosis, a person may develop significant myocardial ischemia which can again lead to arrhythmias or acute pump failure leading to death.

Table 1-3: *Probabilistic approach for attributing ischemic heart disease as the cause of death at autopsy* (adapted from MJ Davies [44])

Pathology	Approximate probability of death due to ischemic heart disease
• Pericardial Tamponade • Rupture of Acute Infarct • Coronary Thrombus	100%
• Acute Myocardial infarction/Coronary Thrombus • Coronary Thrombosis Alone • Healed Previous infarct Scar- without thrombus	90%
No Thrombus – No Scars • X 3 vessels with >75% stenosis • X 2 vessels with >75% stenosis • X 1 vessels with >75% stenosis	70%
• X 1 vessel with <50% stenosis	Less than 50%

Determining the contribution of coronary artery disease in causing death is a probabilistic exercise. Depending on the severity of atherosclerosis and thrombosis identified during the autopsy, this probability can range from 50% to 100% as describe by MJ Davies *(Table 1.3)* [44], who's criteria are still widely used by

pathologists and recommended by the guidelines [45]. However, there are several weaknesses in this approach which will be discussed below.

There are several other causes of sudden cardiac death such as primary arrythmias, cardiomyopathies and coronary artery anomalies which are beyond the scope of this work.

1.4.3 Assessment of coronary artery disease at conventional autopsy and its limitations:

As is evident from the discussion above, the assessment of coronary artery disease during a post-mortem examination should be directed to either identify evidence of acute coronary plaque events and/or thrombus, (with or without severe stenosis), or to identify the severity and extent of arterial stenosis potentially contributing to death.

In keeping with this, the UK and European guidelines for post-mortem examination of the heart recommend several specific steps. These include cross sectioning of all the major coronary artery branches along their length at 2-3 mm intervals, inspection of the cross sections for evidence of acute plaque events with thrombus; visual assessment of the severity of any stenosis and to diagnose a severe stenosis by failure to pass a 1 mm probe through the lesion. Also, decalcification is strongly recommended where necessary as otherwise attempts at cross section would cause damage to the vessel wall and lumen architecture making subsequent assessment difficult. Finally, selected sections, it is recommended, should be considered for detailed tissue processing and histological examination to identify microscopic evidence of acute plaque rupture or fissuring which would not be visible otherwise. The myocardium should also be examined macroscopically and histologically to identify evidence of necrosis or other myocardial pathology.
Following such detailed investigation, the probability of death from an acute coronary event can range from a certain 100% to as low as 50% based on the Davies' criteria (Table 1-3) [44–46].

1.4.4 Pitfalls in the current method

There are some major limitations in the current approach in assessment of coronary artery disease at routine coronial autopsy.

1.4.4.1 Very limited use of histology

Studies have shown that 2/3rds of acute myocardial infarction occurs at the site of previously non-significant stenosis. Subsequently, studies on victims of sudden cardiac death have shown that the precursor lesions most commonly associated with coronary events and deaths is thin cap fibro atheroma (TcFA) which usually causes <50% diameter stenosis in nearly three-quarter of the cases [47–49]. Therefore, if histology is not routinely performed, many of these lesions will not be considered as significant unless a macroscopically visible thrombus is present during post-mortem examination. The presence of post-mortem clots also increases the risk of misinterpretation if only naked eye assessment is performed.

The National Confidential Enquiry into Patient Outcome and Death (NCPEOD) report[36] showed that the use of histology in coronial autopsy is very limited, occurring in approximately 16% of cases, despite the recommendations by experts and guidelines. A lack of routine histology might cause a systematic underdiagnosis of coronary artery disease as a cause of death despite an invasive post-mortem examination.

1.4.4.2 Poor assessment of the severity of stenosis and its impact on death

If acute plaque events are not identified, death still can be attributed to coronary artery disease depending on the presence and severity of stenosis. This approach has several major limitations and requires thorough scrutiny.

1.4.4.2.1 Visual and non-quantitative assessment of stenosis severity

Visual assessment of stenosis severity of a cross section of post-mortem coronary artery is highly variable. This weakness has been acknowledged before [50,51] and the

chart developed by Champ and Coghill [50] is often available in mortuary for some standardisation despite some evidence that such approach doesn't improve subjective variation among different observers [51]. It has also been clearly demonstrated that a more objective approach such as stereological point counting and computer aided planimetry are more accurate and reproducible and should be used for assessing coronary stenosis severity during post-mortem [52].

1.4.4.2.2 The effect of depressurisation on vessel and stenosis morphometry

After death, several post-mortem changes affect the morphometry of the coronary artery [46]. In the absence of blood flow, the depressurised vessels will collapse. Also, the presence and extent of atherosclerosis and calcification in different segments of the vessel may cause local variations in compliance. The interplay of all these factors, therefore, may lead to a very different post-mortem impression of stenosis severity compared to life. Even following the guidelines very stringently would not resolve this issue and this therefore remains a permanent draw-back of current post-mortem techniques. The literature suggests that there was a nearly 30% discrepancy between early coronary angiography findings with autopsy which was largely attributed to underestimation of stenosis severity by angiography [53].
However, Davies and colleagues strongly argued that many of those differences are due to assessment of stenosis in a collapsed vessel at autopsy. Therefore, they recommended routine post-mortem angiography of the heart using pressurised radiopaque solution to address the issue [54]. Also many authors used pressurised solution of fixatives using various techniques and protocols before performing histological studies, the idea being that this would distend the coronary arteries to their physiological dimension and results would be more accurate [53–57]. However, they never attempted to measure the intra-coronary pressure to demonstrate what pressure was actually achieved inside the vessels.

1.4.4.2.3 Variable effect of tissue processing

If the guidelines are strictly followed and more histology is performed, this introduces a new challenge. Siegle et al. demonstrated that the effect of fixation and tissue processing is variable on different segments of coronary artery wall depending on the severity of pre-existing disease [58] and leads to a different interpretation of stenosis severity compared to the non-processed state. This highlights the fact that even microscopic assessment of coronary stenosis is not free from systematic error.

1.4.4.2.4 Inability to determine functional significance of stenoses

Another fundamental and inherent weakness of the 'gold standard' autopsy is its inability to determine the true functional or haemodynamic significance of a coronary stenosis. A probabilistic approach is used to determine the impact of a coronary stenosis as recommended by the Davies' criteria. [44]. But the authors themselves agreed that there was no real way of understanding the haemodynamic significance of a stenosis at death and therefore the proposed criteria were an attempt at standardising the assumptions among pathologists based on observation and experience only. However, in clinical cardiology, it is now well established through robust research that the functional significance of a lesion correlates poorly with the apparent severity of the anatomical stenosis [59–63]. This is a critically important point and conventional autopsy has no current means to deal with this issue.

All these points discussed above highlights that the current post mortem gold standard of invasive autopsy has a number of fundamental limitations to accurately diagnose the presence and significance of coronary artery disease. Assessing coronary artery stenosis at autopsy in a collapsed vessel and using techniques such as passing a 1 mm probe to identify a severe stenosis seems a crude approach compared to the contemporary practice of clinical medicine. While this might be with the intention of simplifying the already lengthy and complex autopsy examination, this has to be balanced against the significant prevalence of coronary disease and the manifold implications of getting an accurate assessment at autopsy.

1.5 Argument for a change

The above discussion and evidence highlight that a large number of autopsies are still being carried out for medico-legal purpose which fall short in terms of any educational or scientific value. As stated in the Luce report in 2003, "such an extensive intervention done in such a large volume still lacks any empirical evidence to support its ongoing use in its current form. In case of any clinical intervention, this would have resulted in a large body of evidence to justify and modify any such procedures" [35,36]. Despite that report, there still has been no major change in the system.

Therefore, the whole autopsy process requires detailed re-examination in terms of its quality, suitability in modern society and its primary purpose. Also, public debate is required about the laws regarding autopsies, in particular to those in non-suspicious circumstances. It is not very plausible that the hospital autopsy rate will increase despite all the potential benefits if it still remains a lengthy, expensive and invasive procedure. Even many pathologists don't consider autopsy as a valuable tool anymore due to other competing interests in the field. [24,64,65] It is, therefore, important to modify and modernise autopsy to make it less invasive, more accurate and acceptable and still useful with regards to its clinico-pathological, epidemiological, educational and research value as well as serving the medico-legal purpose.

However, to establish any new approach over and above an existing gold standard, it has to be comparable, if not superior, to established methods. As coronary artery disease remains one of the major causes of death around the world, any new approach to autopsy has to be able to identify this condition with high accuracy compared to current techniques.

1.6 Minimally invasive autopsy: a new frontier

1.6.1 Overview

To overcome various challenges and limitations of conventional autopsy and to continue the practice of post-mortem examination for its manifold importance, there has been considerable interest in recent years in various minimally invasive and non-invasive methods with a view to either replace or complement the current approach to autopsy [8].

Among the alternative approaches to conventional autopsy, there is so called 'verbal' autopsy, or more appropriately termed post-mortem clinical case review; 'view and grant' system where external examination is conducted by a pathologist without dissection of the body; minimally invasive procedures like post-mortem endoscopic examination of certain organs of interest; needle biopsy of organs with or without ultrasound guidance and cross-sectional imaging using modern computed tomography (CT) and magnetic resonance imaging (MRI) scanners[66–71].

All the various approaches mentioned above have certain benefits and would often allow avoidance of complete dissection of the body in exchange for accepting a probable cause of death. Their application is often influenced by socio-economic, cultural and legal factors as well as research interest [8]. Amongst all of them, cross sectional imaging such as CT or MRI has the greatest appeal due to the non-invasive nature and perceived simplicity and speed of this approach together with the potential benefits from having permanent records with a view to audit and in training and education.

1.6.2 Post-mortem cross-sectional imaging:

There has been considerable interest in post-mortem cross-sectional imaging techniques and over the last decade, a lot of research has been done in this field. Dirnhofer et al. first used the term 'VIRTOPSY' to refer to their work in post-mortem

imaging using both CT and MRI [72]. Since then, various publications used different terms to describe variations in these techniques. Recently a group of experts in this field have suggested some standardized terminology *(Table 1.4)* for clarity and simplicity.

Table 1-4: *Terminology used for various modalities and approach in post-mortem cross-sectional imaging* (adapted from Rutty et al [73])

Abbreviation	Elaboration
PMCT	Post-mortem computed tomography
PMCTA	Post-mortem computed tomographic angiography- either whole body PMCTA or targeted PMCTA for the heart
PMMR	Post-mortem magnetic resonance imaging
PMMRA	Post-mortem magnetic resonance angiography
VPMCT	Ventilated post-mortem computed tomography
VPMMR	Ventilated post-mortem magnetic resonance

The majority of the early literature on post-mortem cross sectional imaging was based on single cases or small series of cases[74–83]. Roberts and colleagues published one of the earliest validation studies of post-mortem imaging in a series of 182 cases looking at the accuracy of PMCT and PMMR independently and in a combination against the actual cause of death as determined by subsequent invasive autopsy in adults in the UK [84]. They concluded that PMCT was superior to PMMR as a preferable modality with major discrepancy rate of 32% and 43% respectively when compared to standard autopsy. Even when the radiologist was confident that no autopsy would be required, the major discrepancy rate was still 16% and 21% respectively. The findings highlighted the fact that even in a controlled research environment, PMCT

or PMMR still could not provide a 'probable' cause of death in a significant proportion of cases, which is the minimum legal requirement for coronial autopsies. Failure to accurately identify cardiovascular disease, which was the commonest (40%) cause of death in the series, was one of their major weakness [85]. The researchers have however pointed out that this discrepancy rate is similar to discrepancy rates in death certificates [86] and a case has been argued in favour of PMCT on that ground. While there is some validity to this argument, there needs to be much more extensive debate about the importance of finding the accurate cause of death in all circumstances to make this a genuinely valuable process.

A major point of debate is whether the invasive autopsy is the best comparator. As mentioned above *(section 1.5.3)*, the gold standard itself has various pitfalls, sources of variability and lacks robust evidence base. Therefore, it raises the question whether some of the discrepancy is due to failings of the autopsy reference rather than failings of the imaging. This is a very challenging yet very important research question to investigate before imaging-based autopsy can be considered as a truly viable alternative. Therefore, it is of potential concern that PMCT is already being used with accuracy rates claimed as high as 99% while there is as yet not nearly enough scientific study to back that data [87].

1.6.3 Assessment of Ischemic heart disease in minimally invasive autopsy

To overcome the difficulty and to correctly identify coronary artery disease with significant luminal stenosis or thrombus, there have been further refinements of PMCT to incorporate contrast enhanced angiography or PMCTA. This idea has been strongly encouraged by development of multi-slice CT coronary angiography in a clinical setting which is now the recommended the investigation of first choice for most patients with suspected coronary artery disease [88]. Moreover, CT scanning of a deceased body has some advantages compared to the living. An immobile heart allows simpler scanning protocols. There is no concern about radiation-induced disease in the subject as long as staff are protected, and also non-sterile and relatively toxic materials can be potentially used as contrast. All these have led to significant interest in research and further development of PMCTA. Therefore, it is important to have some detail understanding of the various PMCTA techniques and

how well suited they really are to meet the challenges in accurate post-mortem diagnosis of coronary artery disease.

1.7 Post-mortem computed tomographic angiography

The pioneering work in this field has been done by the 'Virtopsy' group in Switzerland. They, for the first time, demonstrated contrast enhanced PMCT angiography of a whole adult body [89]. Since then, they have modified their technique in various stages using different protocols[89–93]. Subsequently, there have been two single centre UK based studies including more than 300 cases where the accuracy of PMCTA was directly compared against conventional autopsy [84,94].

1.7.1 Current state of evidence in support of PMCTA

The two studies mentioned above looked at the accuracy of targeted PCMTA in routine coronial autopsy cases in the UK and whether their implementation would reduce the number of invasive autopsies. Both of them used previously published targeted PMCTA techniques validated in smaller cohorts of patients [95–97].
The earlier study by Roberts et al. had 60 cases with PMCT and 60 cases with targeted PMCTA. The results showed that the need for invasive autopsy to identify a probable cause of death reduced from 62% in the PMCT group to 30% for those which had additional angiography. Importantly, PMCTA identified 82% of cases with ischemic heart disease compared to 15% with PMCT [98].

The recently published study by Rutty et al. [94] was a more structured study of more than 200 cases where there was a direct comparison made for the final cause of death identified by a consensus PMCTA report against that by coronial autopsy. They showed a high accuracy rate for PMCTA in identifying cause of death with 92% of cases correlating with autopsy and with major discrepancy rate of only 6%. Importantly, they reported a sensitivity and specificity of 97% and 99% for PMCTA in identifying a cardiac cause of death.

In terms of more detailed assessment of accuracy at a lesion level, there is currently only one study by Morgan et. al which showed good specificity but relatively weak sensitivity of PCMTA against histology based stenosis grading [99]. Once several modifications were applied to the gold standard of histology, sensitivity improved to 85%. However, there has been no replication of these results and while the findings are promising, it needs to be demonstrated whether they can be replicated in more routine practice outside the context of research.

1.7.2 PMCTA techniques

There are two main PMCTA techniques which have been established by various research groups, 'Whole body PMCTA' and 'Targeted PMCTA [96,100]. Each technique has certain advantages and disadvantages.

Targeted PMCTA is a relatively simple method, requiring only a few additional materials and personnel beyond a PMCT . A catheter, such as a "large balloon" Foley urinary catheter, inserted by cut down and simple dissection to the left carotid or axillary artery, which can be done by a pathologist or a trained mortuary technician. The catheter can then be progressed and will generally pass into the ascending aorta and a "bounce' will then be felt when the catheter meets the aortic valve. The contrast pump injector is standard equipment in CT suites and the contrast is routinely available so studies can be performed dynamically, i.e. the scans take place during active injection of contrast, allowing scanning under a certain amount of pressure within the vessel lumen [96].

However, the angiography is limited to only the heart and coronary arteries and therefore any other vascular pathology cannot be identified with any accuracy beyond PMCT. Also, the contrast extravasates rather rapidly and therefore the injection of contrast and scanning has to be done within a short period.

In contrast, the mostly commonly used whole-body angiography technique, multiphase post-mortem CT angiography (MPPMCTA) [93] requires complex and relatively expensive equipment and specially trained personnel to use a modified

heart lung machine (although a simple peristatic pump may produce similar results). Also, the paraffin and lipophilic contrast used are proprietary adding to further cost. However, as contrast enters the vasculature of the whole body and stays there for relatively long period, much more information about general vascular pathology can be gathered beyond the coronary arteries [97,101,102].

1.7.3 Limitations of current PMCTA approaches

The two studies [94,103] detailed above currently form the largest body of evidence in support of targeted PMCTA as a relatively reliable technique in identifying coronary artery disease at coronial post-mortem. However, there are some important limitations and the findings have to be taken into account with caution.

Firstly, both of these studies were single centre studies with modest numbers compared to the very large number of coronial autopsies performed every year [34]. Secondly, they lack a control arm to diagnose the cause of death based on demographics, history, events leading to death. As those factors play a significant role in making a diagnosis of probable cause of death, such a control arm is essential to determine the added value of PMCTA.

Thirdly, PMCTA was compared mostly to the naked eye assessment of coronary disease by the pathologist. The very limited use of histology in HM Coroner invasive autopsy practice in England and Wales remains a major drawback, as this should be used as the gold-standard for coronary artery disease, tissue ischaemia and myocardial infarction.

Fourthly, both studies refer to example images of coronary artery disease and concluded that the occlusion or stenosis of a coronary artery associated with hypo perfusion of the adjacent myocardium was a confirmatory sign of acute myocardial infarction leading to death. Also, the presence of severe stenosis in the absence of calcification has been considered as an acute plaque rupture event. However, there has been no histological confirmation of the presence of acute infarction or plaque rupture in any of these cases, which is the ultimate gold standard [45,46]. There is, as

yet, no major literature to support that these PMCTA findings are indeed diagnostic of those features as proposed.

Finally, the reported high accuracy rate of PMCTA requires further analysis. In a clinical setting, despite fewer confounding factors, the sensitivity and specificity of CT coronary angiography (CTCA) in identifying significant coronary artery stenosis compared against invasive angiography is in the range of 85-90% at most [104,105]. Therefore, the 96% accuracy rates in the series by Rutty et al. might reflect some selection bias and confirmation bias of the reporter due to the deceased's background history. It is also likely that the disease is more prevalent in those who have died compared to clinical cohorts. Therefore, while the findings are very encouraging, they still need to be replicated in larger cohorts, in case-controlled trials with routine use of histology, with an additional 3rd arm as mentioned above and outside specialist research centres.

While the conclusions and cause of death from these PMCTA studies would still be accepted by the coroner as a probable cause of death, there is a wider debate about the importance of higher accuracy of the diagnosis at post-mortem. As discussed above *(Section 1.2)*, autopsy played a vital role in development of modern medicine and imaging-based autopsy might provide us with a new opportunity to revive that role. With better social and cultural acceptability, faster turn-around time and permanent records of the findings, it can create a whole new paradigm of learning for clinicians. But that will be possible only if a more stringent approach is taken towards establishing an accurate cause of death. Without that, there is a real risk of replacing one inaccurate approach with another one.

Some of the current limitations are potentially due to the fact that routine coronial autopsies, which is usually the gold standard, themselves fall short of answering complex scientific questions such as the importance of non-obstructive plaque ruptures or the functional significance of lesions. Therefore, one might even argue that as there is likely to be a degree of repressurisation of the vessel in PMCTA during contrast injection, stenosis severity might be better assessed by imaging

compared to current approach in invasive autopsy *(Section 1.5.3)*. Also with further research, features such as acute plaque ruptures/ high risk plaques might be more recognisable on PMCTA as is the case with clinical CTCA [106].

However, this still would not resolve the problem entirely if the current gross anatomy based probabilistic approach is not modified to reflect the knowledge and body of evidence in clinical cardiology confirming repeatedly that the plaque vulnerability and functional significance of a stenosis is much more important than anatomical severity of stenosis in a significant proportion of cases.

Therefore, a new paradigm of culprit and functionally significant lesions, however challenging, is essential if we are looking towards autopsy using imaging as a new gold standard for the 21st century.

1.8 Clinical cardiology investigative tools: Do they have a role in autopsy?

As we have discussed, given the challenges of both the gold standard invasive autopsy as well as novel PMCTA techniques in accurately identifying and attributing coronary artery disease as a cause of death, it is important to review some of the other clinical cardiology tools and whether they might have a role in addressing these issues in an autopsy context.

It is crucial for the development of a more modern and scientifically robust post-mortem examination to see if these techniques might have any role in better understanding:

- The effect of depressurisation in post-mortem vessels

- The pathological importance of non-obstructive coronary artery disease to enable identification of sites of plaque rupture or erosion leading to coronary thrombosis without recourse to histology and
- The functional significance of a stenosis identified in an autopsy setting
- With a view to that, we briefly discuss the use of optical coherence tomography (OCT) and invasive and non-invasive assessment of functionally significant coronary stenosis using fractional flow reserve (FFR) and computational fluid dynamic (CFD) techniques respectively.

1.9 Optical coherence tomography (OCT)

Optical coherence tomography (OCT) is an intracoronary imaging technique and is considered to be highly accurate in identifying coronary artery lesions of various types and severity due to its sub-millimetre resolution [107,108]. It has been considered as a potential tool in autopsy by many experts in the field though more research is still needed. Therefore, we will discuss this imaging modality in further detail to explore its potential use in minimally invasive autopsy.

1.9.1 Overview

Optical coherence tomography (OCT) uses near infrared light-based technology and is capable of showing tissue microstructure. In 1991, Huang et al. first demonstrated its potential in medical imaging using ex-vivo examples of retina and human coronary artery [109]. Since then, OCT systems have gone through significant development. Real time imaging of coronary arteries using the intra-coronary OCT catheter in patients is now an established technique in clinical cardiac catheter laboratories [108,110].

In the following sections, the basic principle of OCT and its use in clinical cardiology are briefly discussed and its potential role in minimally invasive autopsy is examined.

1.9.2 Theoretical aspects

- Image formation

OCT uses reflection or backscatter of light from target tissue for creating an image in a manner similar to use of sound wave echo in ultrasound. However, the speed of light makes it difficult to directly measure the actual time of reflection from target. To overcome this, OCT uses interferometry. Light from the source passes through the interferometer and splits into two beams called the reference arm and sample arm. The reference arm signal is back reflected from a mirror at a fixed distance; hence the time delay is known. The sample arm signal is back reflected from different tissue structures. These two signals, when recombined, create an interference signal. The intensity and amplitude of the interference signal varies according to the type and depth of the tissue respectively. This is called an A scan. When a series of A scans across a tissue are combined, two dimensional (2D) images or B scans are produced. With addition of multiple B scans along the length of a tissue, a three dimensional image of the tissue can be constructed [108,109,111]. Use of near infrared light which has very small wavelength (1280-1350 nm) underpins major strengths and limitations of OCT.

- Different types of OCT system

The earlier OCT systems were called time domain OCT (TD-OCT). TD-OCT could only detect signal from a single point at a time making them unsuitable for imaging a significant section of coronary artery in a beating heart, where blood also has to be displaced with injection of other fluids. The new generation of OCT called 'Fourier' or 'Frequency' domain OCT (FD-OCT) systems can detect signal from multiple points simultaneously and are significantly faster. Hence, FD-OCT is currently used in cardiac catheter laboratories with a few commercial systems available [111,112].

1.9.3 Strengths and weakness of OCT and comparison with other modalities:

- Resolution

The very short wavelength of the near infra-red light used in OCT gives the technology a very high axial and lateral resolution of 10-15 micron and 20-40 micron respectively which is 10 times higher than intra vascular ultrasound (IVUS). This allows detailed imaging of tissue microstructure, only rivalled by cellular imaging techniques such as con-focal microscopy and histology [113].

The temporal resolution of the new FD-OCT systems is in the range of 200 frames/second, making this very suitable for real time imaging of long segments of the coronary arteries within a few seconds. This is also an advantage over IVUS where ECG gating is required for acquiring images from the same phase of the cardiac cycle for any volumetric assessment [114].

- Pre-requisite to displace blood

Another important technical point pertaining to OCT and other infrared optical imaging techniques is their inability to acquire images in presence of blood as the red blood cells absorb the light from the image source. This is a particularly important point when considering imaging of blood vessels in the living [109,114]. In clinical practice, this is easily achieved by forceful injection of angiography contrast into the coronary artery which automatically triggers image acquisition by the OCT catheter once blood has been sufficiently displaced. However, this occasionally limits the use of OCT in patients when contrast volume has to be kept as low as possible due to poor renal function or if there is risk of fluid volume overload.

- Depth of tissue penetration

This is limited to 1.5-2 mm due to optical characteristics of the tissue and the short wavelength of infra-red light. In contrast IVUS has a penetration depth of up to 10 mm. Hence, OCT has emerged as a preferred tool for assessing luminal morphology, luminal plaque characteristics and guidance on stent sizing and apposition in coronary intervention whereas IVUS retains its role in assessment of total plaque burden and assessment of aorto-ostial lesions in left main and right coronary artery

where displacement of blood is difficult and the penetration depth of OCT often proves to be inadequate [109–111].

- Risk of damage to the coronary artery

In clinical cardiology, the safety concerns related to OCT are primarily due to mechanical factors, as the very low energy delivered to the tissue is considered harmless. Like any intra-coronary device, there is risk of dissection, ischemia etc. Studies show that transient ischemic ECG changes, chest pain and ventricular ectopic activity are seen in 10-30% of cases with OCT which resolves as soon as the image is acquired [111]. With the shorter imaging duration of newer FD-OCT systems, these occurrences are significantly rarer.

1.9.4 Use of OCT in clinical cardiology:

Due to its various strengths, OCT has become a popular tool in clinical interventional cardiology. It allows assessment of coronary artery disease from various perspective to guide further management plans.

- Quantitative assessments of vessel dimensions and guiding coronary intervention

Modern OCT systems can measure the lumen and stenosis dimensions of a coronary lesion in a semi-automated and automated manner [111]. Due to its high resolution, speed and ability to image 75-100 mm length of coronary artery within seconds, OCT is now the modality of choice for accurately measuring the coronary arterial lumen and precisely sizing stents which has significant clinical implications [115].

- Imaging of coronary plaque

A large systematic study investigating histological correlates of different OCT findings performed on 357 cadaveric samples of diseased vessels demonstrated high sensitivity and specificity for several different types of tissue micro-structure encountered in an atherosclerotic plaque such as fibrous, calcific, fibro-calcific and lipid rich plaque. The sensitivity and specificity was 71% to 79% and 97% to 98% for

fibrous plaques, 95% to 96% and 97% for fibro-calcific plaques, and 90% to 94% and 90% to 92%for lipid rich plaque respectively [116]. Other studies have shown similar accuracy [117,118].

Plaque rupture, erosion and presence of thrombus are the main lesions seen in post-mortem studies of sudden cardiac death (SCD) [43]. OCT can identify these features with very high accuracy compared to any other imaging modalities. Also, OCT is the only clinical imaging modality which can identify thin cap fibro atheromas (TcFA) due to its very high spatial resolution. TcFAs are more prone to rupture leading to acute coronary events and associated with sudden cardiac death as shown in histopathological studies [40,119]. Thus, OCT essentially has become the tool of choice for performing virtual histology of coronary artery in living subjects and used widely in all relevant research.

Many of the studies mentioned above were performed using explanted coronary arteries, in whole or in sections, during post-mortem examinations to validate the OCT findings. As the accuracy of OCT findings have now been well established, this now holds potential to be useful in the autopsy setting and novel research is required in this area. If proven to be useful, it will potentially reduce the need for time consuming and expensive histology examinations during autopsy. Also as these assessments with OCT are done in an intact vessel, it also avoids the risk of any distortion that may arise from tissue sectioning and histological preparation and might prove to have some unique advantage [107,120].

1.9.5 OCT for post-mortem assessment of coronary artery disease during autopsy: Prospects and potential challenges

While the possible use of OCT in the context of autopsy had been considered in the past [121], the technology was still in the developmental stage and there has been no research towards this goal before the second decade of 21st century.

In 2013, Adlam et al.[122], for the first time, published a proof of concept paper demonstrating OCT of the right coronary artery in a cadaver in the context of

minimally invasive autopsy. This work suggested that it might be possible to use a cardiac catheter laboratory-based approach in the cadaver when performing 'in vivo' OCT of the coronary artery as part of minimally invasive autopsy. Also, it showed that OCT of these post-mortem vessels without any circulating blood provides a good quality image suitable for further analysis.

While this is an exciting prospect, there are several technical and logistical challenges which need to be overcome for using OCT in the autopsy context.

- Getting to the site of interest (coronary artery catheterization)

To pass an OCT catheter into the coronary artery, the coronary ostium has to be reached with a coronary guiding catheter and then a coronary wire has to be inserted into the coronary artery. In the cardiac catheter laboratory, this process is performed under fluoroscopic guidance with relative ease, significantly aided by the flotation of catheter in the blood vessels with an active circulation. However, it is not the case in the autopsy setting and Adlam et al. [122] report significant difficulty of catheter manipulation, requirement of very long fluoroscopy time and the need of a specially trained multidisciplinary team.

- Passing OCT catheter into the coronary artery

The next step is passing a fine wire inside the coronary artery, over which the OCT catheter can be passed. This again is aided by fluoroscopy and blood flow into the coronary artery, without which this would be extremely challenging and might result in significant iatrogenic injury to the coronary artery.

- Collapsed post-mortem coronary arteries

In the case of Adlam et al., the OCT was performed within a collapsed and de-pressurised coronary artery as is the case in routine pathological examination. The limitations of such assessment of depressurised coronary arteries have already been discussed. Therefore, it needs to be investigated how this can be overcome to get the most accurate data using OCT.

- Requirement of multidisciplinary team

Conventional autopsy is performed by mortuary assistants and pathologists. However, using OCT, in particular in an intact cadaver, will require involvement of personal trained in using fluoroscopy, cardiac catheter manipulation and interpretation of OCT. While mortuary technicians and pathologists can be trained to perform these procedures, the benefit of OCT or other minimally invasive approach has to be well established before advocating for such measure.

- Time and cost implication

In clinical setting, OCT is expensive and the cost is primarily driven by single-use of catheters. In cadaveric settings, these catheters can be re-used which reduces the cost significantly. OCT will be most useful where coronary artery disease is thought to have had significant contribution to death. Therefore, once the techniques are developed and validated, selective use of OCT as an adjunct to conventional autopsy for a virtual histology of coronary artery disease might be suitable and beneficial.

Therefore, while the work of Adlam et al. is encouraging, much more systematic research is required to establish any meaningful role of OCT in routine post-mortem examinations.

1.9.6 OCT as a useful tool in post-mortem research

The potential use of OCT during conventional autopsy is therefore still unclear. However, given its various strengths as discussed above, OCT can play a significant role to further research in this area. The effect of depressurisation in post-mortem coronary arteries, identification of high risk or culprit plaques and functional significance of a coronary stenosis are unresolved questions in post-mortem assessment of coronary artery disease. OCT can have a unique role as a research tool in this context as discussed below.

- Investigating the effect of depressurisation in cadaveric coronary arteries:

As discussed in *Section 1.5.3.2.2,* there are several effects of depressurisation of a coronary artery after death which potentially can lead to an erroneous conclusion by a pathologist [123]. However, there is no systematic study looking into the effect of depressurisation and thereby any potential benefit of repressurisation of cadaveric coronary arteries. To study that, accurate measurement of in-situ coronary artery dimensions is required as explanting the arterial architecture from the heart muscle might introduce systematic error. OCT of in situ re-pressurised cadaveric coronaries could be the perfect tool for such investigation on how the coronary arterial dimensions alter with and without pressure and how its compliance might vary across its length involving normal and diseased segments.

- Identification of 'culprit' lesions

OCT has the ability to identify 'culprit' and high risk coronary atherosclerotic lesions in the living associated with cardiac death. If techniques used during the histopathological correlation and validation studies of OCT [116,124,125] are modified and applied in the context of minimally invasive autopsy, this could potentially lead to significant improvement over and above the current approach.

- Assessment of stenosis severity

Anatomical severity of a coronary stenosis still remains one of the key questions investigated during autopsy despite its various weakness. OCT can measure luminal stenosis in a highly accurate and objective manner. Therefore, If OCT examinations could be performed in otherwise intact and re-pressurised coronary arteries, this could significantly improve the accuracy of the assessment of stenosis severity [115].
The above discussion highlights that the various advantages of OCT could prove significantly useful if used in the autopsy setting, by improving accuracy, and quality of post-mortem examinations as well as providing a permanent record for training and audit. However, there are also challenges including training, time and cost implications and most importantly a lack of adequate evidence in this context. Therefore, given the potential, there is a strong argument to direct research in this field to see if these challenges can be overcome and to assess whether OCT really has a role in the mortuary.

1.10 Functional assessment of coronary artery disease

As mentioned in *Section 1.5.3.2.4,* one of the fundamental limitations of the current gold standard of invasive autopsy is its reliance on anatomical severity of coronary stenoses rather than functional significance.

In stable coronary artery disease in the living, the risk of a myocardial infarction or death is much more strongly related to the burden of myocardial ischemia than to the anatomical severity of stenosis. Hence, a range of non-invasive diagnostic tests have been developed to assess myocardial ischemia. This includes exercise tolerance test, stress imaging such as stress echocardiography, myocardial perfusion scan and stress cardiac magnetic resonance imaging (MRI). All these non-invasive investigations can provide valuable information regarding the total burden of myocardial ischemia and longitudinal studies have shown correlation of high ischemic burden with poor outcome. However, invasive fractional flow reserve (FFR) is the first and the current 'gold standard' modality which can directly identify ischemic potential specific to individual coronary artery lesions during invasive angiography[126,127].

1.10.1 Coronary fractional flow reserve (FFR)

Pijls et al. first showed that, under certain conditions, the ratio of intra coronary pressure across a coronary stenosis correlates with downstream flow and hence predicts the extent of ischemia in the relevant myocardial territory. In an in-vivo animal model, they measured both pressure and blood flow through different grades of stenosis and at different ranges of blood pressure and very elegantly demonstrated a very strong correlation between the two. This confirmed the method's ability to identify lesion specific ischemic potential. The measure is now defined as fractional flow reserve (FFR) [128,129].

Based on the experimental model, FFR has gone through extensive clinical validation. Of the many studies, the randomized and multicentre FAME trial (Fractional Flow Reserve versus Angiography for guiding Percutaneous Coronary Intervention) provided the most conclusive evidence for the superiority of FFR over the anatomical assessment of a lesion for decision making in coronary intervention. The international guidelines have given FFR guided decision making in coronary intervention the highest recommendation[130,131].

Therefore, if such measures could be applied in the autopsy setting, the post-mortem assessment of coronary artery disease severity would become more functionally relevant and objective compared to the current subjective and largely probabilistic approach.

However, translation of FFR in the autopsy setting will pose a number of significant challenges. Some of those challenges will be technical, but most importantly the absence of physiological blood flow is potentially a fundamental limitation. But, as FFR in the living is independent of haemodynamic parameters, it is still reasonable to investigate if artificially generated flow could be used in the cadaver to derive parameters similar to clinical FFR to assess physiological significance of a coronary stenosis. Alternatively, options which would not necessarily require such complex invasive measures but still might provide a reliable functional assessment should be explored.

1.10.2 Computational flow dynamic (CFD) modelling and virtual fractional flow reserve (FFR)

The use of invasive FFR is limited to the cardiac catheter laboratory and requires a trained interventional cardiologist. This limits its use to patients who have already been assessed to have potential for significant coronary artery disease on the basis of clinical assessment and may require coronary intervention. As a result, many patients have to undergo a relatively invasive procedure who often don't have a suitable target for coronary intervention following FFR assessment.

To overcome this issue and to reduce the number of patients having invasive procedures, investigators have been looking into the possibility of non-invasive or less invasive FFR derived from imaging studies, largely obtained by CT scans (non-invasive) or from conventional diagnostic coronary angiogram (less invasive) only. Such an approach has been largely possible due to development of techniques known as computational fluid dynamics modelling [132–134].

Due to its non-invasive nature and the fact that the data required is derived from CT coronary angiography, adaptation of CT-FFR to autopsy practice might prove an important adjunct to PMCTA such that a stable coronary stenosis identified during post-mortem imaging can be assessed for its potential functional impact during life in an objective and reproducible manner.

As there is increasing evidence to support the use of PMCTA, translation of the CFD techniques for predicting an FFR equivalent value could be a more realistic approach than some of the invasive techniques (OCT and FFR) described above.

1.10.2.1 What is CFD and FFR_{CT}

Computational fluid modelling is based on the application of the knowledge of fluid dynamics in a certain environment to predict the behaviour of flow and its variations within that environment. The flow of fluid or air through the model is predicted using the governing equations of fluid dynamics, collectively known as Nevier Stokes equation [134,135]. The principles of CFD are universally applicable to various circumstances such as airflow in a jetliner or flow of blood within a vessel.
To model flow of blood through a vessel, the pre-requisites are: an anatomical model of the blood vessel; boundary conditions at the inlet, outlet and luminal aspect of the model; and finally the CFD modelling calculations. CFD modelling requires considerable knowledge and skills in fluid dynamics and computation, solving millions of equations simultaneously using powerful computers to provide a numerical output which then requires subsequent processing and visual representation.

1.10.2.2 Role of CFD in coronary artery disease assessment

Kim, H. J. *et al.* elegantly demonstrated a computational model that can predict coronary blood flow under various physiological conditions such as rest and exercise. This concept has been applied to 3D coronary anatomy derived from non-invasive CTCA to determine flow variation through normal and stenosed coronary artery segments. Subsequently, in a number of validation studies, it has been shown that a numerical value can be generated reflecting the flow of blood through coronary arteries and through various grades of stenosis.

The term FFR_{CT} has been coined as based on the modelled flow and pressure, a numeric figure similar to invasive FFR can be calculated. Studies have shown a good correlation between true invasive FFR and FFR_{CT} making it a potentially useful non-invasive alternative. A number of clinical studies have been performed comparing the accuracy of identifying significant stenosis using this computational derivative, FFR_{CT}, against conventional CTCA and invasive FFR, which is the gold standard in predicting ischemia in a coronary artery territory. FFR_{CT} performed significantly better compared to CTCA alone in identifying significant coronary lesions as identified by invasive FFR. While this is not a replacement for invasive FFR, FFR_{CT} significantly improved the positive and negative predictive value of CTCA for intermediate lesions using a non-invasive dataset and potentially improved patient selection for invasive treatment strategies. Due to the highly promising clinical trial data, the use of FFR_{CT} in day-to-day practice is now being evaluated through a national programme funded by NHS improvements. All the clinical trials highlighted the fact that FFR_{CT} is certainly superior and more reproducible compared to CTCA alone, which is subject to the interpretation of the reporting clinicians [136].

1.10.2.3 Potential role of CFD modelling in Autopsy setting

Although invasive autopsy is the current gold standard for post-mortem assessment of coronary artery disease, the approach has several limitations (section 1.4.4) to an extent which arguably would not be acceptable for a diagnostic paradigm in the living. PMCTA, on the other hand, has some potential advantages as it is non-invasive, creates a permanent record allowing for better training, audit and

improvements and so far, the evidence shows good correlations compared to conventional autopsy. However, PMCTA is also primarily focussed on identifying anatomical severity of a stenosis and therefore, has the same drawbacks as conventional autopsy by failing to ascertain true functional significance of a stenosis.

Therefore, while PMCTA has certainly some attractions, that alone will not be able to meet the level of objectivity, reproducibility and clinico-pathological correlation consistent with current clinical understanding of coronary artery disease. On the other hand, CFD modelling of coronary flow using the PMCTA dataset holds great potential. Currently these techniques have mostly been used in the context of clinical research and only very recently, has widespread commercial use really started. Given the potential improvement that can be achieved in the accuracy of post-mortem assessment of coronary artery disease, it is very important to direct research in this area along in parallel with further development of PMCTA.

1.11 Conclusion

Based on the discussion above, it is apparent that there is a strong case for the current post-mortem practice to be overhauled and modernised as a whole and in particular with regards to assessment of coronary artery disease given its high prevalence. Current knowledge about non–obstructive coronary culprit lesions as well as the importance of functional rather than anatomical significance of stenoses needs to be taken into account. It is apparent that even the 'gold standard' approach recommended by guidelines and experts would not always be sufficient to answer some of these issues. As a new technique, PMCTA seems a very promising area with the potential to compete and even outperform the conventional autopsy in many of these cases.

However, further research and innovation is required to address many of the issues discussed above. Several potential advantages are apparent if clinical coronary artery disease assessment techniques can be reliably translated into minimally

invasive autopsy practice. Tools and techniques such as PMCTA, OCT and CFD modelling of blood flow need to be rigorously tested for their suitability, reliability and reproducibility for the primary purpose of identifying a cause of death at autopsy more accurately. Also, their additional value in creating permanent records that can be used for training, audit and educational purpose as well as their contribution to new knowledge in understanding the pathophysiology of the disease also requires scrutiny.

1.11.1 Aim and objectives of this research

This thesis aims for a systematic experimental approach towards achieving some of these goals with the following aims and objectives:

Aim: A systematic study on the post-mortem assessment of coronary artery disease by investigating the accuracy of PMCTA and the effect of repressurisation on post-mortem coronary arteries using clinical cardiology tools.

Objectives:

1. Systematic comparison of PMCTA and histology in the assessment of coronary artery stenosis
2. Developing and validating a repressurisation technique and testing the feasibility of using optical coherence tomography (OCT) in re-pressurised porcine coronary arteries
3. Systematically investigating the effect of repressurisation on vascular dimensions in re-pressurised porcine coronary arteries using OCT
4. Systematic investigation of the effect of repressurisation on cadaveric coronary arteries using OCT
5. A proof of concept study investigating the feasibility of computational fluid dynamic (CFD) modelling techniques using post-mortem imaging data from PMCTA and OCT

2 Chapter 2: Methodology

2.1 Introduction

In this chapter, general aspects of the methods and techniques used in this thesis are discussed. *Appendix B* contains a full list of the equipment used for this study. Specific methodology pertaining to different sections of the study are discussed in relevant chapters.

2.2 Study design

A planned experimental design was used for all the different elements of the study with the exception of the proof of concept work on application of CFD modelling of blood flow in the post-mortem context. This work is therefore presented in a descriptive manner.

2.3 Ethical considerations and consenting:

The project had ethical approval from the Leicestershire regional ethics committee. (REC ref. 04/Q2501/64). Also, the Leicestershire coroners were in full agreement for the study.

For the cadaveric experiments, a specially trained nurse specialist obtained consent from the next of kin via telephone. This process has been used for post-mortem research in the department and has been well validated [31]. A detailed information pack was also sent to the next of kin by fax or post where requested. A consent form detailing the experiments for which consent was obtained was signed by the

consenting nurse on behalf of the relatives and filed in the pathology department for future records and audits.

Where applicable, specific consent for tissue retention was obtained. All the specimens for histology were processed and stored in a Human Tissue Act (HTA)[137] authorised laboratory within the forensic pathology department.

2.4 Case selection

2.4.1 Porcine heart model

- Source:

The pig heart was used as a model of the post-mortem human heart, given the challenges associated with obtaining suitable human material, to allow method development and refinement. Porcine hearts were collected with permission from a local abattoir. The animals were killed for human consumption as per the national regulation. The hearts were dissected out with a small portion of the aorta and pulmonary trunk. They were collected in a closed box at room temperature and transported to the laboratory within 1 to 2 hours from time of death.

- Preparation and storage:

In the laboratory, excess tissue was dissected, and the hearts were rinsed in tap water. The ventricles were emptied of any blood clot. Hearts which sustained significant injury to the coronary arteries or ventricles were discarded.

During the initial set-up phase, hearts were stored in sealed containers in the fridge for up to 3 days or the freezer for longer depending on the planned experiment dates and where required, they were defrosted 6-12 hours before the experiment.

Once the technique of OCT of the re-pressurised vessels was fully developed, a set of 4 hearts was used for the systematic study on the effect of repressurisation on vessel

dimension. These specimens were stored at 4^0 C for up to 3 days until the experiment was performed.

All the specimens were disposed of using the animal tissue disposal process as required by the University of Leicester.

2.4.2 Cadaveric study

The cadaveric cases were selected from the daily referrals for autopsy to the department of pathology by the regional coroners. Only cases of non-suspicious unexplained death were considered. On designated study days, the cases were selected where the background history did not preclude the planned experiments (e.g. major trauma to the chest/heart from road traffic accident) and informed consent from the next of kin could be obtained before the end of the working day 17:00 hours.

- Time scale

The timing of the whole research process was co-ordinated with the standard workflow of the post-mortem process. Most coronial post-mortems are carried out the following morning after receiving the referral from the coroner's office. Therefore, once referral for a post-mortem was received by the mortuary, a member of the research team screened the referral and identified potentially suitable subjects by mid-day. The referral was then forwarded to the consenting nurse who had to contact the next of kin and obtain a consent by 5 pm of the same day. If successful, PMCTA was undertaken in the evening by the research team. Invasive post-mortem was then carried out the following morning and experiments on the cadaveric heart had to be completed by mid-day to allow completion of the post-mortem examination before the end of the day. This restricted any experiment time on cadaveric hearts to 3-4 hours at most.

- Location of experiments

The PMCTA catheter was inserted in the mortuary area prior to moving the body for scanning. PMCTA was undertaken in the clinical CT scanner ensuring appropriate transportation and full confidentiality as required by the local guidelines and the Human Tissue Act. [137] All the repressurisation and OCT experiments were performed in a designated room within the mortuary. *Figure 2.1* below shows the various steps of the research process:

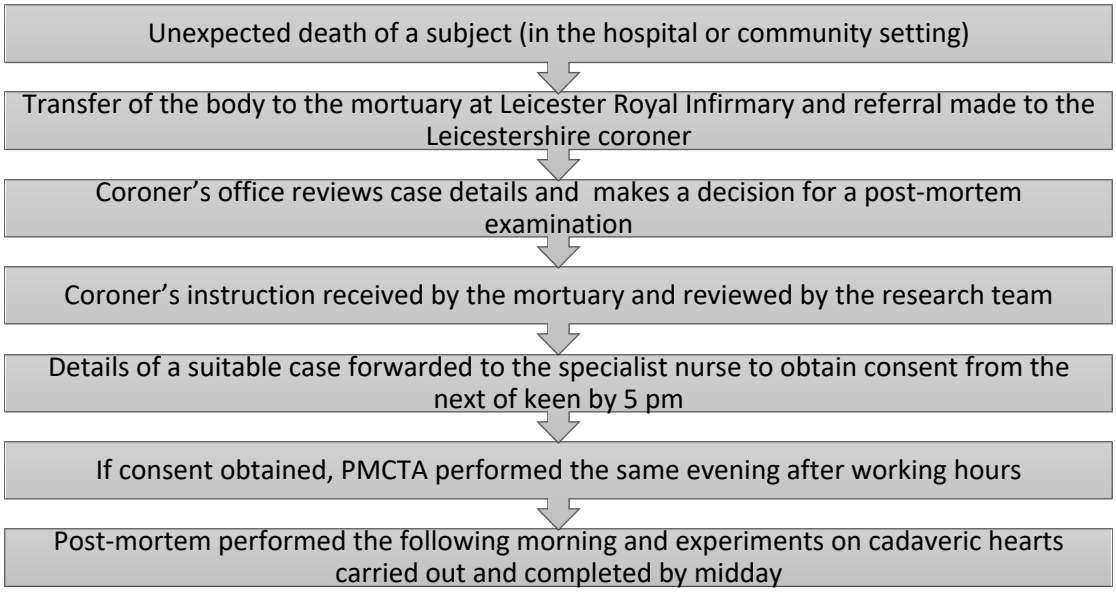

Figure 2-1: A flow diagram of the research pathway

2.5 Repressurisation

The principles used for repressurisation of the post-mortem porcine and cadaveric vessels was similar to those of others[138–140] where a pressurised fluid was passed into the coronary artery through a conduit. However, the method of pressure generation and the technique for pressure recordings were different.

2.5.1 Pressure generation

Clinical sphygmomanometers, used for measuring arterial blood pressure, were used where the cuff of the sphygmomanometer was wrapped around a bag of fluid used as

the perfusate. Plain tap water or saline was used for the porcine experiments and normal isotonic saline was used for the cadaveric studies.

An infusion set connected to the bag of fluid was attached to the proximal end of a 6 Fr. Size coronary guide catheter. The guide catheter was then attached to the proximal end of the desired vessel fixed with an external suture. This prevented any leak of fluid and kept the catheter fixed in place.

2.5.2 Pressure recording

A pressure transducer was placed between the tubing of the infusion set and the guide catheter. A coronary pressure wire *(PressureWire™ CERTUS™, ©Abbott, Maidenhead, UK)* was passed through the guide catheter into the coronary artery to record pressure within the vessel. Both the pressure transducer and the pressure wire were connected to a *RadiAnalyzer*™ pressure wire system (©*Abbott, Maidenhead, UK*). This set up provided pressure readings from three different points, namely the sphygmomanometer, the pressure transducer and the pressure wire.

The pressure in the sphygmomanometer reflected the pressure within the bag. The pressure at the transducer reflected the exact pressure of the fluid column entering the guide catheter. The pressure reading from the pressure wire reflected the exact pressure within the coronary artery. *Figure 4.10* shows the set-up with a porcine heart.

2.6 OCT

2.6.1 Equipment

Two commercially available unit (*ILUMIEN™ and ILUMIEN™ Optis, ©Abbott, Maidenhead, UK*) were used for performing the OCT studies in the porcine and cadaveric hearts respectively. Two different models of OCT catheters were used (*C7 Dragonfly™ and Dragonfly™ Duo Imaging Catheter, ©Abbott, Maidenhead, UK*) depending on availability.

2.6.2 Catheter preparation and placement

The catheter was flushed through its side channel as per the manufacturer's instruction using saline or plain tap water. This cleared any air bubbles within the OCT catheter. This was then connected to the dock of the OCT machine, which ran automated tests and to confirm the functionality of the catheter. If this test showed any error, the catheter was discarded and a different one was used. The built in autocalibration process of the OCT system was used before every measurement. In this step, the known diameter of the OCT catheter is used by the system to calibrate all further measurements.

The tip of the catheter beyond the entry point of the monorail wire was also cut short to allow more distal placement of the OCT lens. This allowed imaging of a relatively longer length of vessel. A catheter was reused for multiple recordings till there was a mechanical failure or any visible degradation in the image quality.
Once the pressure wire was in the desired location within the coronary artery, the OCT catheter was passed over the pressure wire through the guide catheter into the coronary artery using the over the wire monorail system.

2.6.3 Image acquisition

Once the vessel was re-pressurised to the desired level, an automated pullback was performed. This imaged 54 mm of the coronary artery and guide catheter in a retrograde manner. The pullback speed was set at 20 mm/second for all recordings. This allowed for high-resolution 'virtual histology' imaging. The OCT lens automatically went back to its original position at the end of each recording allowing repeat imaging of the same section of the vessel at different intra-vascular pressures.

2.6.4 Data collection and analysis

All the OCT images were stored on the machines hard drive. The repressurisation data and the timing of the OCT for each recording were documented in a data sheet for cross-referencing.

For the porcine study, the OCT images were analysed using established in-built software of the vendor and all the necessary data were collected on data sheets for further analysis.

For the cadaveric study, all the OCT image data were downloaded from the St. Jude's OCT system to a generic hard drive. They were initially reviewed by an open-source DICOM reader (*Osirix MD, © Pixmeo SARL, Switzerland*) for image quality, artefact, and presence and extent of atherosclerosis in selected frames. For measuring vessel dimensions across the length of the vessels, which constituted several thousands of frames, a proprietary software (*QCU CMS ™, Medis Medical Imaging System, Netherlands*) by our collaborators was used which allowed automated extraction of the vessel measurement data from the RAW OCT data files, unlike the vendors inbuilt system which would have required going through each frame and manually documenting the figures. Thus, the vessel cross sectional area and diameter for each pair of OCT pullbacks at low and high pressure were collected.

2.7 PMCTA

Two well validated PMCTA techniques were used for investigating the accuracy of PMCTA in assessing coronary stenosis compared to histology. Both these techniques have been well described in literature.

2.7.1 Targeted PMCTA

This technique was developed and published by the forensic pathology and radiology research group at University of Leicester [96,102]. This allowed a CT coronary angiogram of the deceased using both air and water-soluble radiopaque contrast, delivered through a Foley catheter, inserted directly into a cut down preparation of the carotid artery. An automated power injector was used for injection of both air and the contrast medium. A total of 5 scan sequences were performed, starting with air as a negative contrast medium and subsequently injecting radio-opaque contrast.

2.7.2 Whole body PMCTA

Whole body PMCTA was performed using a technique called Multi-phase Post mortem computed tomographic angiography (MPPMCTA) developed in Laussanne, Switzerland with the support of *Fumedica AG, Luzernerstrasse,Schweiz* [100,141,142] . A modified heart lung machine was used for delivery of paraffin oil and contrast medium through femoral artery and vein. In the first step, the vascular tree of the body was flushed with paraffin oil to remove any post-mortem clots and to causes micro-embolization to prevent extravasation. In the second step, a radio-opaque contrast agent was delivered. Subsequently a whole-body CT scan was performed.

Studies have shown this method to be able to identify both coronary artery disease and thrombus when compared to conventional autopsy. [90]

2.8 Autopsy and histology

A number of pathologists who were all fully trained, and part of the research team performed the autopsies according to guidelines *(Section 1.5.2)* [45].

For the comparative case series of PMCTA and histology, procedures similar to those described by Morgan et al.[99] were used. The vessels were dissected out along with the proximal part of the aorta and formalin fixed after being photographed. They were decalcified in 10% formic acid if necessary, as per guidelines. They were manually sectioned at a distance of every 2-3 mm. Microscopic slides stained with haematoxylin and eosin were then prepared from their proximal cut surfaces. One senior and highly experienced forensic pathologist scored each slide for the severity of coronary stenosis.

2.9 CFD

PMCTA images of several cases were reviewed and a vessel was selected which could be easily segmented from the full dataset without any artefact and CFD modelling technique was applied. Chapter 7 describes this proof of concept work in detail.

2.10 Statistical analysis

Data was entered into Microsoft Excel© (*Microsoft Corporation, One Microsoft Way, Redmond, USA*) spreadsheets and was subsequently transferred to SPSS© version 22.0 (*IBM corporation, Armonk, New York, USA*) for all statistical analysis. Data is presented as mean +/- standard deviation or median and range where data is not normally distributed. Independent sample T test and one-way ANOVA was used for comparing parametric data and Mann Whitney U test was used for comparing non-parametric data. Pearson's correlation test was used for continuous variables. Cohen's kappa statistics was used to test the strength of agreement between different PMCTA reporters on severity of stenosis of each segment. P value of <0.05 was considered to be significant.

3 *Chapter 3: Post-mortem assessment of coronary artery stenosis using post-mortem computed tomographic angiography (PMCTA): A systematic comparison with histology*

3.1 Introduction

In section 1.7.1, the role of PMCTA in assessing coronary artery disease during post-mortem examination and the current evidence base in the field has been reviewed. While there seems to be a good correlation between PMCTA findings with conventional coronial autopsy, there are still various limitations which have been identified (*section 1.8.3*). If PMCTA is to replace conventional autopsy in any meaningful way, its accuracy in assessing coronary stenosis has to be validated in a more stringent manner. Therefore, we conducted a systematic study of 10 PMCTA cases using two different techniques with the following objectives:

- To determine the diagnostic accuracy of PMCTA for identifying different degrees of coronary artery stenosis when reported by trained individuals with varying background, compared to histological grading of stenosis by a pathologist
- To indirectly compare the accuracy of two different PMCTA techniques
- To determine the level of agreement among different observers on the severity of coronary artery stenosis seen on PMCTA

3.2 Materials and methods

Chapter 2 detailed the ethics and consenting process, logistics and the principles of the PMCTA techniques used in this study.

3.2.1 PMCTA examination

3.2.1.1 Scanning

The 10 PMCTA scans were conducted on 9 different days over a period of 13 weeks. The first five cases underwent targeted PMCTA and the next five cases underwent whole body PMCTA as discussed in section 2.7. A *Toshiba Aquilion* 64 slice CT scanner was used for the scans after 5 pm on the designated study day.

3.2.1.2 PMCTA analysis and reporting

Three independent reporters, trained in cardiac CT with varying experience and background (reporter 1-a cardiology specialty registrar; reporter 2- an advanced forensic practitioner; reporter 3- a cardiac radiology consultant with experience of PMCT reporting), reported all the scans separately. All of them used the same raw dataset. The first 2 reporters used an opensource software platform (*Osirix, Pixmeo, Switzerland*) and the third reporter used commercial clinical cardiac CT reporting platform (*Syngo via, Siemens AG, Germany*) both of which are validated and routinely used. Subsequently, reporter 1 and 3 had a joint second reading for a consensus report. This consensus PMCTA report was used for all subsequent analysis.

For each case, all the three major coronary arteries were analysed. The left main stem (LMS) was analysed as part of the left anterior descending artery (LAD). The left circumflex (LCx) artery distally extended either into the atrio-ventricular (AV LCx) or obtuse marginal (OM) branch whichever was the larger. The right coronary artery (RCA) included the posterior descending artery (PDA) distally.

A visually estimated stenosis grade at 10% interval (0% to 100%) was assigned to each millimetre length of the vessel. The origin for each artery was determined by identifying the earliest point in longitudinal view where a full cross section of the vessel could be viewed and the endpoint was determined distally, beyond which the

reporter could not identify the vessel with confidence. Presence of calcification, large side branches, and blood clots were also identified for each mm of vessel. All the reporters were blinded to the autopsy report and histology.

3.2.2 Autopsy

3.2.2.1 Post-mortem examination and coronary artery histology

The post-mortem examination was performed the following morning after the PMCTA following a standard protocol for coronial autopsy. Assessment of coronary artery disease and subsequent histology was performed as described in section 2.8.

3.2.2.2 Coronary artery histology and reporting

Each histological section was approximately 2-3 mm apart. Two experienced forensic pathologists reviewed the slides and visually assigned a degree of stenosis to each section of the coronary artery. For each section, they also commented on presence of calcification, sidebranch and clots.

3.2.3 Co-registration of PMCTA and histology

3.2.3.1 Vessel identification

Data for each vessel was recorded in a separate spreadsheet. The PMCTA and histological stenosis grading for each vessel were aligned on a spreadsheet along with information about calcification, clot and presence of side branches.

3.2.3.2 Mathematical adjustment for length

PMCTA stenosis was graded for every millimetre of vessel as mentioned above. However, for histology, each vessel was initially sectioned and processed into a number of paraffin blocks which were subsequently sliced in a microtome 2-3 mm apart and numbered from proximal to distal on to the slide. To adjust for this difference, the stenosis severity for every 3 mm of PMCTA was averaged and compared against 1 corresponding histology section.

3.2.3.3 Anatomical adjustment

PMCTA and histology reports for each vessel were reviewed side by side along with information about origin, calcification and branching. As the vessel was intact during PMCTA, with regards to vessel length the histology was adjusted according to that. If there was an obvious mismatch in corresponding vessel section based on the calcification and branching pattern, the histology report was either adjusted proximally or distally along the length of the CT report to ensure accurate co-registration.

3.2.4 Qualitative grading of stenosis severity

The percentage stenosis of each 3 millimetre of the PMCTA and histology sections were converted to qualitative grading according to the SCCT guideline for severity of coronary artery stenosis. (*Table 3.1*) [143] with one modification. Both normal and minimally diseased segments were graded in the same category as it was assumed that PMCTA is unlikely to be able to differentiate between these two very close categories and also that this modification would not alter the ultimate objective of identifying significant lesions by PMCTA. Both grade 3 and grade 4 were considered as significant stenosis (50-99%) for determining specificity and sensitivity of PMCTA.

Table 3-1: *Grading of stenosis severity in CT* (adapted from SCCT guideline [143])

Stenosis severity	Grading
<25%	Grade 1/ normal or minimal disease
25-49%	Grade 2 / mild disease
50-69%-	Grade 3 / moderate disease
70-99%	Grade 4/ severe disease
100%	Grade 5/ total occlusion

3.2.5 Segmentation of the vessels for comparison of stenosis severity

For the purpose of comparison, each vessel was divided into several 1 cm (approximate) segments, representing every three to four original histology sections (9 to 12 mm). Each of these segments in both modalities was then compared for agreement or disagreement for the maximum stenosis within that segment. *(Table 3.2)*

3.2.5.1 1 cm segments

There were 317 segments of approximately 1 cm length (9.28±1.21 mm) of which 252 segments were successfully co-registered between PMCTA and histology. The rest were excluded as one or the other modality did not have results available for corresponding sections. *Figure 3.1* shows a flow chart detailing the numbers of cases and eventual segments analysed.

Table 3-2: *Criteria for agreement or disagreement between PMCTA and Histology*

Agreement status	Agreement grade
The same maximal stenosis in both PMCTA and Histology	Complete Agreement
1 grade of difference between the two modalities without severe stenosis or occlusion on histology	Minor disagreement
2 grades difference between the two modalities without severe stenosis or occlusion on histology	Moderate disagreement
3 grades difference between the two modalities, or any disagreement involving a severe stenosis on histology	Major disagreement

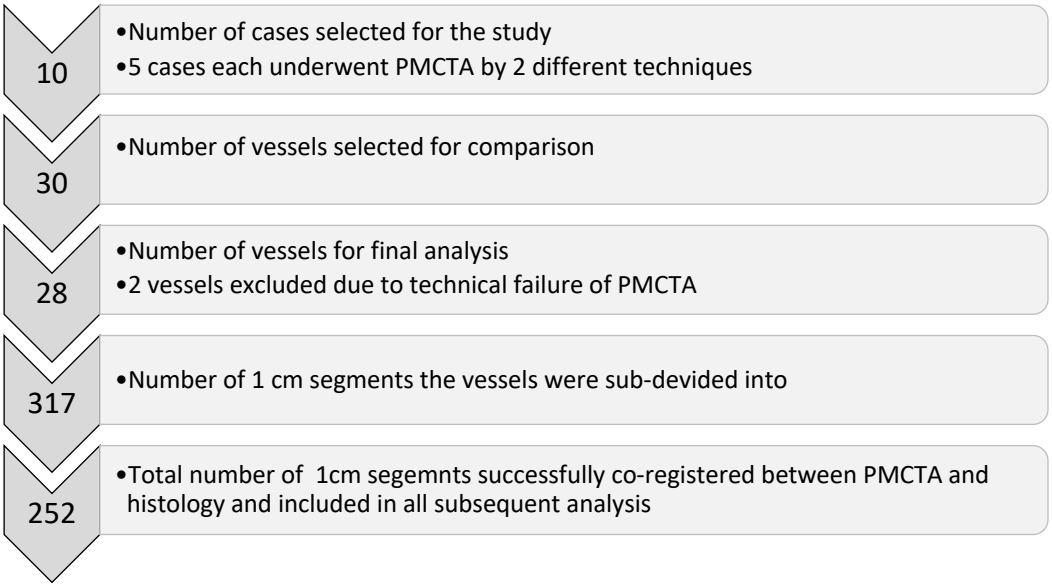

Figure 3-1: *Flow diagram showing the numbers of vessels and segments analysed in this study*

3.2.6 Inter-observer agreement on PMCTA reporting

2 cases constituting 20% of the total study population were selected for this analysis. The cases were identified using an online random number generator, one from the targeted PMCTA group and one from the whole-body angiography group.

All the segments identified by each pair of reporters were used for the analysis. Also, each reporter's PMCTA report was compared with the consensus PMCTA report. Inter-observer agreement was checked for identifying all four grades of stenosis as well as for identifying significant stenosis (moderate to severe) only. Cohen's kappa statistics was used.

3.2.7 Comparison of final cause of death by PMCTA and conventional autopsy

An expert forensic radiologist reviewed all the available information from the autopsy request including medical history where available and the PMCTA scans and provided a cause of death. This was compared against cause of death provided by conventional autopsy.

3.3 Results

3.3.1 Baseline characteristics

Table 3.3 shows the demographic and other characteristics of the group undergoing PMTCA

Table 3-3: *Patient characteristics and time elapsed between death and PMCTA.*

Characteristics	Total Study Population, N=10, %
Age at death (median, range)	79, 31-93
Male	6, 60%
Female	4, 40%
PMH	
• Unknown	6, 60%
• Cardiovascular disease or risk factors	3, 40%
• Mental health issues	1, 10%
Hours elapsed between death and PMCTA (median, range)	51, 8-160

3.3.2 Number of vessels and length analysed

Histology was available for the whole coronary tree of all the 10 cases and as described in the methodology, the 3 major epicardial coronary arteries for each case were selected to compare with PMCTA. Out of the 30 major epicardial vessels, 28 were clearly identified by both PMCTA and histology, which were selected for further analysis (10 LAD and RCA, 8LCx). In 2 cases, the contrast injection did not open up the LCx during the whole-body angiogram and were excluded and classed as technical failure.

The total length of the 28 vessels analysed by PMCTA was 2710 mm and by histology was 2562 mm. The mean length of the vessels in PMCTA was 96.79±37.12 and in histology was 91.50±35.96 mm, which was not significantly different (p=0.298).

3.3.3 Baseline characteristics of the 2 groups scanned using different PMCTA

The cases in the whole-body angiography group were older. But otherwise, the two groups were largely similar in baseline characteristics. There was technical failure on 2 occasions for the whole-body angiography approach where 2 small calibre LCX vessels were not opened up by the contrast injection. *Table 3.4* shows important characteristics of the different PMCTA groups.

Table 3-4: *Case characteristics for targeted and whole body PMCTA*

Characteristics	**Targeted PMCTA**	**Whole body PMCTA**
No. of cases	5	5
Age at death- years, (Median, range)	61,31-82	84, 66-93
Sex (Male, %)	3, 60%	3, 60%
Known Cardiovascular disease/ Risk factors	1, 20%	2, 40%
Time elapsed between death and PMCTA – hours, (Median, range)	50, 8-54	56, 29-160
No. of vessels analysed	15	13
Technical failure	0 vessel	2 vessels
No. of segments analysed	157	160
No. of co-registered segments	120	132

3.3.4 Calcification

72 (65%) of the 115 segments with histological calcification were correctly identified by PMCTA. Histology identified more length of vessels with calcification than PMCTA

in all the vessels within the co-registered segments and also when the non co-registered segments were included. (829 mm vs. 519 mm).

3.3.5 Analysis of different degree of stenosis

Data in Table 3.5 shows the number of segments with different grades of stenosis as identified by histology and PMCTA. There was more agreement on mild stenosis or normal segments between the two modalities compared to more significant stenosis.

Table 3-5: *Number of segments with different degree of stenosis as identified by PMCTA and histology.*

Grade of stenosis by PMCT	Different grades of true stenosis by Histology				Total
	None / Minimal	Mild	Moderate	Severe	
None / Minimal	113	66	25	5	209
Mild	6	9	4	1	20
Moderate	1	4	10	1	16
Severe	1	2	4	0	7
Total	121	81	43	7	252

Complete agreement in green; disagreement- minor=yellow, moderate=grey and major=red as defined in table 3.2.

3.3.6 Agreement between PMCTA and histology on severity of stenosis

216 (85.7%) of the 252 co-registered segments showed either complete agreement or only minor disagreement on severity of stenosis identified and graded by PMCTA and histology. *Table 3.6* shows a complete breakdown of different levels of agreement and disagreement as per the classification detailed in *Table 3.2*.

Table 3-6: *Agreement and disagreement between PMCTA and Histology*

Agreement level	Segments, N (%)
Total agreement	132 (52.4)
For minimal or no disease	113 (44.8)
For mild disease	9 (3.6)
For moderate disease	10 (4.0)
For severe disease	0 (0)
Minor disagreement	84 (33.3)
Moderate disagreement	28 (11.1)
Major disagreement	8 (3.2)

Among the 8 segments where there was a major disagreement, 7 involved severe stenosis grading by histology which was under-reported by PMCTA as mild disease.

3.3.7 Specificity and sensitivity for significant stenosis

PMCTA identified 11 segments in total having either moderate (N=10) or severe (N=1) stenosis. However, histology identified total 50 segments of significant stenosis (43 moderate and 7 severe stenosis). Therefore, the sensitivity and specificity, positive predictive value (PPV) and negative predictive value (NPV) to identify coronary artery disease with significant (moderate to severe) stenosis by PMCTA were as follows: Sensitivity 30%, specificity 96%, PPV 65% and NPV 84%.

3.3.8 Comparison of findings between the two PMCTA techniques

Table 3.7 shows the extent and severity of coronary stenosis between the two PMCTA groups. The cases undergoing whole-body angiography had more calcification. The sensitivity and specificity, PPV and NPV for identifying significant stenosis were not statistically significantly different.

Table 3-7: *Comparison between the two different PMCTA approaches*

Characteristics	Targeted PMCTA	Whole body PMCTA
Segments with different grades of disease (%)		
Minimal or no disease	57.5	39.4
Mild disease	20.8	42.4
Moderate disease	18.3	15.9
Severe disease	3.3	2.3
Segments with calcification (%)	22.5	43.9
Diagnostic accuracy for significant stenosis		
Sensitivity	34.6	25.0
Specificity	95.7	96.3
Positive predictive value	69.2	60.0
Negative predictive value	84.1	85.2

3.3.9 Inter-observer agreement among the 3 PMCTA reporters

All 3 reporters had fair agreement amongst themselves for identifying all 4 different grades of stenosis. However, for significant stenosis only, reporter 1 and 2 had moderate agreement but only fair agreement with reporter 3. When compared with the consensus PMCTA report, the agreement level was fair to poor *(Table 3.8).*

Table 3-8: *Inter-observer agreement among the 3 reporters.*

Reporter 1	Reporter 2	Agreement on different grades of stenosis	
		For all stenosis	For significant stenosis
Reporter 1	Reporter 2	0.245	0.509
Reporter 1	Reporter 3	0.229	0.317
Reporter 2	Reporter 3	0.204	0.320
Reporter 1	Consensus CT report	0.160	-0.032
Reporter 2	Consensus CT report	0.214	0.199
Reporter 3	Consensus CT report	0.331	0.217

Kappa value <0.2= poor agreement, 0.2-0.4=fair agreement, 0.4-0.6 = moderate agreement, 0.6-0.8 = good agreement, 0.8-1.0 = strong to perfect agreement.

3.3.10 Comparison of final cause of death

There was only 1 case where conventional autopsy confirmed ischemic heart disease as the definitive cause of death and that was correctly identified by PMCTA. In 2 other cases IHD was thought to be at least contributary but was not supported by the pathologist. No case of IHD identified at autopsy was missed by PMCTA.

Table 3.9 shows the cause of death (COD) recorded by PMCTA and subsequent autopsy in full detail.

Table 3-9: *Final cause of death from PMCTA and conventional autopsy.*

Radiology COD	Autopsy COD 1a	Autopsy COD 1b
Not IHD	Acute liver failure	Paracetamol toxicity
Not IHD	Dilated cardiomyopathy	N/A
Not IHD	Hanging	N/A
Not IHD	Head injury sustained in a road traffic accident	N/A
IHD likely COD	Ischemic heart disease	N/A
IHD	Hypothermia	N/A
Myocardial insufficiency due to both LVH, AV disease and IHD	Left ventricular hypertrophy	Aortic Valve disease (operated in 2008)
1a) GI Bleed due to liver cirrhosis 1B) IHD	Gastrointestinal haemorrhage	Ruptured oesophageal varices
Infective exacerbation of COPD, Cor pulmonale and IHD	Bronchopneumonia	Chronic Obstructive Pulmonary Disease
IHD and LVH	Left ventricular failure	Hypertensive heart disease

3.4 Discussion

This study was designed to assess if PMCTA is accurate enough in diagnosing coronary artery disease at the level of individual segments of stenosis compared to histology. A number of reporters reviewed the PMCTA images, performed using two different techniques, with a view to assess if some of the previous findings by experts in the field can be more generalized which would be a vital step to move forward with expansion of any diagnostic technique. The methodological aspects and findings are discussed below:

3.4.1 Co-registration of PMCTA and histology and other methodological aspects

The methodology for co-registration of PMCTA and histology for different segments of coronary artery was meticulous as described in section 3.2.3. The origin of the vessel, the distance of any stenosis from the origin of the vessel, presence of branches and calcification were used for co-registering findings in both modalities independently by the reviewers. Co-registering two modalities with such a significant difference in resolution proved challenging. Also, histological processing results in shrinkage of tissue and it is very difficult to measure such changes. While some post-hoc adjustment might have helped to address that, it is not possible to quantify such adjustments and therefore methods and results could become very difficult to replicate and the study could become subject to systematic bias. The aim was to use a method that can be clearly described and replicated. Therefore, unlike one of the previous studies[99], no post-hoc adjustment was allowed to maintain a robust methodological approach.

Case selection was not randomised or blinded but any cases without any major contra-indication to PMCTA were considered. However, the extent of coronary artery disease could have significantly varied among the cases and this factor could not be controlled.

Two established PMCTA techniques, pioneered by well-established research groups in the field, were used. Firstly, this allowed the findings of the study to be more generalized. Also, both these leading techniques could be compared for their performance in identifying coronary artery disease. Therefore, the overall methodology was stringent and appropriate to ensure the objectives were met with high precision.

3.4.2 Baseline characteristics of the study population

The study population was largely an elderly cohort with slightly higher proportion of male subjects. Between the two techniques, subjects undergoing whole body PMCTA were older with more risk factors for IHD.

The median time elapsed from death to PMCTA was more than 2 days though it was as long as a week in one case. Such delays, therefore, would have led to significant decomposition of the body and potentially might affect the outcome of any post-mortem assessment, both through PMCTA and invasive examination.

While PMCTA was largely successful, in 2 cases the circumflex artery was not opacified. Both cases underwent whole body PMCTA. While the numbers are small to draw major conclusion, it raises the possibility that the targeted PMCTA technique might have better chance of opacifying all the coronary arteries as the contrast is delivered at the aortic root rather than in the femoral artery and therefore might have a higher pressure to open up collapsed post-mortem vessels.

3.4.3 The accuracy of PMCTA compared to histology

Despite all the limitations (Section 1.5.3), invasive autopsy and histology still remains the imperfect reference standard against which PMCTA was compared. Section 3.3.6 & 3.3.7 shows analysis of various grades of stenosis as identified by PMCTA and histology. It is encouraging that as a whole, the modalities showed a good level of agreement for nearly 85% of the segments. However, on further analysis, most of the agreement appears to be for segments with mild or no disease. While it might be easier to be able to identify absence of disease more accurately than assessing the severity of stenoses that is present, this is still an important finding and suggests that despite various post-mortem artefacts, it is still possible to identify normal or minimally disease segments in PMCTA with high level of accuracy.

Consequently, the study shows that PMCTA is highly specific (96%) for significant coronary artery disease when compared to histology on a segment by segment analysis. In other words, 96 of the 100 segments identified as significant by PMCTA are confirmed as significant when assessed by histology. Also, it has a very high negative predictive value reflecting the point discussed above. However, the sensitivity of PMCTA in this study was rather poor (30%). So PMCTA will only identify 30% of lesions identified by histology as significant and this is associated with a low positive predictive value (65%). However, when compared against the final cause of

death identified by conventional autopsy, PMCTA assessment by an expert showed full agreement.

3.4.4 The performance of two different PMCTA techniques

The results *(Section 3.3.9)* show that the accuracy for identifying different grades of stenosis and calcium were similar in both groups even though the whole body PMCTA group were older subjects with more calcified segments and with more diffuse and milder disease. This comparison is limited by the fact that both techniques were not applied to the same case. But the very nature of post-mortem imaging using contrast means it is not really possible to have a more paired comparison of techniques in the same subject. Factors such as a lack of active circulation, uncertainty about clearance of the first contrast medium and ongoing degradation of the body poses major challenges which will confound the findings if a second method is applied on the same subject. Therefore, the method used was an appropriate alternative and overall, they seem to perform at the same level. Therefore, although, case number was small to draw a major conclusion, it can be suggested that 'Targeted PMCTA', which is less expensive, less time and labour intensive, might be the preferred option which can be easily expanded across centres. The results in this study suggests it can exclude significant coronary disease with high specificity and sensitivity.

3.4.5 Inter-observer agreement

The aim of this analysis was to determine if PMCTA can be accurately reported by trained personnel who were not necessarily experts in reporting PMCTA but had considerable experience in identifying coronary artery disease by various imaging modalities. 2 of the reporters had level 2 training for reporting clinical CTCA which is one of the requirements to report clinical scans without direct supervision. The 3rd reporter was a highly experienced cardiac radiologist.

The inter-observer agreement between 3 reporters for grading the stenosis severity by PMCTA was relatively weak with modest agreement between reporter 1 and 2 for

identifying significant stenosis. Therefore, it suggests that reporting PMCTA, despite considerable experience in a relevant field, might be challenging and requires dedicated training.

While it can be argued that more experience in PMCTA might improve this agreement level, this may primarily highlight the subjective nature of qualitative grading of a stenosis. Therefore, before any widespread expansion of PMCTA, it is crucial to agree on the standards and ensure that everyone concerned is properly trained. It would be unacceptable if a new technique with many potential benefits is brought into mainstream use with all the weakness of the already established gold standard and without proper exploration of how that could be improved.

3.5 Limitations

The first limitation of this study is the relatively low prevalence of severe coronary artery disease segments. The prevalence of coronary artery disease (CAD) in the adult population in England is 14% [144]. Other studies show that the incidence of fatal myocardial infarction is approximately 10% among those older than 65 years with a lower incidence in women than men [145]. However, the prevalence of significant coronary stenosis in the current study population was <3%. This certainly had a significant impact when PMCTA was compared against histology at lesion level.
It can be argued that if the current study subjects had a higher prevalence of coronary stenosis, the accuracy of PMCTA would be different. On the other hand, at patient level, 1 out of the 10 subjects (10%) had IHD causing death as suggested by autopsy and PMCTA could make that diagnosis correctly which is probably more important from a coronial autopsy perspective.

Secondly, co-registration of the segments between histology and PMCTA proved very challenging as discussed in section 3.4.1. We did not take into account any shrinkage in length after the vessels were fixed in 10% Formalin. Also, there was not enough side branch information on the histology report to correct the co-

registration at multiple points for each vessel. This might have resulted in reducing the level of agreement between some segments.

Thirdly, the stenosis severity measurement was qualitative for both modalities. For histology the chart by Coghill and Chapman was used but that is also a visual assessment. This remains a fundamental limitation of conventional autopsy practice which requires improvement. The possibility for quantitative or semiquantitative assessment using PMCTA should be explored.

3.6 Conclusion

PMCTA has high specificity and negative predictive value in identifying significant coronary artery stenosis at lesion level but lacks in sensitivity as shown in this study. Also, the agreement between different reporters for PMCTA is modest. The technique for obtaining the PMCTA scans doesn't appear to make any significant difference in accuracy for identifying significant coronary stenosis in vessels successfully opacified by contrast. Finally, despite the relatively weak performance at lesion level, the ability of PMCTA in identifying significant coronary artery stenosis as the probable cause of death for individual subjects was good compared to conventional coronial autopsy.

However, this study identifies several areas of weakness that need to be addressed before widespread use can be recommended. Otherwise, PMCTA might become an imperfect replacement for the already imperfect standard of invasive autopsy. Therefore, research must continue in this field and try to explore some of the issues identified here.

A major question is whether the conventional autopsy still can be considered as the gold standard given its many limitations *(Section 1.5.3)*. The lack of any intra-vascular pressure during invasive autopsy and histology might cause systematic over or underestimation of stenosis severity as has been highlighted by experts in the

field for a long time [123]. On the other hand, the contrast agent for PMCTA, which is injected rapidly would generate some pressure and might get the vessel to expand more towards the physiological calibre that assessment might be more accurate for stenosis severity.

Before making such assumptions, research is required to investigate how repressurisation actually affects the post-mortem coronary arteries as that remains a completely unknown territory. Therefore, to understand the effect of repressurisation, we developed some novel methods using intra-coronary pressure measurements and imaging with OCT in porcine and human cadaveric coronaries to investigate this fundamental issue further.

4 *Chapter 4: Development and validation of a repressurisation technique and optical coherence tomography for post-mortem coronary arteries*

4.1 Introduction

As discussed in *Section 1.5.3.2.2*, the issue of depressurisation of post-mortem vessels has been considered by researchers and various techniques have been used to re-pressurise them. However, there is no study which systematically studied the effect of repressurisation on the vessel dimensions. The importance of taking such changes into account when assessing a stenosis severity has been highlighted in the previous chapter where it proves challenging to compare PMCTA with histology due to differential state of intra-vascular pressure during the two methods. Therefore, to address this issue, experiments were designed with the following aims and objectives:

4.2 Aims and objectives:

The aim was to develop and validate a novel technique to repressurise and record pressure and perform intra-coronary imaging of post-mortem vessels
A number of experiments were performed in various phases with the following objectives:

1. Developing a pressure generation and recording technique using a phantom model
2. Translation and validation of the repressurisation and recording system in porcine coronary artery
3. OCT imaging of the re-pressurised porcine coronary arteries

4.3 Materials and methods

Appendix A provides a detailed list of materials used for the experiments in this section. Experiments were carried out in several phases as described below

4.3.1 Phase 1: Developing a pressure generation and recording technique

4.3.1.1 Experiment

- An empty bag of saline was filled with tap water and the inlet was closed
- A sphygmomanometer cuff was wrapped around the bag
- Two coronary pressure wires were inserted into the bag through a wide bore needle after calibration as per the manufacturer's guidelines
- The pressure wires were connected to the pressure wire machine to record the pressure in real time
- The pressure of the sphygmomanometer cuff was gradually increased from 0 to 120 mmHg at 10 mmHg interval
- Repeated small inflation of the cuff was required to maintain target pressure in the bag, especially at the higher range of pressure
- The pressures recorded by the pressure wires at each pressure point were documented
- *Figure 4.1* shows the schematic of the set-up. Panel A and B of *Figure 4.2* shows that in a photograph

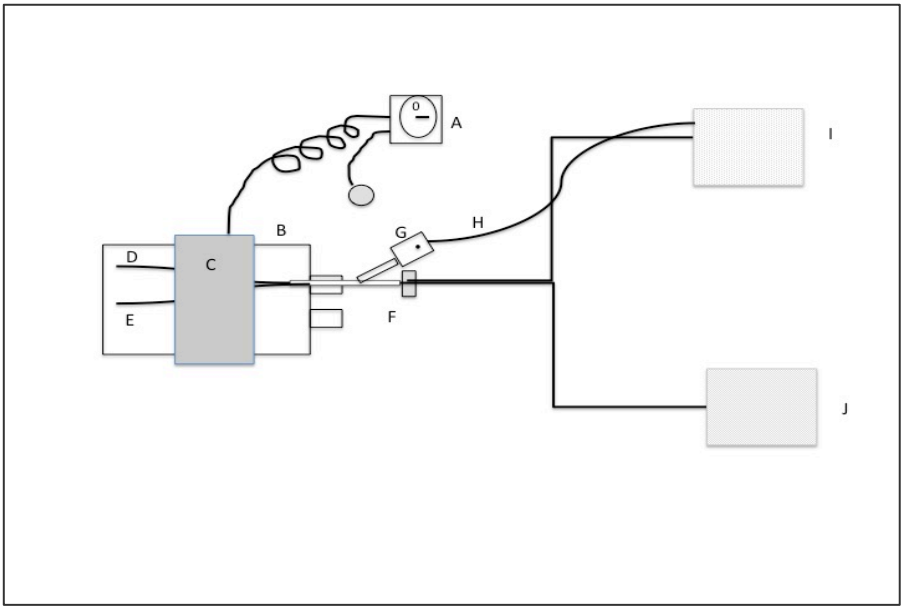

Figure 4-1: *Schematic diagram of the experimental set-up used for pressure generation and recording.* Figure legends: A) Sphygmomanometer B) Bag of fluid filled with tap water C) Sphygmomanometer cuff D) &E) Distal end of pressure wires F) Y connector with haemostatic valve G) Pressure transducer H) Proximal end of the pressure transducer connection I) & J) Pressure wire machines

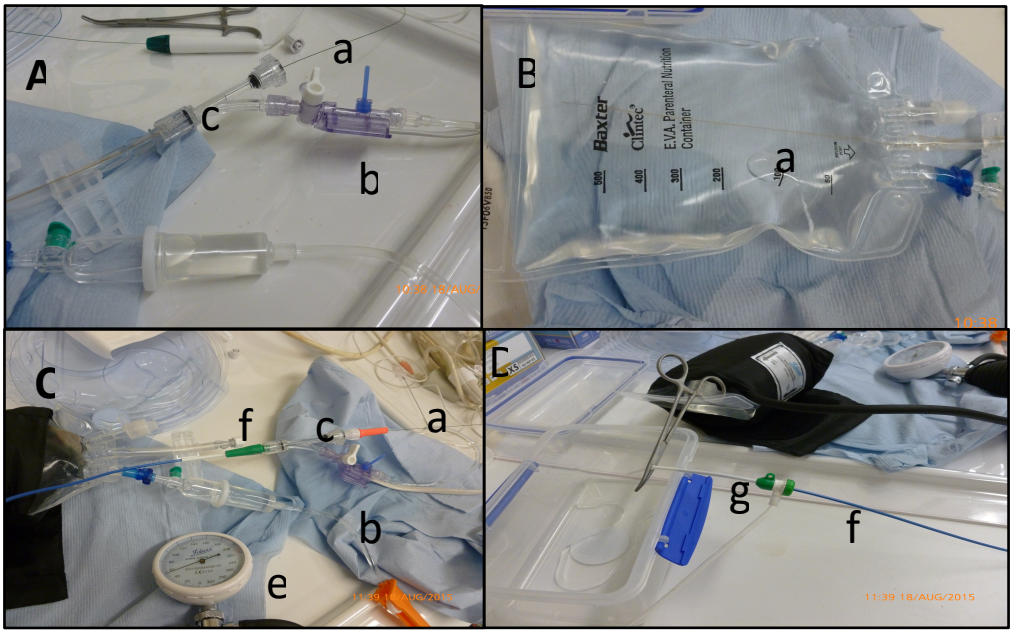

Figure 4-2: *Photograph of different elements of the phantom set-up. Figure legends:* A) pressure wire (a) entering into the bag of fluid through the haemostatic Y connector (c) and pressure transducer connected to the side arm. B) Pressure wire inside the bag of fluid c) Pressure wire connected to the vascular phantom (f) and the cuff pressure being recorded by the sphygmomanometer (e). D) Distal end of the vascular phantom (f) inside a haemostatic sheath (g) distal end of the sheath being clamped for a complete seal

4.3.1.2 Results

- *Figure 4.3* shows that the pressure generated within the bag of fluid is recordable by both the pressure wires
- The pressure recorded by the pressure wire is lower compared to the sphygmomanometer reading, more so at the higher range of pressure. This difference became more marked during the second test.
- There was no reproducibility of this system.

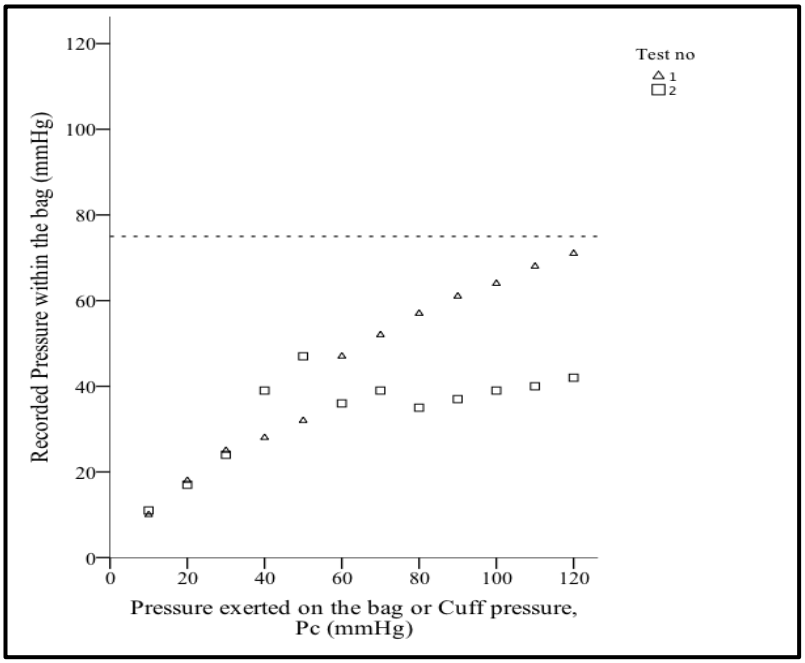

Figure 4-3: *Pressure recorded within the bag by the pressure wire.* Pc- Pressure exerted by the sphygmomanometer cuff on the bag of fluid or cuff pressure. On both occasions the recorded maximum pressure was significantly lower than the cuff pressure.

4.3.1.3 Analysis of the result and modification of the set-up

It was observed during experiment 1 that there was a small leakage of fluid from the bag through the needle hub used for inserting the pressure wires. This explained the reason for increasingly lower pressure recorded within the bag at higher range of pressure during test 1 which became even more apparent during test 2. As the exerted pressure on the bag increased, fluid leaked more rapidly through the needle hub and pressure within the bag failed to rise. While the bag was repeatedly re-

inflated to maintain the target pressure, it became increasingly difficult to do so with more loss of fluid with time.

The setup was modified to address this issue replacing the needle with a Y connector which had a haemostatic valve and the experiment was repeated as before. The Y connector also allowed the addition of a pressure transducer to measure the pressure of the fluid at the outlet of the bag.

4.3.1.4 Experiment

- A Y connector with a haemostatic valve was used to insert the pressure wires inside the bag of fluid sealing off any leakage of fluid and thus creating a closed system.
- A separate pressure transducer was also connected to the second arm of the Y connector to measure pressure of the fluid coming out of the bag.
- Rest of the steps were similar to experiment in 4.3.1.1
- Pressure recoded by both the pressure wire and the pressure transducer was documented.
- The experiment was repeated 3 times to test for reproducibility and inter-device variability

4.3.1.5 Results

- There was strong linear relationship between cuff pressure and the pressure recorded within the bag of fluid by the pressure wire *(Figure 4.4),* as well as pressure of the fluid immediately at the outlet of the bag as measured by the pressure transducer *(Figure 4.5).*
- The Pearson's correlation value for each of the above pairs respectively were 0.987 and 0.991.

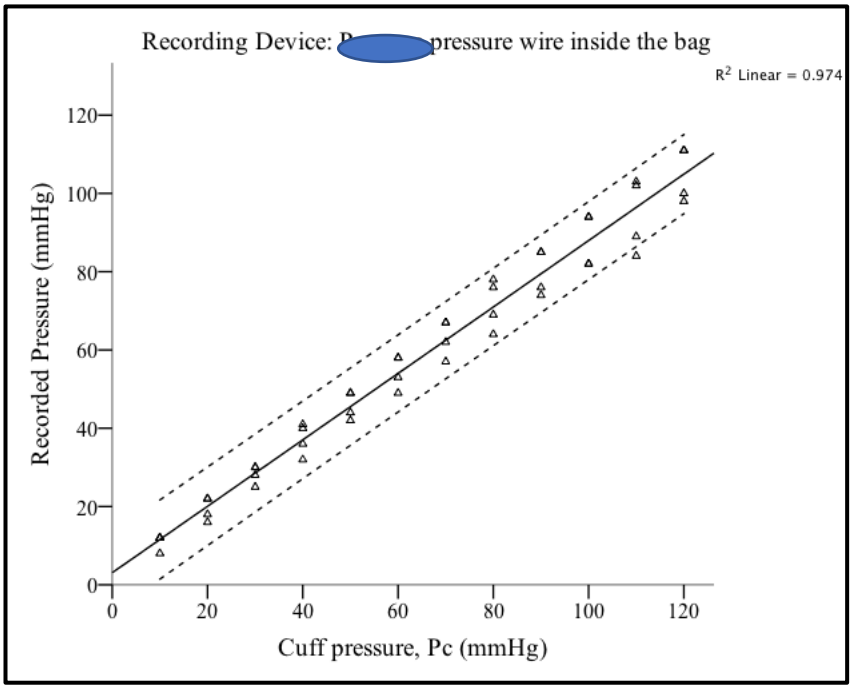

Figure 4-4: *Correlation between cuff pressure (PC) and the pressure recorded by the pressure wire.* Line of best fit for linearity and 95% confidence interval (CI), N=3

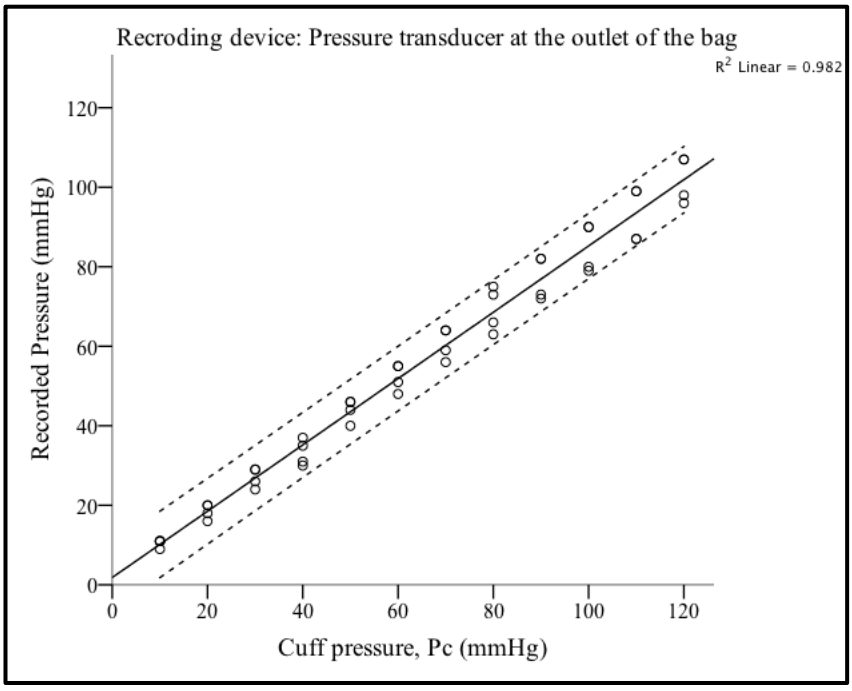

Figure 4-5: *Correlation between cuff pressure (PC) and the pressure recorded by the pressure transducer.* Line of best fit for linearity and 95% confidence interval (CI), N=3

4.3.2 Phase 2: Validating the technique in a phantom model.

4.3.2.1 Experiment 3

The previous system was modified as follows:

- Firstly, the Y connector was connected with the outlet of the fluid filled bag in an orientation that allowed fluid from the bag to go through the Y tube into a coronary guide catheter which served as a phantom model of a blood vessel
- The pressure transducer was connected between the Y connector and the guide catheter, to record pressure of the fluid which came out of the bag and entered the phantom
- The valve of the transducer controlled the inflow of fluid into phantom
- A pressure wire was introduced through the haemostatic valve end of the Y connector into the phantom to very distal end.
- The coronary guide catheter was inserted into a vascular sheath with haemostatic valve, the end of which was clamped. This made the system sealed without causing disruption to the physical integrity of the distal end of the guide catheter.
- Pressure was raised at a 10 mm Hg interval from 0-120 mm Hg within the bag of fluid and simultaneous reading from the transducer at the inlet of the phantom (representing delivered pressure) and from the pressure wire at the distal end of the phantom (representing actual intra-vascular pressure) were recorded
- *Figure 4.6* shows schematic of the set-up with 3 different pressure measurement point. Panel C and D of Figure 4.2 b show photographic representation.
- Pressure recorded by the sphygmomanometer cuff is denoted as P_C. This reflects the original pressure exerted on the bag of fluid.
- Pressure recorded by the pressure transducer at the inlet of the phantom is denoted as P_I. This reflects the infusion pressure of the fluid column into the vascular phantom.
- Pressure recorded by the pressure wire is denoted as P_d. This represents the intravascular pressure within the phantom.
- The experiment was repeated 4 times.

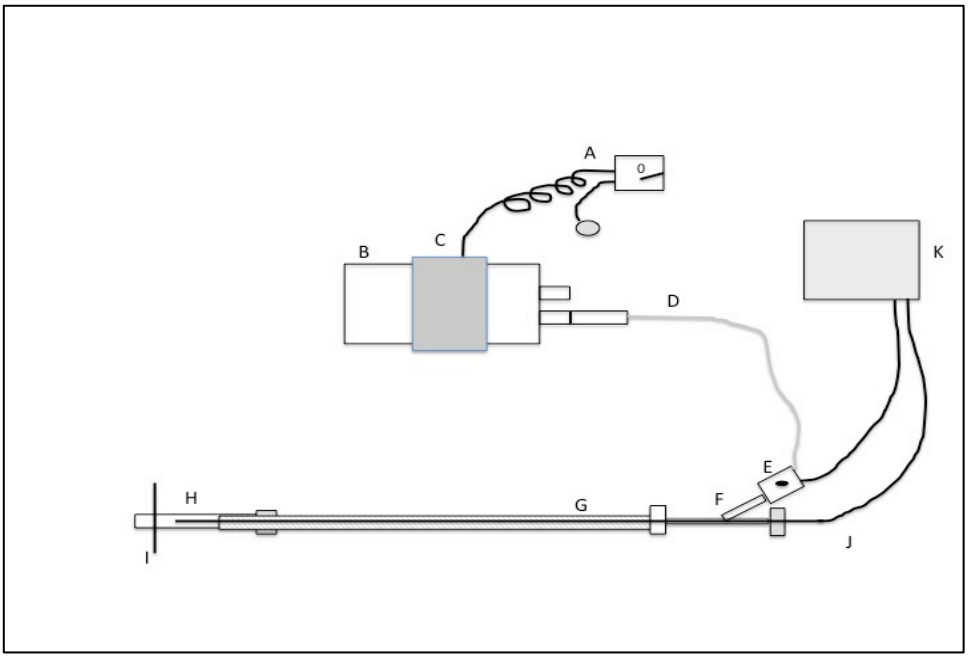

Figure 4-6: *Schematic diagram of the experimental set-up used to re-pressurise a vascular phantom*.

Figure Legends: A) Sphygmomanometer B) Bag of fluid filled with tap water C) Sphygmomanometer cuff wrapped around the bag of fluid D) Outlet of pressurised fluid from the bag into the phantom E) Pressure transducer with a valve at the inlet of the phantom controlling the fluid flow and recording the pressure of the fluid F) Y connector with a haemostatic plug G) Coronary guide catheter used as vascular phantom H) Vascular sheath as the end of the phantom I) Clamp to close the vascular sheath J) Pressure wire inserted into the distal end of the phantom through the haemostatic plug of the Y connector K) Pressure wire machine simultaneously displaying the pressure at the transducer and the tip of the pressure wire.

4.3.2.2 Results

- The pressure exerted on the bag of fluid (Pc) showed strong liner correlation both with the pressure at which fluid entered the phantom (Pi) and the pressure achieved at the distal end of the phantom (Pd) with Pearson's correlation value of 0.992 and 0.991 respectively *(Figure 4.7, panel a & b)*
- The pressure within the distal end of the phantom (Pd), ie. the intravascular pressure was identical to the pressure at which fluid entered the phantom (Pi), ie. the infusion pressure *(Figure 4.7, panel c)* with Pearson's correlation value of 1.

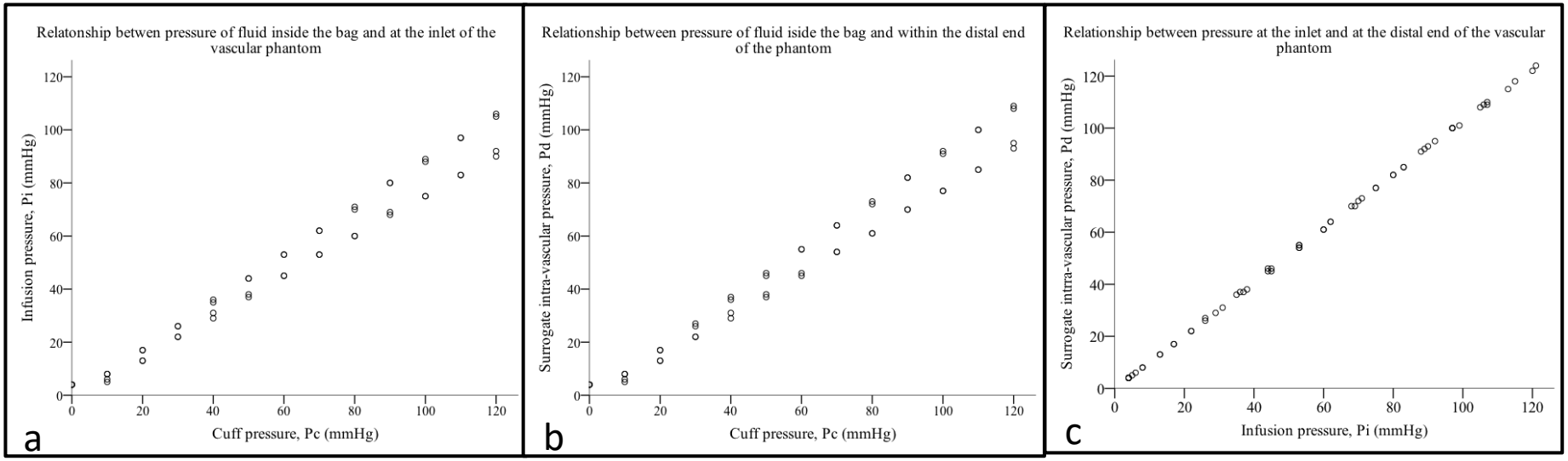

Figure 4-7: *Relationship of pressure recorded at various points of the phantom set-up.*

Pressure within the bag of fluid (Pc) correlates strongly with the pressure at the inlet (Pi or surrogate infusion pressure for a vessel) and at the distal end (Pd, surrogate intra-vascular pressure) of the vascular phantom (figure 4.3a and 4.3b respectively). Pressure at the inlet (Pi) showed complete linear correlation with pressure at the distal end (Pd) with a Pearson's correlation value of 1 (figure 4.3 c)

4.3.3 Phase 3: Re-pressurising post-mortem porcine coronary arteries and recording the intra-coronary pressures

4.3.3.1 Experiment 4

- The materials used in this section have been listed in Appendix B
- The process of collection and storage of porcine hearts has been described in *Section 2.4.1*.
- Connecting the pig heart with the pressure generation system: In an intact porcine heart, the ostium of the coronary artery was identified through the transected aorta and the proximal 0.5-1 cm course of the vessel was dissected clear from its relevant groove on the myocardium. The tip of the guide-catheter was inserted into the ostium of the porcine coronary artery through the aorta and a silk suture was applied around the tip of the catheter to maintain its position. A polystyrene rig was used in which the heart was suspended through the aorta or pulmonary trunk using a supporting bar. *Figure 4.9 and 4.10* show photographs of various aspects of the set-up.

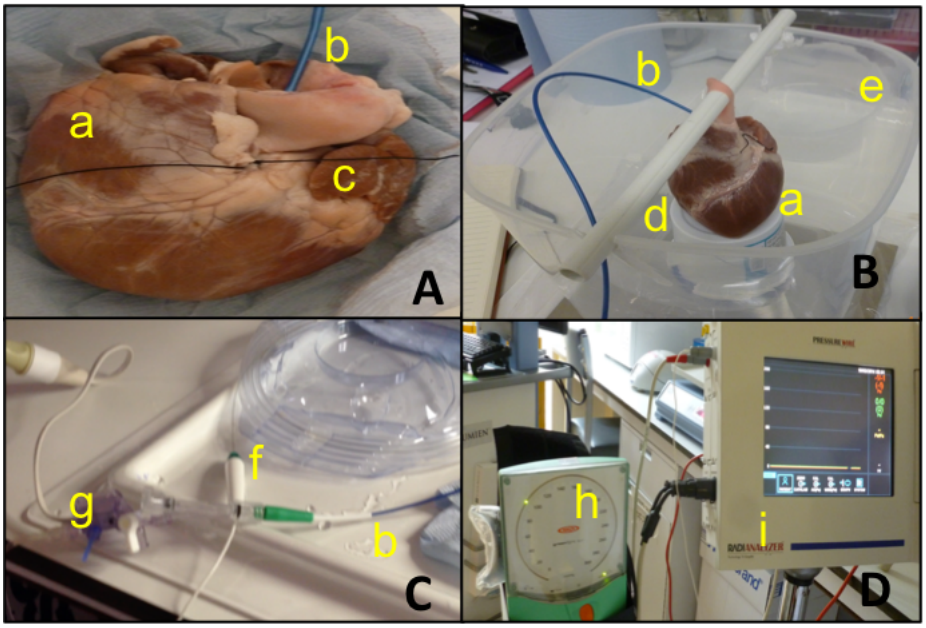

Figure 4-8: *Image of various sections of the initial set-up with porcine heart.*

Figure legends: A) A coronary guide catheter is attached to the ostium of the left coronary artery. B) The porcine hear is suspended through the aortic root in a perspex jar C) Connection of the pressure transducer to the proximal end of the guide catheter through a Y connector. D) The pressure wire machine displays the pressure from the pressure transducer and the pressure wire. a) Porcine heart b) Guiding catheter c)Suture to hold catheter in place d)Perspex rod through the aortic root e)Perspex box to suspend the heart f) The proximal end of the pressure wire g)Y connector and pressure transducer connection h) Sphygmomanometer i) Pressure wire machine

- Creating flow within the post-mortem coronary artery: Once the catheter was attached following the setup described in section 4.3.2, the bag of fluid was pressurised with the sphygmomanometer cuff at 100 mm Hg which entered into the blood vessel through the guiding catheter.
- Wiring the coronary artery with pressure wire: Initial attempts at passing the coronary pressure wire into the distal coronary artery caused damage to the pressure wire or the vessel wall or both. Therefore, the insertion technique was refined to reduce the chances of iatrogenic injury and was thereafter performed in the following steps-

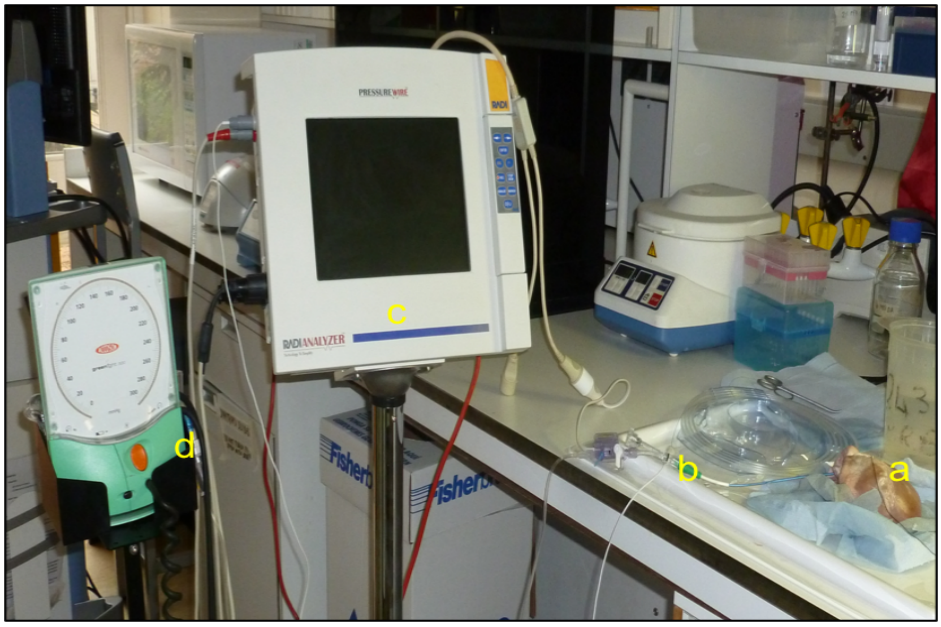

Figure 4-9: *The complete set-up with porcine hearts.*

Figure legends: a) Porcine heart b) The transducer and fluid connection to a shortened catheter c) The pressure wire machine, d) The sphygmomanometer

- Insertion of a hydrophilic coronary angioplasty wire- A hydrophilic coronary angioplasty wire was introduced through the haemostatic end of the Y connector into the guide catheter through which it was inserted into the vessel while the flow was maintained into the vessel. It was advanced into the distal segment of the vessel using direct visualisation through the translucent vessel wall and tactile feedback from the tip of the wire.
- Micro catheter aided exchange into a coronary pressure wire-
  A micro-catheter with a very small calibre was then passed over the hydrophilic wire to the distal vessel. The hydrophilic wire was then withdrawn, and the coronary pressure wire was passed through the micro-catheter to the distal part of the vessel. Finally, the micro-catheter was withdrawn but the pressure wire kept in desired position using the common 'blow out' technique used in interventional cardiology. This involved injecting fluid through the micro-catheter at high pressure and

simultaneously pulling it back using a syringe with luer lock. The pressure of the injected fluid thus kept the wire in position while the microcatheter was manually pulled back using the luer lock syringe.

- Pressure data recording: Once the set-up was complete, this allowed recording of pressure data from 3 different points similar to setup in phase 2 *(Section 4.3.2).*
    - *Pc* (mmHg) was the pressure exerted on the bag of fluid by the sphygmomanometer cuff or the *cuff pressure*
    - *Pi* (mmHg) was the pressure of the fluid entering the vessel from the guide catheter as recorded by the pressure transducer or the *infusion pressure*
    - *Pd* (mmHg) was the pressure recorded within the distal end of the vessel by the pressure wire or the *intra-vascular pressure*

Once recording of intra-coronary pressure was established, further experiments were designed to test pressure-time relationships, to determine the effect of any distal flow and to establish the relationship between cuff pressure, transducer pressure and the intra-coronary pressure as described below:

4.3.3.2 Experiment: Testing for steady state of pressure-time relationship

- Once the vessel was successfully wired with a pressure wire, it was re-pressurised at 100 mmHg.
- All the pressures were recorded at specific time intervals of 7, 15,30 seconds at which point pressurisation was stopped by closing the inlet valve
- Further pressure data was recorded at 35, 40, 45, 60, 75 and 90 seconds to determine time taken to depressurise to baseline pressure.
- The test was repeated 4 times to check for test-retest variability

4.3.3.3 Results:

- Both the infusion pressure (Pi) at the inlet of the guide catheter recorded by the pressure transducer (Figure 4.10, panel A) and the intra-vascular pressure (Pd) recorded by the pressure wire (Figure 4.10, panel B) reached steady state after 7 seconds and dropped rapidly after pressure generation ceased at 30 seconds

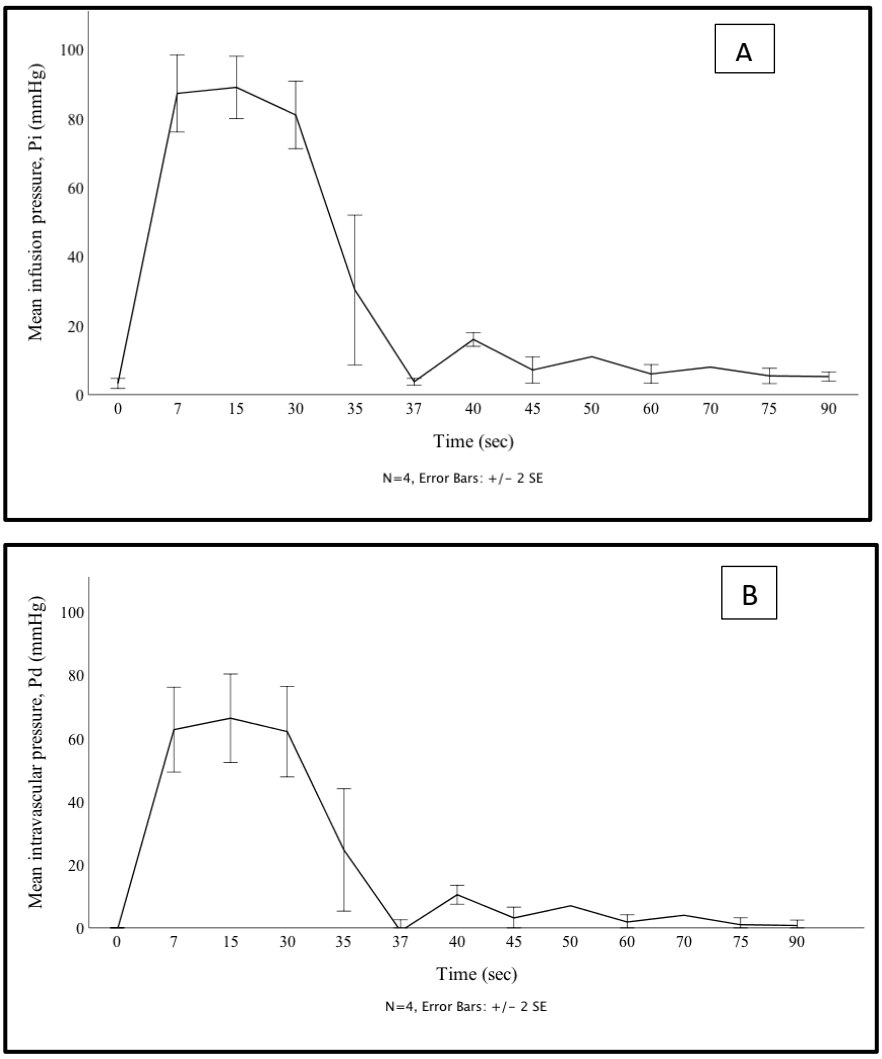

Figure 4-10: *Time-pressure relationship in porcine coronary arteries.* Time in seconds, Pi-infusion pressure (mmHg), Pd- Intravascular pressure (mmHg), N=4, Error bars ±2 SE of mean.

- Both pressure readings reached a steady state after 7 seconds and pressure recorded at 15 seconds showed minimal variability (*Table 4.1)*

Table 4-1: *Mean and standard deviation of infusion and intravascular pressure at 100 mmHg of cuff pressure*

Time point of pressure measurement (seconds)	Infusion pressure (Pi, mmHg) ± St. Dev.	Intravascular pressure (Pd, mmHg) ± St. Dev.
7	92.75 ± 21.81	75.25 ± 20.22
15	100.25 ± 3.87	84.50 ± 4.66
30	93.50 ± 3.11	80.75 ± 3.95

4.3.3.4 Experiment: Determining the effect of significant distal flow

- The previous experiment was then repeated in 2 different porcine heart specimens.
- Specimen 1 was grossly intact with no obvious injury to the coronary arteries or ventricles.
- Specimen 2 had a laceration through the left ventricle transecting 2 of the side branches of the left anterior descending artery.
- Mann Whitney U test was used for comparing mean pressure

4.3.3.5 Results

- Both the infusion pressure (Pi) and the intravascular pressure recorded for specimen 2 was significantly lower than specimen 1 respectively (77.75±5.06 mmHg vs. 100.25±3.86 mmHg, P=0.029 and 48.25±3.95 mmHg vs. 84.5±4.67 mmHg, P=0.029, N=4) as shown in *Figure 4.11*

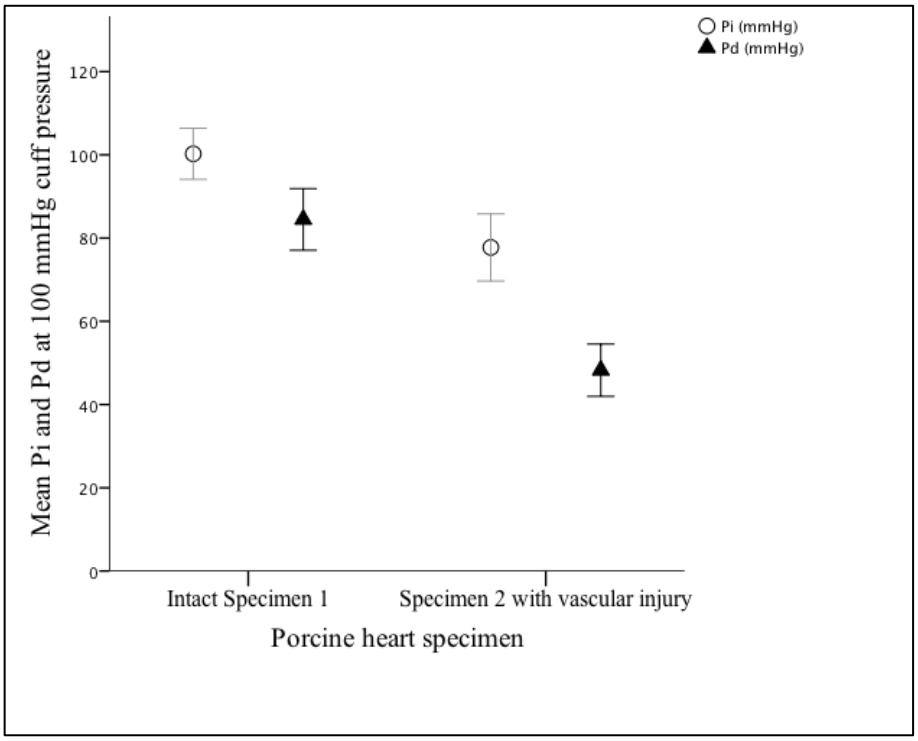

Figure 4-11: *Behaviour of pressure in two different porcine hearts.* Pi- Infusion pressure at the guide catheter inlet; Pd- intra vascular pressure, N=4, Error Bars – 95% CI

4.3.3.6 Experiment: Determining relationship between cuff pressure, infusion pressure and distal intra-vascular pressure

- Tests were repeated in 4 porcine coronary arteries
- The infusion pressure and the intravascular pressure were analysed in relation to the cuff pressure
- The correlation between the infusion and the intra-vascular pressure was also tested.

4.3.3.7 Results

- Pearson's correlation value for the cuff pressure, Pc and infusion pressure, Pi was 0.958 (*Figure 4.13, panel A*)
- Pearson's correlation value for the cuff pressure, Pc and the intravascular pressure, Pd was 0.881 *(Figure 4.13, panel B)*

- The distal intravascular pressure, Pd showed strong linear relationship with the infusion pressure, Pi with a Pearson's correlation value of 0.933 (*Figure 4.13, panel C*)

4.3.4 Phase 4: OCT imaging of the re-pressurised porcine coronary artery

In this final phase, qualitative experiment was performed to demonstrate the following objectives:

- Performing OCT of the re-pressurised porcine coronary artery
- Identify factors that might affect the repressurisation of vessels or quality of the OCT image

4.3.4.1 Methods

- Introducing OCT catheter into the vessel

The OCT catheter was prepared and calibrated as described in *Section 2.6.2*. Once the pressure wire was in position and provided reliable pressure reading, an OCT catheter was passed into the vessel lumen over the pressure wire using the monorail system. The infra-red light of the OCT catheter was visible through the vessel wall which allowed accurate determination of position *(Figure 4.14).* Multiple OCT recordings were performed using each catheter until there was loss or degradation of image or other mechanical failure.

- Performing OCT of the re-pressurised vessel

The vessel was re-pressurised as described in Phase 3 (*Section 4.3.3*) after insertion of the OCT catheter. At each point of repressurisation, after steady state was achieved at 15 seconds, OCT recording was obtained using automated pullback of the machine. Repressurisation was stopped after OCT pullback was completed.
Both the repressurisation and OCT pullback at each point of repressurisation was performed twice to check for variability.

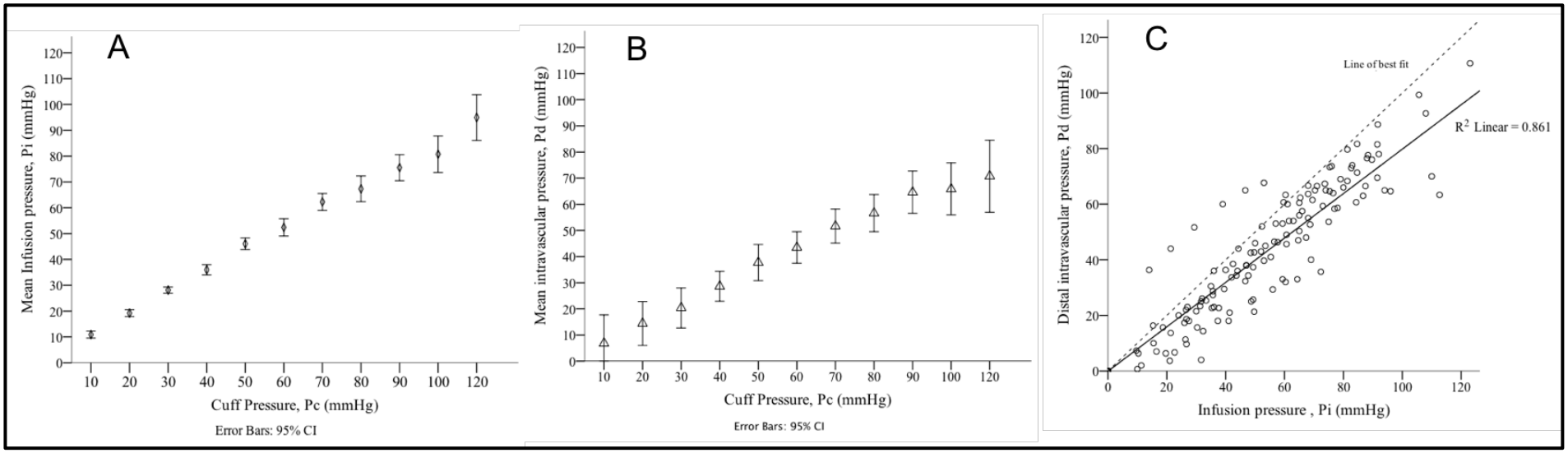

Figure 4-12: *Relationship between pressure of fluid at different points in the porcine model set-up.* Pressure expressed in mmHg. Pc- cuff pressure, Pi- Infusion pressure, Pd- intravascular pressure. Error bar in panel A and B- 95% CI

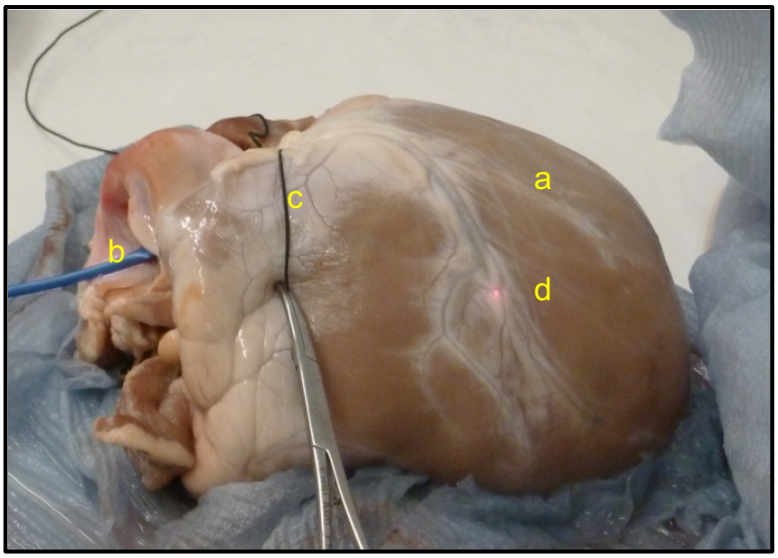

Figure 4-13: *OCT catheter in the distal left anterior descending coronary artery.*

Figure legends: a) Porcine heart b) Guiding catheter containing pressure wire and OCT catheter c) Suture to hold guiding catheter inside coronary ostium d) Infrared light of OCT lens inside the coronary artery

4.3.4.2 Modification to the OCT catheter

- The lens of the OCT catheter is located 2 cm proximal to the tip of the catheter, both of which have radio-opaque markers to allow precise positioning of the OCT catheter in a coronary artery in relation to a fluoroscopic X-ray in a clinical setting.
- In this experiment, the additional 2 cm of the catheter distal to the lens prevented passing of the OCT lens as distal as possible in relatively short porcine coronary arteries. Therefore, the tip of the OCT catheter was cut short by 1.5 cm using a scalpel, just distal to the entry of the monorail port which allowed imaging additional length of distal coronary artery.

4.3.4.3 Removal of air bubbles through irrigation

- Several initial OCT pullback images showed presence of significant amount of air bubble within the vessel

- They were removed through backward irrigation using a large amount of saline through the tip of the OCT catheter while keeping the Y connector valve open at the distal end of the guide catheter
- This allowed air bubbles to be irrigated backwards from the point of the tip of OCT catheter through the guide catheter and Y connector

4.3.4.4 Measuring the cross-sectional area of the vessel

- The OCT images were used for measuring cross sectional area of the vessel using the generic software of the OCT machine.
- A frame was chosen 1 cm distal to the tip of the guide catheter, avoiding any dissection or artefact
- The luminal area was measured and recorded at that point at different infusion pressure ranges
- The OCT images were analysed qualitatively for image quality and artefacts to establish the suitability of this approach for subsequent systematic studies.

4.3.4.5 Results:

- The images were of adequate quality to clearly visualise the vessel lumen and the wall of the porcine coronary arteries as seen on *Figure 4.14.*

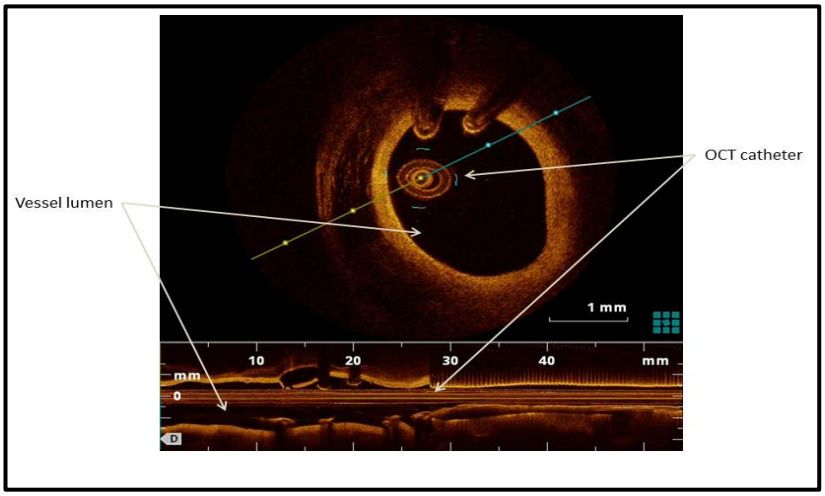

Figure 4-14: *OCT of a re-pressurised porcine coronary artery.* The lumen and the vessel wall are clearly seen in both transverse and longitudinal view

- Artefacts such as air bubbles and dissection flaps of the vessel were clearly seen on the OCT images *(Figure 4.15)*

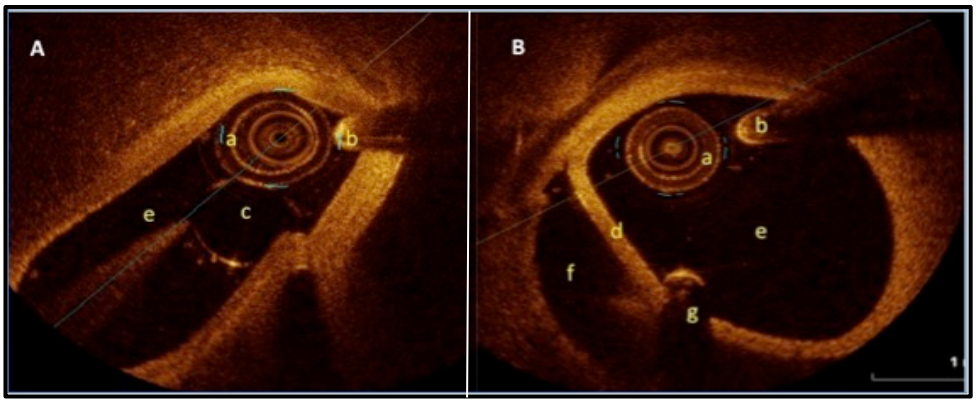

Figure 4-15: *Artefacts noted during OCT of post-mortem porcine coronary arteries.* Figure legends: Panel A- air bubble and Panel B- coronary artery dissection (B). a) OCT catheter b) Guide wire and associated optical shadow c) Air bubble d) Dissected coronary intima e) True coronary artery f) False lumen g. Looped distal end of the guide wire

- There was a qualitative change in the intra-vascular dimension with repressurisation *(Figure 4.16)*
- The image quality was adequate for quantitative intra-vascular measurements *(Figure 4.17)*

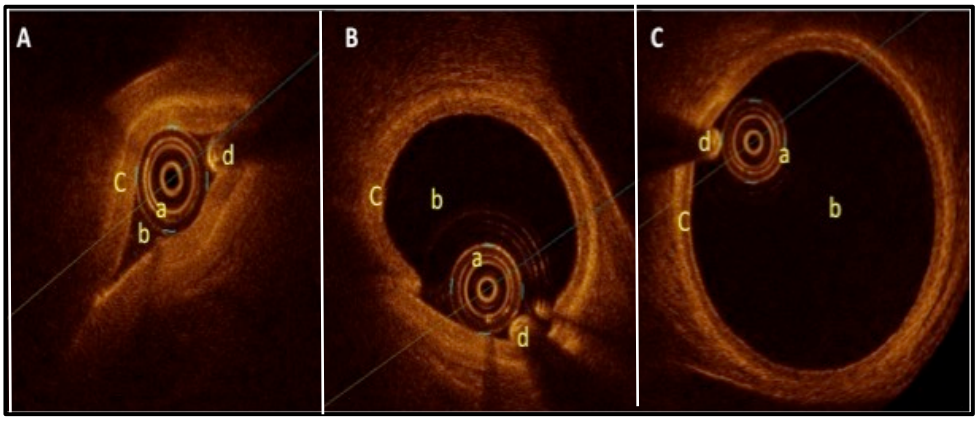

Figure 4-16: *OCT of porcine coronary artery at different range of infusion pressure.*

Figure legends: a) OCT catheter b) Vessel lumen c) Vessel wall d) Guide wire and associated optical shadow. Pressure in A) <5 mmHg, B) 26-36 mmHg & C) 86-95 mmHg.

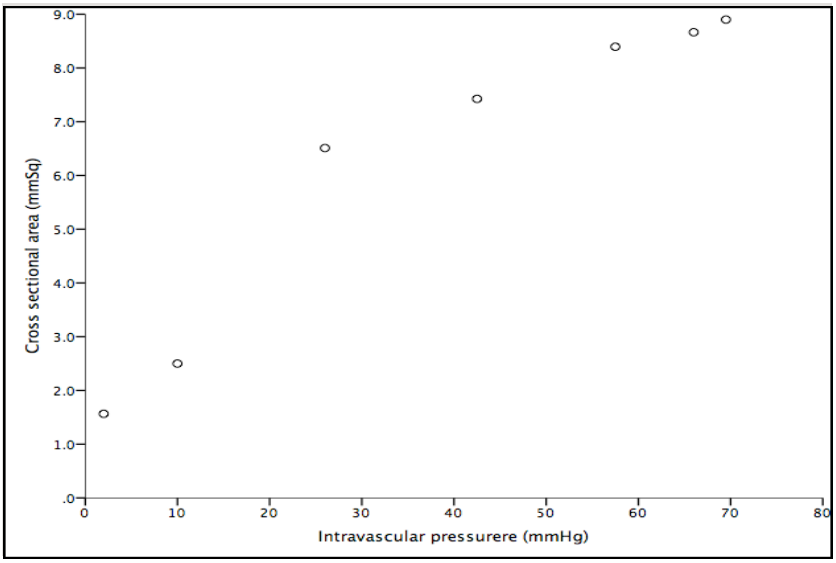

Figure 4-17: *Change in vessel dimension with repressurisation. As the intra-vascular pressure rises, the vessel CSA also increases as measured by OCT.*

4.4 Discussion and Conclusion:

4.4.1 Technical challenges

4.4.1.1 Lack of fluoroscopy and challenges during wiring of the vessels

For intra-coronary imaging, it is essential to pass a coronary wire into the vessel on which the OCT or IVUS catheter can travel using a monorail system. A coronary pressure wire can also be used for the same purpose which can also provide data regarding pressure. In a cardiac catheter laboratory, this is done under fluoroscopic guidance and with the aid of radio-opaque contrast where necessary.

In a post-mortem setting, those facilities are expensive, time and labour intensive and have various ethical and regulatory issues. In fact, for a vessel which has been dissected out of the heart, this can be achieved relatively easily without fluoroscopy as described by some authors [116,124]. However, in an intact heart model it becomes more challenging as the wire has to negotiate the curvature and branches of the vessel. There is only one research group who performed OCT in an intact heart model and used fluoroscopy, but this was not available for this study. This led to

significant challenges and caused physical damage either to the pressure wire or to the wall of the porcine coronary arteries or both. Therefore, a different approach was used to mitigate the problem.

Firstly, a hydrophilic wire was used which was much easier to advance within the vessel with the aid of the infusate. This was subsequently exchanged for a pressure wire with the aid of a microcatheter. Secondly, the left anterior descending artery and the proximal half of the right coronary artery were preferred for experiments due to their relatively straighter course.

Both these measures significantly reduced the incidence of damage to the porcine coronary arteries or the pressure wire during the wiring process despite no use of fluoroscopy.

4.4.1.2 Dealing with air bubbles within the vessel

This was another technical challenge for which a novel technique was used. If there were large air bubbles seen on OCT, the OCT catheter sitting at the distal end of the vessel was flushed with fluid which irrigated the air bubble out proximally through the open valve of the Y tube. Thus, the possibility of the air bubble going downstream and causing embolism of the microvasculature was reduced.

4.4.2 Implications of the findings

The above experiments demonstrate the development and validity of a novel repressurisation and intra-coronary imaging technique. Unlike other researchers [53,54], an intact heart model was used to re-pressurise the post-mortem coronary arteries with the ability to record the exact intra-vascular pressure. Several crucial observations were made at different phases of the experiments.

It was noted that, in a completely closed system, this technique can generate and record pressure with a high level of accuracy. There was no significant test-retest

variability. The fluid leaving the bag remained at high pressure as detected by the pressure transducer which could be introduced in a vessel to re-pressurise it.

When a vascular phantom was used, the system was able to deliver pressurised fluid into the phantom and could record the intra-vascular pressure through the pressure wire. As this was a largely closed system with no obvious route of fluid outflow. However, there was a small pressure drop from the point of the bag of fluid to the inlet of the phantom and this gap in pressure gradually widened at higher ranges of pressure. On the other hand, there was virtually no pressure drop within the proximal and distal end of the phantom throughout the pressure range in this model. Therefore, the infusion pressure at the inlet of the phantom, Pi, was a better predictor of the intravascular pressure, Pd.

Subsequently, the set-up was able to generate pressure in ex-vivo porcine hearts. The cuff pressure or Pc, the inlet pressure or Pi and the pressure generated within the vessel or Pd could be reliably measured by the system. It was noted that the pressure within an intact vessel rapidly reached a steady state which was maintained as long as repressurisation continued. The pressure dropped soon after stopping continuous infusion of fluids. It was more difficult to re-pressurise specimens with vascular injury as the outflow was relatively higher in those than intact specimens leading to rapid drop in pressure as noted during the initial experiments with phantom models.

The intra-vascular pressure had a strong linear relationship with the infusion pressure, therefore allowing the latter to be used as a predictor of the former. Overall, the set-up allowed predictable and measurable repressurisation of the vessels and further experiments could be carried out using this set-up. Subsequently, it was demonstrated that OCT of re-pressurised porcine coronary artery could be performed in an intact porcine heart while measuring the intra-vascular pressure by using coronary pressure wire. OCT images also demonstrated presence of artefacts such as air bubbles and dissection which were dealt with in subsequent

modifications of the set-up. Finally, the OCT images allowed quantitative measurements within the vessel lumen.

Therefore, the experiments in this section demonstrated the successful development of a novel system which could be used to study the effect of repressurisation on post-mortem vessels by using modified clinical cardiology techniques and tools. They also identified challenges including difficulty of re-pressurising an 'open system' where there is significant outflow which could be due to vessel wall injury, diffusion through the tissues or distal flow through the vascular bed, all of which are potential confounders in the cadaveric setting when repressurisation is considered.

In the following chapters, a systemic study on the effect of repressurisation using post-mortem porcine heart models followed by the effect of repressurisation on cadaveric coronary arteries is discussed using this novel technique. The various challenges and implications of the findings will be discussed in chapter 8.

5 *Chapter 5: A systematic study of the effect of repressurisation of post-mortem coronary arteries in porcine models*

5.1 Introduction

The novel techniques described in the previous chapter allow to pressurise and directly measure intra-vascular pressure in real time. With the addition of high-resolution OCT, this system potentially allows to determine the effect of repressurisation on vessel dimensions in a precise manner. This hypothesis will be tested in this chapter on porcine models.

5.2 Aims and objectives:

The aim of this work was to study the effect of repressurisation on ex-vivo porcine coronary arteries using intracoronary OCT in a systematic manner. The main objectives were:

1. To re-pressurise the post-mortem coronary arteries and record the exact pressures
2. To measure any change in vessel dimension with various degrees of repressurisation

5.3 Materials and methods

The previously developed system of repressurisation and OCT was used. All materials used are listed in Appendix A. Details of the process of collection and storage of porcine hearts as well as statistical methods used were discussed in *Section 2.4.1.*

5.3.1 Selection of specimens

- For this element of the study, 4 hearts were collected on which experiments were completed the same day within 3-8 hours from the time of death to avoid any significant degradation of tissue.
- Total 6 coronary arteries (4 LAD, 2 RCA) were used for experiments (the rest of the RCAs were unsuitable due to injury sustained at the slaughterhouse during the harvesting process or during cannulation and wiring)

5.3.2 Repressurisation and OCT recording

- Graduated repressurisation was performed by inflating the sphygmomanometer cuff from 0 to 120 mmHg at 10mmHg intervals and OCT pullback recordings were then performed after 15 seconds of repressurisation achieving steady state
- The cuff pressure was denoted as Pc(mmHg), the infusion pressure recorded at the pressure transducer was denoted as Pi (mmHg) and the distal intra-vascular pressure recorded by the intra-coronary pressure wire was denoted as Pd (mmHg)
- Repressurisation was stopped once OCT pullback was complete
- Each step was repeated twice and the mean of the two pressure values was used in analysis
- OCT recordings were obtained from the proximal 5 cm of the vessels excluding the very first 1-1.5cm where the catheter was attached

5.3.3 Analysis of the OCT images

- The OCT images were subsequently analysed offline using the proprietary software of the manufacturer. The automated luminal mean diameter and cross-sectional area data were collected at a proximal, mid and distal point of each vessel
- Frame number, side branches, micro-vessels in the coronary wall and distance from the tip of the guide catheter were used to ensure that measurements were made at the same points for the same vessel at different pressure range
- Luminal dimensions of the completely de-pressurised coronary arteries were difficult to measure due to their slit like appearance and presence of artefacts.

(*Figure 4.16).* Therefore, vessel dimensions at infusion pressure range of 26-35 mmHg, which were more consistent were considered as baseline.

- The OCT images showed only 1 of the vessels (an RCA) sustained some distal dissection by the coronary wire. However, the proximal 5 cm of the vessel was intact and suitable for all intended measurements.
- The luminal diameter and cross-sectional area of the pre-specified proximal, mid and distal point of the vessels was analysed against both the infusion pressure (Pi) and the intra-vascular pressure (Pd)

5.4 Results:

5.4.1 Analysis of the pressure data

- As shown in *(Figure 5.1),* there was a strong linear relation between infusion pressure (Pi) and the intra-vascular pressure (Pd) with Pearson's correlation value of 0.861

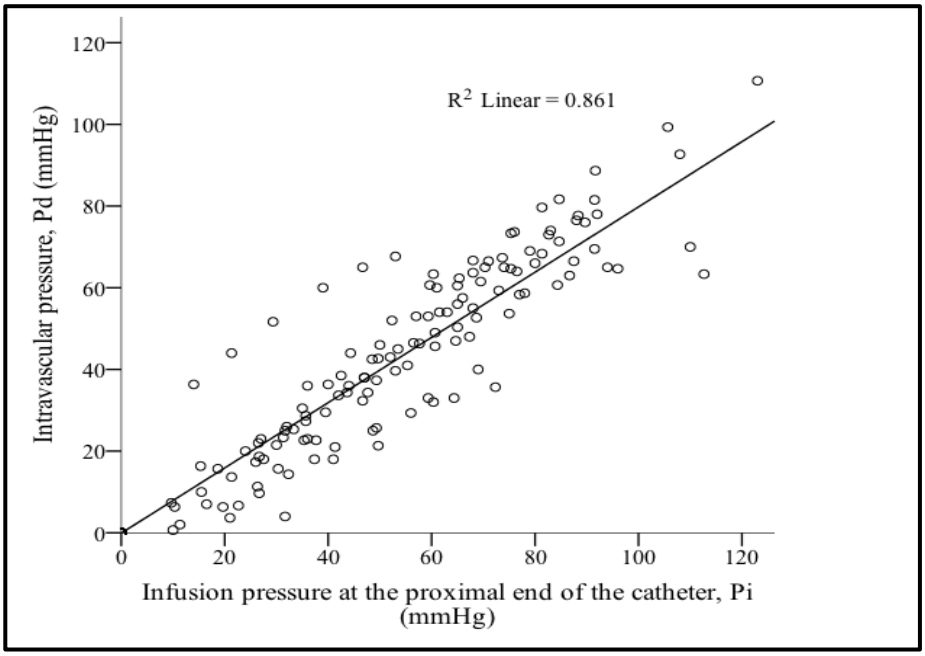

Figure 5-1: *Relationship between the infusion pressure (Pi) and intra-vascular pressure (Pd)*

- However, the intra-vascular pressure was lower than the infusion pressure and the gap between the pressures widened at higher ranges of infusion pressure. *(Figure 5.2)*

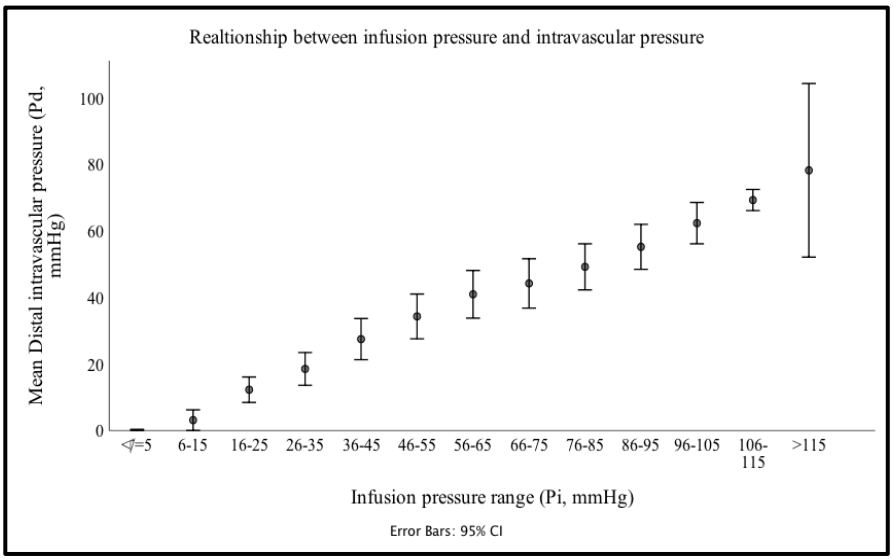

Figure 5-2: *Relationship of porcine coronary intravascular pressure and infusion pressure.* Pi- infusion pressure of fluid in mmHg; Pd – intravascular pressure recorded by the pressure wire in mmHg, N=6, Error Bars – 95% CI. As the infusion pressure range increases, the intravascular pressure increases but significantly less in magnitude

5.4.2 Analysis of the OCT data

- The vessel area increased as the repressurisation range increased.
- When compared against different infusion pressures, vascular dimensions steadily increased in proximal, mid and distal measurement points as the pressure rose within the vessels as shown in *Table 5.1* and *Figure 5.3*

Table 5-1: *The mean absolute cross-sectional area of the 6 vessels at different ranges of infusion pressure (Pi).* Vessel area expressed as Mean ± 2 SD, N=6.

Location of measurement of vessel area	Intravascular pressure range			
	26-35 mmHg	46-55 mmHg	66-75 mmHg	86-95 mmHg
Proximal vessel area	3.3 ± 0.5 mm^2	5.0 ± 0.9 mm^2	6.6 ± 1.5 mm^2	8.4 ± 1.5 mm^2
Mid vessel area	3.1 ± 0.9 mm^2	4.8 ± 1.7 mm^2	6.8 ± 2.5 mm^2	8.9 ± 1.4 mm^2
Distal vessel area	2.5 ± 1.0 mm^2	3.9 ± 1.7 mm^2	5.2 ± 2.3 mm^2	7.4 ± 2.1 mm^2

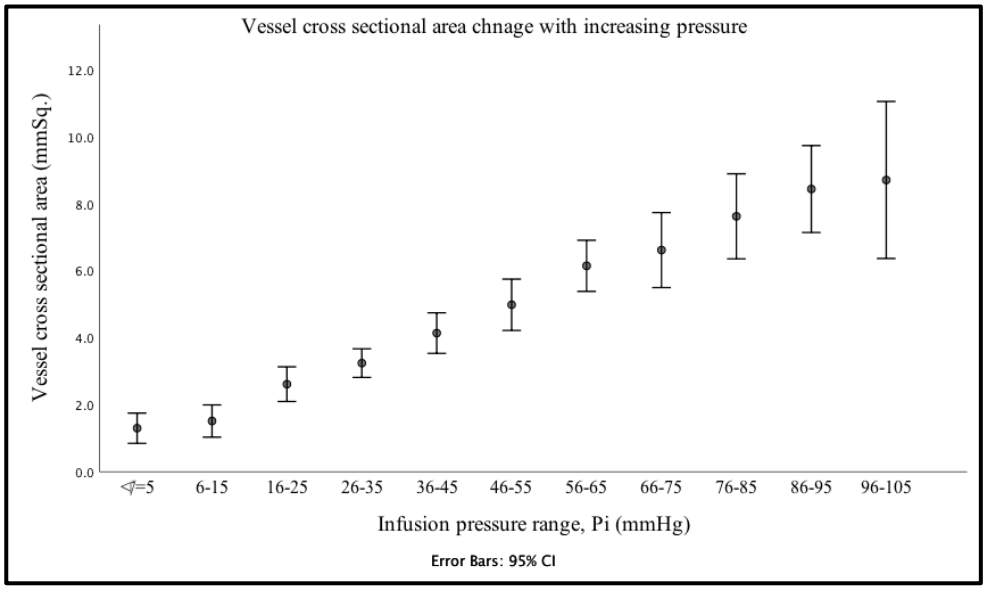

Figure 5-3: *Relationship of vessel size and infusion pressure (Pi) at progressively increasing pressure range.* Pi- infusion pressure in mmHg; Vessel CSA in mm^2; N=6; Error bars: 95% CI.

5.5 Discussion and conclusion

This study confirms that porcine coronary arteries can be systematically re-pressurised and studied using a novel technique. Also, the intra-vascular pressure was consistently lower than the infusion pressure across a range of pressures even in these relatively fresh post-mortem samples with no significant physical injuries. Therefore, the assumptions in many studies that the post-mortem vessels expand to physiological caliber when vessels were pressurised with fluids infused at the physiological range of pressure appears to be incorrect. This is the only study where an intra-coronary pressure device has been used which clearly showed that was not the case.
This study also, for the first time, objectively demonstrates that there is significant increase in post-mortem vessel dimension with various degrees of repressurisation compared to its de-pressurised state. While this was in a porcine model with coronary arteries free of atherosclerosis, similar changes might also occur within human coronary arteries and requires investigation.

Therefore, this study raises serious concern about previous post-mortem studies where coronary arteries were re-pressurised for various reasons but mainly with the aim to preserve their physiological caliber. Thus, this study created strong grounds to proceed with a study of human hearts in a systematic way using this validated technique to explore those questions further.

6 *Chapter 6: Effect of repressurisation on cadaveric coronary arteries- a systematic optical coherence tomography study*

6.1 Introduction

In the previous chapter, it was demonstrated that repressurisation of post-mortem coronary arteries, using the techniques developed in this research, significantly changes the cross-sectional area of porcine coronary arteries compared to their usual de-pressurised state after death. However, porcine coronary arteries are usually free of atherosclerotic disease and do not necessarily reflect the changes which might be encountered in human coronary arteries, especially if there is atherosclerosis. Therefore, there is a need to repeat similar experiments in human post-mortem coronary arteries.

6.2 Aims and objectives

The aim of this study was to re-pressurise and investigate the effect of repressurisation on cadaveric coronary arteries with the following objectives:

1. To determine if cadaveric coronary arteries can be re-pressurised using the developed methods
2. To record intra-coronary pressure of the re-pressurised cadaveric coronary arteries
3. To determine the effect of repressurisation on the coronary artery dimensions and determine if there is a differential effect of repressurisation depending on the presence and extent of atherosclerosis

6.3 Materials and methods

All the materials and equipment used in this study are listed in Appendix B. *Chapter2* describes the ethics, case selection, consenting, study protocol and statistical methods used for the study in detail.

A total of 16 cases were recruited during the study period of which 13 cases were included in this study. Following PMTCA on the evening of Day 1, a designated pathologist commenced the autopsy in the mortuary between 09:00-10:00 hours of Day 2. The heart and coronary arteries along with a portion of the ascending aorta were explanted from the rest of the body and were taken to the designated room within the mortuary for the planned experiments. Specific care was taken by the pathologist not to cause any injury to the coronary arteries which might preclude successful repressurisation and OCT of the coronary arteries. Experiments were then performed in a designated room within the mortuary after which the heart was returned to the pathologist who completed the rest of the autopsy examination, including dissection of the heart and coronary arteries. Histology sections were collected if that was necessary for the autopsy examination or in selected cases, if specific consent for histology for research purpose was obtained from the next of kin. The process has been illustrated with a flow diagram in *Figure 2.1.*

6.3.1 Repressurisation of the cadaveric coronary arteries

The technique of repressurisation was similar to that described in the previous chapter with porcine hearts *(section 5.3.2)* but there were necessary adaptations as human hearts are different in size and the extent of atherosclerosis compared to porcine hearts. First the heart and the coronary arteries were examined for any gross abnormality including injuries during harvesting. Some of the post-mortem clots at the root of the aorta were removed for better visualization and access to the coronary artery ostia. Both left and right coronary ostia were identified and examined for any obvious thrombus and macroscopic atherosclerotic disease. Subsequently, OCT of both the ostia was performed.

Intubation and wiring of the selected coronary artery were performed in the same manner as described for the porcine hearts *(Section 4.3.4)*. However, wiring the human coronary arteries, without the aid of fluoroscopy, was much more challenging due to presence of atherosclerosis and calcium and resulted in dissections in many occasions as shown in *figure 6-1* .

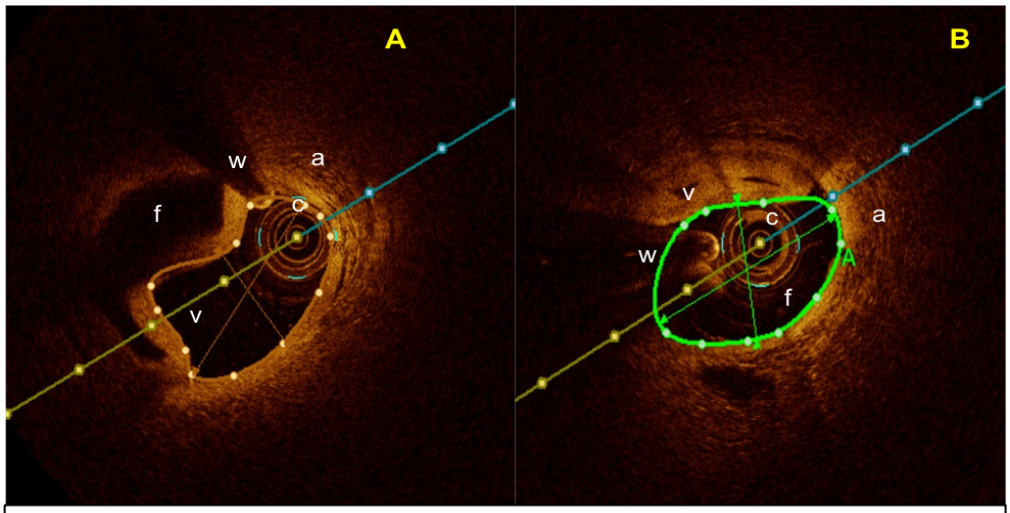

Figure legends: a- adventitia, c- OCT catheter, v- true vessel lumen, f- false lumen , w- wire artefact

Figure 6-1: Examples of dissected cadaveric coronary arteries during wiring. Partial dissection of the vessel wall, B) Complete dissection of the vessel wall and collapse of the true lumen, the software erroneously tracing the false lumen for measurement.

Due to time restriction, experiments could only be conducted on 2 of the three main branches of the coronary artery system in most cases. The LAD and RCA were selected first for their relative ease to wire without fluoroscopy. The LCX was more difficult to wire due to the acute angle with the LMS. However, if time permitted or any of the other vessels were unsuitable, experiments were conducted on LCX.

6.3.2 Recording of pressure data and OCT images:

The pressure recorded at the transducer (which was displayed on the pressure wire machine) or the infusion pressure (Pi) was used to guide graduated repressurisation. The pressure recorded within the coronary artery by the pressure wire was recorded as the intra-vascular or intra-coronary pressure (Pd). Once the infusion pressure

reached the target for each pre-defined pressure point (0 to 120 mmHg at 10 mmHg intervals) and equilibrium was reached, an OCT pullback recording was performed. All pressure data were recorded simultaneously on a data collection sheet and OCT images were stored in the hard drive with appropriate labelling. The exact timing of OCT pullbacks at each pressure point was also recorded on the data sheet to cross check the electronic labelling. This ensured that the correct image for the relevant pressure data was analysed.

6.3.3 Analysis of OCT images

6.3.3.1 Selection of OCT pullbacks for further analysis

Each OCT pullback performed at a defined infusion pressure range, recorded a combined length of 54 mm (540 frames) of coronary artery and guide catheter.
For each vessel, a pair of OCT images were selected for analysis. OCT pullbacks of the completely depressurised vessel or at very low intra-coronary pressure were excluded as they mostly showed a slit like coronary artery with no clear lumen definition similar to porcine coronary arteries. *(Figure 4.16).*

Therefore, OCT pullbacks at the lowest intra-coronary pressure with lumen border clearly identifiable were selected as the baseline pressure image and the pullback at the highest intracoronary pressure range was selected as the peak pressure image. These paired OCT images of the same vessel were used for subsequent analysis.

6.3.3.2 Classification of frames according to the presence and extent of atherosclerosis

All the selected OCT pullback images were reviewed frame by frame for presence and absence of atherosclerotic changes as well as presence or absence of calcium and divided into subgroups as shown in *Table 6.1.*

6.3.3.3 Dividing vessels into segments

As the OCT pullback frame separation is very small (100 micron), it was hypothesized that there is potential for significant interaction between neighbouring frames in

terms of vessel compliance. To minimise such interaction, the OCT images of each vessel were sub-divided into segments. Each segment constituted 50 frames giving a length of 0.5 cm per segment. The segments were classified into subgroups based on presence and extent of atherosclerosis as shown in *Table 6.2* below.

Table 6-1: *Subgroup classification of OCT frames according to presence and extent of atherosclerosis*

Frame Subgroups	Description
Group A- Normal frames	Intimal thickening may be present, but the tri-layer structure of the vessel wall is preserved
Group B- Atherosclerosis without calcification	Loss of tri-layer structure of the vessel wall with involvement of media but no calcification seen
Group C1- Atherosclerosis with mild to moderate calcification	Calcification present, ≤ 2 quadrants
Group C2- Atherosclerosis with significant calcification	Calcification present, > 2 quadrants

Table 6-2: *Criteria for different subgroups of segments*

Segment Subgroups	Description
Group A - Predominantly normal segment	>50% of the frames showing normal vessel wall or intimal thickening only
Group B - Predominantly atherosclerotic without calcification	>50% of frames with atherosclerosis but no calcification
Group C - Predominantly atherosclerotic with calcification	>50% of frames with atherosclerosis and calcification

The flow chart in *Figure 6.2* shows how many segments and frames were finally analysed.

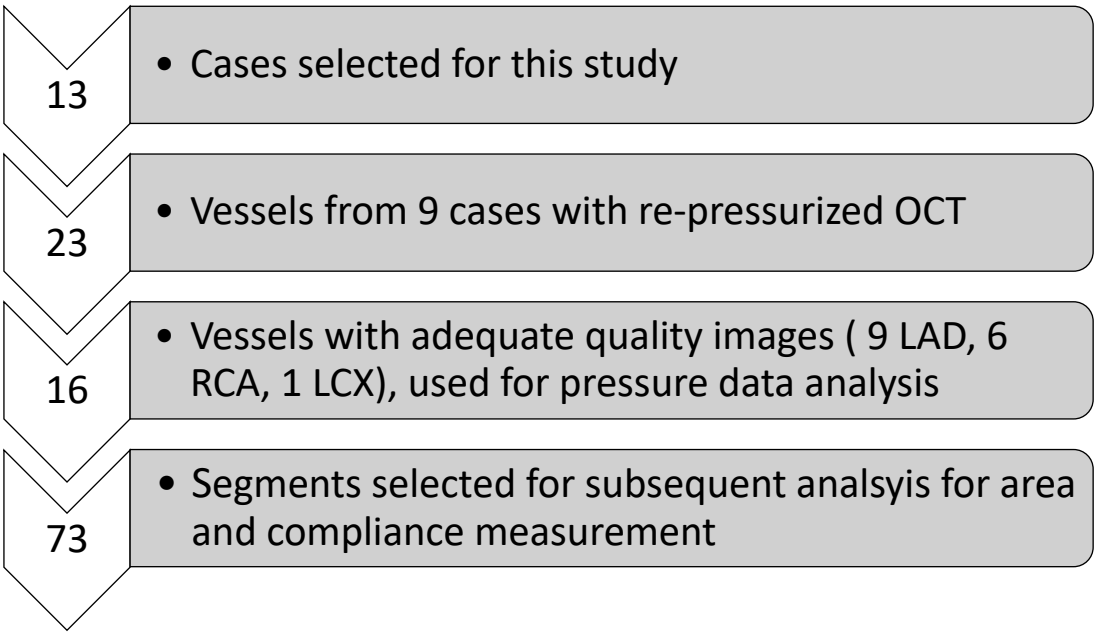

Figure 6-2: *Flow diagram showing selection of regions*

6.3.3.4 Cross sectional area (CSA) measurement

Cross sectional area for each frame at both baseline pressure and peak pressure was calculated automatically by the QCU CMS software *(QCU CMS ™, Medis Medical Imaging System, Netherlands, section 2.6.4).* For each segment defined as 50 consecutive frames, the CSA was averaged for all the frames.

6.3.4 Calculations:

- 'Compliance' was calculated as absolute area difference (ΔA) / pressure difference (ΔP)
- To adjust for different vessel sizes, this was further calculated to 'normalized compliance' as follows-
  - Relative change in CSA (%ΔA) / pressure difference (ΔP), expressed as $mmHg^{-1}$ x1000.

6.4 Results

6.4.1 Repressurisation of the vessels

- The infusion pressures (Pi)

  The baseline infusion pressure was 19±10 mmHg (N=16) and peak infusion pressure was 80 ± 31 mmHg (N=16).

- The intra-coronary pressure (Pd)

  The baseline intra-coronary pressure was 7±4 mmHg (N=16) and peak intracoronary pressure was 25±11 mm Hg (N=16).

- The intra-coronary pressure difference or wall stress (ΔP)

  The intracoronary pressure difference achieved between baseline and peak level of repressurisation was 18±10 mmHg.

6.4.2 Cross sectional area (CSA) and compliance measurement of the cadaveric coronary arteries:

- Available image and regions for analysis:

The total length of vessels with OCT pullback images was 630.90 mm. After excluding for dissected vessel segments and artefacts, 382.40 mm or 3824 frames were available for initial analysis. 80 frames (2.1%) showed a negative change in area with higher pressure which were excluded and finally 3744 frames or 374.40 mm of vessel image was analysed.

- Distribution of atherosclerosis and calcification:

Out of 73 segments (5 mm each), 63 had at least 50% length of atherosclerotic plaque compared to only 10 segments with <50% length with plaque. However, only 18 segments had calcified plaque of more than 50% length.

On frame-based analysis, only 457 frames (12 %) were completely free of any atheroma. The 3287 frames had some atherosclerosis. 1001 of these atherosclerotic frames had calcium present, out of which 284 frames had more than 2 quadrants (>180-degree arc) of calcium and 717 frames had less than 2 quadrants of calcium.

- CSA at baseline and peak pressure

For the segments, mean CSA at peak pressure was higher than that at baseline pressure (5.74 ± 5.38 mm^2 vs. 4 .26 ± 3.47 mm^2, P<0.001). Individual frame-based analysis showed similar results with baseline mean area of 4.35± 3.38 mm^2 and peak mean area of 5.56±3.62 mm^2 which was significantly higher (P<0.001). Vessel sections without any atherosclerosis had more absolute change in area than those sections with atherosclerosis as shown in an example in *figure 6-3.*

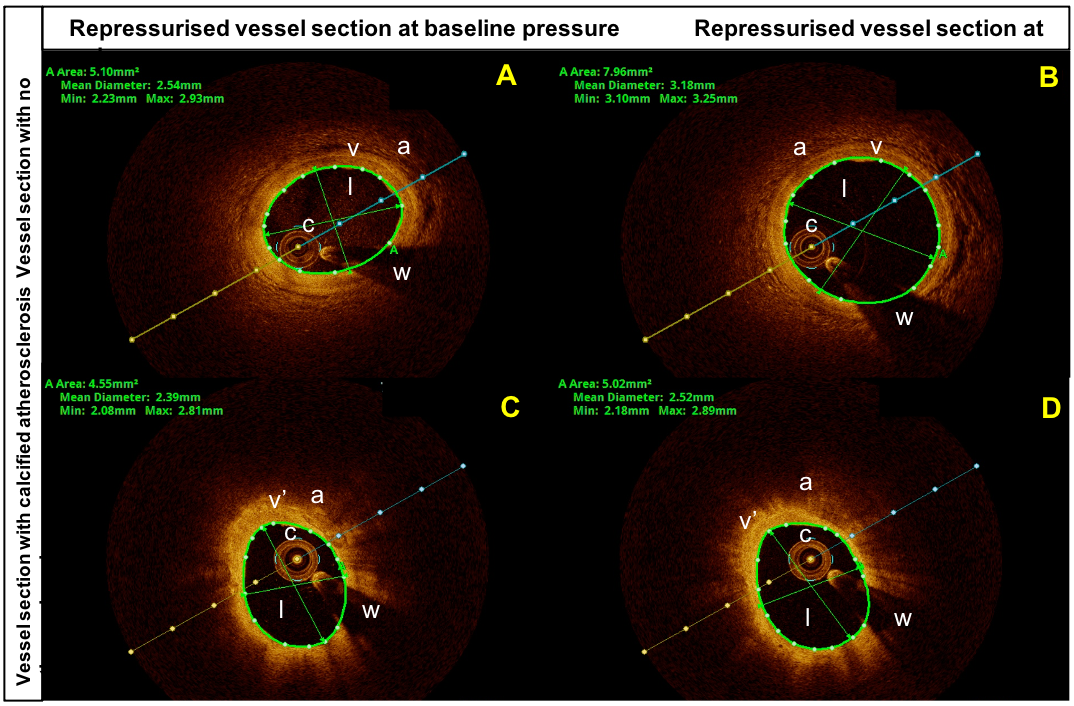

Figure legends : a- adventitia, v- vessel wall without atherosclerosis, v'- vessel wall with calcium and atherosclerosis, c- OCT catheter, l- vessel lumen, w- wire artefact

Figure 6-3: Example images of differential expansion of vessel sections with repressurisation. Vessel section with no atherosclerosis had significant increase in cross sectional area (A&C). Vessel section with heavy calcification and atherosclerosis had very small increase in cross sectional area (C&D)

- Normalised Compliance

Vessel compliance was significantly higher for segments with no atherosclerosis compared to those with atherosclerosis (30.94±20.77 vs. 20.30±15.30 $mmHg^{-1}$ x1000, P=0.006). Within the atherosclerotic segments, those with significant calcification were less compliant than those with no significant calcification, though this did not reach statistical significance (14.87±13.76 vs. 22.47±15.48 $mmHg^{-1}$ x1000, P =0.07).

For frame-based analysis, the significantly atherosclerotic frames and the significantly calcified frames, both groups, were less compliant than their counter parts. *Table 6.3* below shows the results of different groups of segments and frames as described in *Sections 6.3.3.2& 6.3.3.3*.

6.5 Discussion and conclusion

6.5.1 Direct measurement of pressures at different points

This is the first time, pressure was recorded at a number of points during repressurisation of post-mortem human coronary arteries. A number of investigators previously used different repressurisation techniques on animal or cadaveric coronary arteries for a variety of research purposes [58,138,140,146–152]. But none of them recorded the pressure within the cadaveric coronary artery to confirm the degree of repressurisation. These measurements facilitated a better understanding of the repressurisation process with objective data to review if previous studies were correct in their assumptions and also to perform a number of analyses about post-mortem vessel dimension and its change in response to repressurisation.

6.5.2 The variation of pressure within the system and the post-mortem vessels

The data identified two important points with regards to re-pressurising cadaveric coronary arteries.

Firstly, there was lack of uniformity of pressure throughout the system. Previous studies, as mentioned above, only mention about the delivery pressure of the infusate, which in this study is the pressure exerted on the bag by the sphygmomanometer cuff. Those studies assumed that the pressure of delivery of the infusate is the pressure achieved within the vessel. However, in this study, in both human and porcine hearts, it is clearly demonstrated that the cuff pressure (Pc) was a poor predictor of the actual intra-coronary pressure.

Table 6-3: *Variable compliance of cadaveric coronary arteries.* Normalized compliance expressed as ($mmHg^{-1}$ x1000).

		Segment/Frame groups	N	Mean normalised compliance	St. Dev. (±)	P value
Segment based analysis	Based on Atherosclerosis	Predominantly normal (A)	10	35.94	20.77	0.006
		Predominantly atherosclerotic (B+C)	63	20.30	15.30	
	Based on Calcification	Predominantly non calcified (B)	45	22.47	15.48	0.074
		Predominantly calcified (C)	18	14.87	13.77	
Frame based analysis	Based on Atherosclerosis	Frames with no atherosclerosis (A)	457	38.07	17.45	<0.001
		Atherosclerosis, non-calcified and calcified (B+C)	3287	20.20	16.53	
	Based on presence Calcification	Atherosclerosis but no calcification (B)	2286	21.08	16.04	<0.001
		Atherosclerosis and calcification (C)	1001	18.18	17.45	
	Based on extent of Calcification	Frames with <2 quadrant of calcification (C1)	717	17.30	15.70	0.024
		Frames with >2 quadrant of calcification (C2)	284	20.42	21.20	

We therefore considered the pressure at the inlet of the guide catheter, recorded with a separate pressure transducer, as the infusion pressure (Pi) of the infusate. While it had relatively better correlation with the actual intra-coronary pressure recorded by the pressure wire (Pd), the former was still much higher than the latter for a given range of pressure. An infusion pressure (Pi) of approximately 80 mm Hg could only achieve an actual intra-coronary pressure (Pd) of 25+/- 11 mm Hg. This suggests that the only reliable way to determine the extent of repressurisation achieved is by direct measurement of intra-coronary pressure.

6.5.3 The pressure recorded in this study

The actual intra-coronary pressure recorded was significantly lower than the physiological pressure (section 6.4.1). In other words, only partial repressurisation of the vessels could be achieved. Therefore, any absolute measurements of the vessel dimensions still might not reflect the true physiological calibre. This suggests that, in the absence of such stringent methods of pressure measurement, previous studies have significantly overestimated the intra-coronary pressure as detailed above.
On the other hand, while the pressure range was not adequate to measure true coronary artery dimension and stenosis severity, the experiments still provided important data which in conjunction with the high resolution OCT images, could be used to measure vascular compliance and its regional variability as discussed in the next section.

The measure of compliance and its variability is crucial to have a better understanding of the potential errors that can be made by post-mortem assessment of coronary stenosis in a depressurised state as is the current autopsy practice.
Nevertheless, this limitation to achieve a physiological intra-vascular pressure needs to be addressed in future research and will be discussed later.

6.5.4 Measurement of vessel dimensions with OCT

- The segmental and frame-based approach

During histological examinations of coronary arteries, cross sections of vessels every 2-3 mm apart are analysed after they have gone through various stages of tissue processing with its various implications. On the other hand, OCT provides us with cross sectional information on vessel dimension and structure across every 100 microns of the length without any distortion that may arise from vessel sectioning and tissue processing. This makes the OCT measurements more accurate in many respects. However, areas within an intact vessel will have interactions with the adjacent part in terms of dimensions and compliance. 100 micron might be too high a resolution to study such changes in dimensions and compliance independently. Therefore, in this study, while we analysed the OCT images for each of the frames of 100 micron, we also used an arbitrary segment of every 0.5 mm of the vessel to minimise the interactions between adjacent frames.

- CSA and compliance measured by OCT

The results in *(Section 6.4.2)* show that there is a significant (60 ± 35 %) increase in the luminal cross-sectional area (CSA) of the cadaveric vessels with increased intra-vascular pressure. It is known that vessels collapse and lose their dimension after death. However, this is the first time it has been demonstrated how much the vessel expands under pressure.

The extent of expansion in luminal CSA was significantly different between different segments (as defined above) as well as frames of the vessel depending on presence and extent of atherosclerosis and calcification. As a result, compliance in post-mortem vessels appeared to be widely variable across regions of atherosclerosis. This has particularly significant implications as when a collapsed vessel is assessed for severity of stenosis at autopsy, the less compliant calcified sections might appear more patent as they are likely to maintain their architecture whereas more complaint segments will collapse more creating a relatively more significant stenosis. Therefore, assessment of these collapsed vessels can easily lead to systematic errors.

These findings provide more evidence to support the notion that current approach at post-mortem assessment of coronary stenosis, even with a gold standard approach, has significant limitations. Firstly, in a depressurised vessel, a severe stenosis, measured by failure to pass a 1 mm probe, could be much less severe in real life when the vessel is expanded. Secondly, when the stenosis severity is assessed in cross sections, the different compliance of different segments might render them subject to systematic over and under-estimation depending on disease burden and plaque characteristics. Finally, while histology remains the best method to identify plaque rupture/erosion etc, for stenosis severity it faces similar limitations, further compounded by the effect of histological tissue processing and fixation [148]. Therefore, to assess stenosis severity, repressurisation of the vessel and high-resolution quantitative assessment with OCT will provide accurate and objective information on disease burden, stenosis severity as well as plaque characteristics.

However, even with the approach mentioned above, one of the key issues of haemodynamic significance of a stenosis will remain unresolved. Therefore, a different approach is needed should we intend to have a reliable quantitative assessment of haemodynamic significance of coronary stenosis at post-mortem. Therefore, PMCTA based computational fluid dynamic modelling might be a more practical area for further exploration. This suggests that pathologists might have been systematically overestimating the severity of coronary stenosis in a conventional way even if they adhere to best practice guidelines [46,153].

While the above is likely to effect macroscopic assessment of stenosis, Siegel et al. already demonstrated that similar systematic error would result from microscopic assessment of stenosis as well due to variable effect of tissue processing on different degrees of stenosis [58]. Hence, it is reasonable to say that both macroscopic and microscopic assessment techniques of luminal stenosis severity of the coronary arteries at post-mortem are potentially subject to a significant degree of systematic error. If true, this has a major influence on the judgement of presumed contribution of coronary artery disease to the cause of death by the pathologist. Therefore, there is need for further research in this area.

- Implications in the field of bioengineering

The results regarding the compliance and stiffness index are also comparable with the findings of several clinical studies on coronary artery compliance [154–157]. This further supports our methodology and opens up potential for more extensive research in this area. There are many biomechanical questions which are important for understanding atherosclerotic process but a clinical setting limits such investigations [154]. Hence, investigators have largely used animal models which are not subject to natural atherosclerotic change [147,148]. Our model, on the other hand, will be suitable for many of those studies with the added superiority of high-resolution OCT and human vessels with atherosclerosis and might provide more accurate data.

6.5.5 Limitations of the experiments

- Length of adequate quality OCT pullback images

Nearly half of the vessel length which was imaged by OCT had to be discarded as vessel walls were dissected during the wiring process and in some cases, there were artefacts due to presence of large air bubbles or malrotation of the OCT catheter. While this reduced the total available length of vessel for analysis, the high resolution of OCT still allowed comparisons which were of high statistical significance. Use of fluoroscopy would have reduced such dissections but also would have made the whole process more complicated without any additive value to the objectives for this study. However, future studies looking at the accuracy of assessment of stenosis severity at autopsy by conventional methods and OCT should take this factor in account and in such cases, more precise wiring under fluoroscopy will be very important.

- Global rather than regional intra-vascular pressure measurement

Pressure was measured within the coronary artery with a high-fidelity pressure wire. This was placed in relatively distal part of the vessel and was static in position. Thus, we had an estimate of global pressure within the vessel rather than the exact pressure

for the regions for which area and compliance were subsequently measured. This measurement is still more robust than all the studies discussed above. However, there is a reasonable argument that measuring pressure precisely at the site of measurement of the vessel dimension might provide a more accurate data. Such experiments would require use of fluoroscopy which was not the remit of this project but certainly something to be considered for further research.

- Lack of systematic pre-conditioning of the vessel

Investigators in the field of bioengineering have demonstrated that there is a degree of tissue hysteresis during repressurisation of blood vessels and several cycles of repressurisation is ideal to obtain a stable pressure volume curve [152,158]. While, such preconditioning of vessel was not a standard part of our protocol, the vessels were subjected to a few cycles of repressurisation at high pressure to facilitate cannulation, passing of the pressure wire and OCT catheter as well as irrigation of the air bubbles. Thus, this issue has been addressed to a certain extent.

6.5.6 Conclusion

In this study, for first time, the actual intra-coronary pressure was measured within re-pressurised post-mortem vessels. The experiment showed that it is possible to at least partially re-pressurise cadaveric coronary arteries using the techniques described. With the aid of the repressurisation and OCT, it has been demonstrated clearly that there is a significant increase in vessel dimensions even with a very small change in intra-coronary pressure and different regions expand to different degrees depending on presence and extent of atherosclerosis in the vessel wall. The regions without calcified plaque are significantly more compliant. The fundamental information obtained from this study is unique. The findings and their implications with regards to post-mortem assessment of coronary artery disease and any limitations of this study will be discussed in detail in chapter 8.

However, these experiments were technically challenging and adequate repressurisation to physiological range could not be achieved. While some

modifications of techniques might improve that, it is unlikely that such approach would be suitable for day to day work in the mortuary. Therefore, a technically simpler approach is needed. With that view, some early proof of concept work on the potential role of CFD and virtual functional assessment of a stenosis in the post-mortem context is discussed in the next chapter. As PMCTA is already being used by many as an alternative to invasive autopsy, further CFD modelling is possibly the more natural progression in improving the quality and accuracy of post-mortem assessment of coronary artery disease.

7 *Chapter 7: Development of computational fluid dynamics (CFD) modelling technique using post-mortem imaging*

7.1 Introduction: CFD

The previous chapters in this thesis identified the weakness of current approaches to assess coronary artery disease at autopsy. Emerging evidence makes PMCTA an attractive option. However, both the invasive autopsy and PMCTA are limited by a number of factors, including assessment of stenosis severity in depressurised or partially pressurised state of the vessel and their inability to assess the functional significance of a stenosis in an objective manner. Therefore, applying CFD modelling techniques using PMCTA images could be the solution for the objective assessment of both anatomical and functional significance of a coronary stenosis at autopsy, in the same way as current practice in a clinical context.

Therefore, a proof of concept work was undertaken with following the objectives:

- Segmentation and co-registration of coronary artery anatomy derived from PMCTA and PM-OCT images
- CFD modelling of blood flow using the vessel anatomy derived from above and predicting blood flow related parameters

7.2 Methods:

The work was a joint collaboration with department of radiology and imaging at the University of Leiden, The Netherlands and the in-house software and mechanical engineers at the department of cardiovascular science of University of Leicester. The image processing and CFD analysis was performed following the steps as detailed below:

1. A PMCTA dataset, free of major artefacts, was selected from the cases

2. A proprietary software, *QAngio CT RE (Medis Medical Imaging Systems, 2316 XG Leiden, The Netherlands)* was used to reconstruct the coronary arterial tree from the PMCTA dataset.
3. A proximal sub-section of the left anterior descending artery was segmented from the full dataset.
4. The corresponding OCT data set was analysed using a proprietary software, *QCU-CMS (Medis Medical Imaging Systems, 2316 XG Leiden, The Netherlands)*.
5. The PMCTA and OCT datasets were then co-registered to derive a vessel centreline and luminal contours respectively.
6. A 3D surface mesh, representing the selected segment of the coronary artery, was generated
7. This dataset was transferred to a CFD modelling software platform, *ANSYS Fluent. (©Ansys Inc., PA, USA)*
8. A volumetric mesh was produced using finite element analysis (FEA) methods.
9. Newtonian steady-state flow was assumed and the following standard boundary conditions were applied:
    - Inlet Plug flow was considered. Velocity inlet with axial velocity of 0.5 m/sec
    - Outlet Pressure outlet with 100 mmHg pressure
    - Wall No slip boundary condition was imposed
10. CFD solutions were then run in the university supercomputer. An additional geometric length was added to the true vessel model to allow the CFD solution to work
11. The result of the numerical CFD solutions were visually displayed on ANSYS Fluent platform.
12. Only qualitative analysis of the CFD output was performed. Figure 7.1 below shows pictorial examples of the various steps

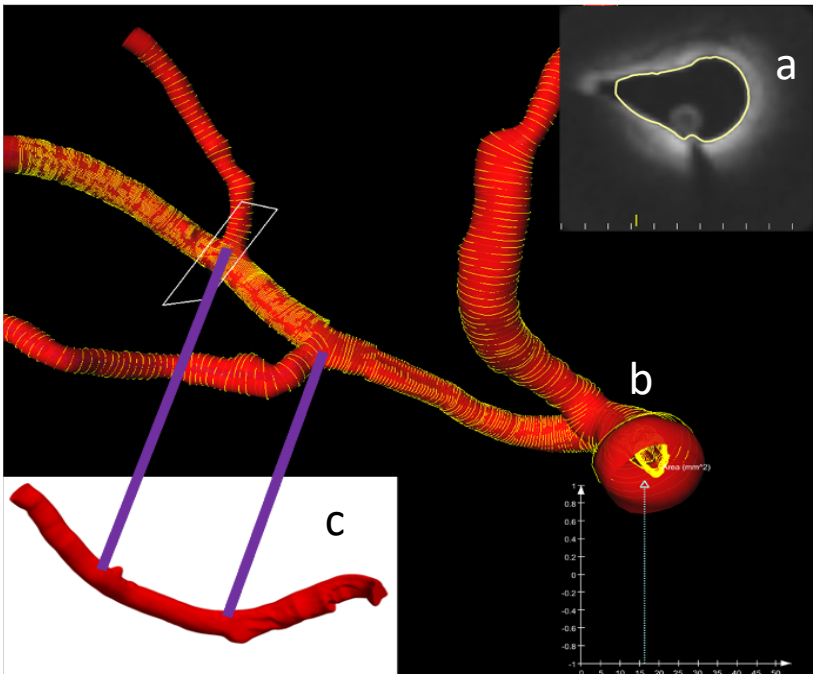

Figure 7-1: *CFD processing of blood flow using post-mortem images.*

Figure legends: a) Cross section of the co-registered PMCTA-OCT image at the vessel origin b) Surface anatomy generated using luminal contour (yellow circles) and centreline from PMCTA c) Selected segment of the vessel for further CFD analysis

7.3 Results:

From the CFD modelling output provided, qualitative and quantitative information on wall shear stress, pressure and velocity of blood flow within the coronary artery were derived.

7.3.1 Wall sheer stress (WSS):

Figure (7.2, panel A) shows the wall sheer stress pattern throughout the vessel wall. The level of wall stress is colour coded from dark blue to eventually red for low to high wall sheer stress. It shows that there is a small increase in WSS at the distal segment of the artery.

7.3.2 Pressure:

Figure (7.2, panel B) shows the behaviour of pressure, colour coded from high to low using bright orange to green colour respectively. It suggests there is a very small drop in pressure within the coronary arterial flow at the outlet compared to the inlet. Also, there was <5 mm Hg pressure difference between the inlet and outlet pressure in both the samples with no obvious coronary stenosis. This suggests that the modelling predicts the pressure parameters as would be expected in real life.

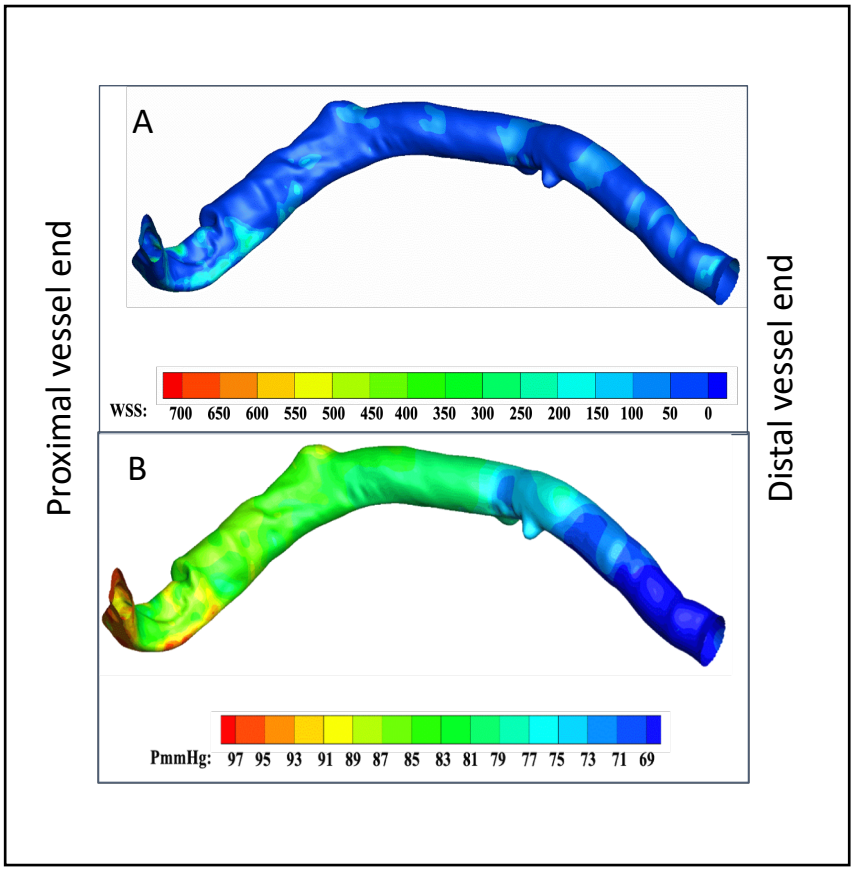

Figure 7-2: *Graphic representation of CFD output depicting wall sheer stress and pressure within the coronary artery.*

Figure legend: Panel A- WSS suggests slightly turbulent flow at the proximal segment but overall no significant variation. Panel B – Only mild change in intra-vascular pressure as would be expected in a coronary artery with no significant stenosis. The last 1 cm of the model is an artificial extension and doesn't represent true vessel.

7.4 Discussion and conclusion

The work above is a preliminary proof of concept. A full working model will require significant computations skills, knowledge of CFD techniques and additional resources which were beyond the scope of this project. The main aim here was to demonstrate that it is possible to obtain necessary raw data and images from post-mortem imaging techniques to perform such analyses. Therefore, this is certainly promising and encourages more research in this area given this has the potential to be the realistic option to assess functional severity of stenosis in a post-mortem coronary artery.

8 Chapter 8: Discussion

8.1 Introduction

The historical role of invasive autopsy has been discussed at the beginning of this thesis and it has been acknowledged that a change in the practice is required for a multitude of reasons. PMCTA overcomes many of the societal and religious barriers to invasive autopsy and provides opportunities for better audit, education and training. Studies suggest good accuracy of PMCTA to identify cause of death compared to conventional autopsy. However, there is not yet enough evidence to show if PMCTA can overcome many of the limitations inherent to invasive autopsy in relation to assessing coronary artery disease.

Therefore, this study was designed with a series of experiments with the following aims:

1. To investigate the lesion specific diagnostic accuracy of PMCTA compared to histology
2. To investigate the importance and practicality of repressurisation of post-mortem coronary arteries and its implications
3. To investigate the feasibility of OCT as an alternative to histology for routine autopsy
4. To investigate the potential for assessing functional significance of a coronary stenosis during autopsy using CFD based approaches.

8.2 The main findings:

The accuracy of PMCTA in identifying significant coronary stenosis compared to histology was modest due to low sensitivity. However, at a patient level, it was still able to identify ischemic heart disease as the potential cause of death as confirmed by conventional autopsy.

A novel repressurisation technique was developed with highly accurate recording of pressure at different points. This showed that repressurisation is challenging in post-

mortem state, more so in the cadaveric vessels than porcine models, presumably due to different anatomical structure and more degradation due to the greater time elapsed. It objectively demonstrated that vessels expand significantly even with partial repressurisation and conversely vessels are significantly smaller in calibre in depressurised post-mortem state compared to life. It also showed the significant variation in compliance across various regions of the same vessel depending on presence and extent of atherosclerosis and calcification, which would potentially have a paradoxical effect on the vessel calibre and stenosis severity at depressurisation after death. Finally, a very preliminary proof of concept work demonstrated that potentially, with further research, reliable CFD modelling of blood flow could be generated using PMCTA images. If that is possible, this might overcome one of the most significant scientific barriers in post-mortem assessment of coronary artery disease by providing an objective, functional paradigm of the effect of stable coronary stenosis which is considered the gold standard in the living and significantly superior to the current anatomical and probabilistic approach as demonstrated in numerous clinical follow up studies.

8.3 Implications of the findings:

Rutty et al. and Roberts et al.[94,98] have shown in their respective studies that PMCTA could be a viable alternative to conventional autopsy and, in conjunction with available previous medical history, can identify probable cause of death with high level of accuracy. However, the current study identified that PMCTA still has many challenges to overcome. When the accuracy of PMCTA is assessed at a higher resolution at the level of the lesion and a more generalisable approach is taken using different techniques and reporting by trained non-experts, the performance against histology is inadequate.

The comparison with histology is inherently difficult due to the problems associated with co-registration *(Section 3.4.1)* and the unquantifiable effect of tissue processing during histology. However, these factors are non-modifiable. While others [99] have shown better accuracy for PMCTA against histology, they had methodological differences (section 3.4.1) with post-hoc adjustments to improve the co-registration and a using a ‘modified gold

standard'. Such adjustments and modifications were avoided in the current study to maintain the reproducibility of the methodology which is crucial if the findings need to be generalised. The previous study population also had higher prevalence of coronary stenosis which possibly influenced their specificity and sensitivity more favourably. The reporting of stenosis severity in all the studies for both PMCTA and autopsy and histology were qualitative and subjective which also might have influenced the results. The weakness of such subjective reporting is demonstrated by the poor to weak agreement amongst reporters in this study. Therefore, one could argue about the validity of such a comparison in the first place. But among all the factors, the very different loading or pressurised state of the vessels during PCMTA and histology is one of the most crucial factors which might have contributed to most of the discrepancies as it has been subsequently shown how significantly and variably repressurisation affects vascular calibre and compliance.

Therefore, if conventional autopsy is considered as gold standard despite all its pitfalls *(section 1.5.3.),* PMCTA could be a promising alternative to identify probable cause of death. However, the broader question is whether it is appropriate to replace an imperfect method by another or whether other options should be explored so that autopsy assessment can be brought in line with current clinical assessment of the severity and significance of coronary artery disease. As autopsy still has numerous potential benefits *(Section 1.2.)*, there is a strong argument to modernising the current practice in a more scientifically robust manner so that it can complement and enhance clinical knowledge and practice.

Subsequently, this study addressed the issue of depressurisation of coronary arteries after death and investigated novel methods of repressurisation. The finding suggests that it is not possible to make assumptions about the intra-coronary pressure in a post-mortem state and a direct measurement as done in this study is the preferred option for accurate data. Therefore, the assumption by many authors that the vessels were distended at physiological pressure based on the pressure of delivery of the infusate is unlikely to be correct and their findings might have had systematic errors as a result [56,57,159].

However, this might also depend on the study set-up. If an isolated arterial preparation was used with all the side branches ligated, that is essentially a closed fluid filled tube. As a

result, the pressure within the whole system in such a setting will equalize after a certain time. Hence, the delivery pressure of the infusate and the intravascular pressure could be similar. Hence, it is possible that experiments using excised blood vessels might have achieved the degree of repressurisation intended [160].

In comparison, a 'whole heart' model as used in this study, behaves like an open system and makes repressurisation very challenging (Section 4.4.2). Therefore, even though there might be no visible leak from the epicardial branches of the coronary arteries, essentially this is a dynamic state and pressure can only reach equilibrium throughout the whole system once the infusion flow rate has reached above and beyond the distal flow rate. Due to highly variable post-mortem changes, there cannot be any defined rate in that state even if that could be measured which is also not possible.

Another point of consideration is the nature of the infusate used, and whether this might affect the repressurisation. Some post-mortem studies have used viscous substances such as colloids [54] arguing that they have less extravasation and therefore stay longer within the coronary arteries. While this is possibly true, and these substances might have performed better in this regard, merely their longer presence within the coronary arteries doesn't provide any information about the true extent of repressurisation. These substances are also more challenging to use within the laboratory setting and often require complex custom-made devices for delivery, which is another limitation.

Some authors took the distal flow into consideration in their study [152] and used "Cab-o-Sil" which is known to block circulation in small vessels. But whether that strategy was successful or not can't be verified without an actual record of intra-coronary pressure at the same time.

Therefore, it is unlikely that repressurisation can be used in the autopsy setting as a routine practice or even for selected cases. As a result, the issues of depressurisation potentially affecting the assessment of stenosis persists for invasive autopsy. On the other hand, if it can be demonstrated that PMCTA achieves a degree of repressurisation, that might be a superior approach. At present it is unknown if and to what degree the contrast achieves

repressurisation during PMCTA and whether there is any consistency between subjects. Designing such experiments could be challenging but the principles and the fundamental techniques developed in this study could be modified to such end. Given the significance, this is an area where further research would be certainly beneficial.

Despite some limitations, the partial repressurisation of the cadaveric vessels still facilitated a novel systematic study on the effect of repressurisation on vascular dimensions which was one of the main objectives of this research project. The high-resolution OCT images at different states of intra-vascular pressure also made it possible to quantitatively study the vessel compliance and its variability across the vessel. The findings provided some crucial insight into the debate of whether it is appropriate to assess stenosis severity in a depressurised vessel or not.

It was clearly demonstrated that compliance varies significantly within the same coronary artery with heavily calcified and diseased segments being much less compliant than segments with mild disease. Therefore, when pressure is lost after death and the vessel collapses, there is going to be a paradoxical effect resulting in the lumen within the non-compliant diseased segments looking potentially larger than more complaint but less diseased segments. This has significant implications for both invasive autopsy where vessels are clearly de-pressurised and PMCTA where the extent of pressurisation is not known. This is the fundamental debate in this thesis and needs to be addressed to avoid systematic error in post-mortem assessment of coronary artery stenosis.

One of the other objectives of the study was to investigate the potential of OCT in the mortuary to avoid the need for histology which, in any case, is very rarely done in routine coroner autopsies in England and Wales. However, with all the technical challenges discussed above, it doesn't seem to be a viable option for routine autopsy. However, it still might play a role in further research.

It is apparent from the discussion above that neither PMCTA alone nor use of OCT appear to be a suitable alternative to improve current autopsy practice. Also, there is no potential for assessing functional significance of stable coronary stenosis. Also, the challenges with

pressurisation of the post-mortem vessels makes it difficult for using techniques similar to fractional flow reserve (FFR) in clinical practice. Although partial repressurisation might be adequate for FFR, as it is pressure independent, the uncertainty of the nature of the flow in cadaveric state would make the measurement of pressure gradient across a stenosis an unreliable method. It will not be possible to use any standard value as each cadaveric heart might behave differently. Therefore, given PMCTA can provide good quality images of the post-mortem coronary arteries, addition of computational modelling to derive virtual functional significance seems to be the more realistic approach to address this complex issue.

Subsequently, the work detailed in chapter 7 demonstrates that, similar to a clinical context, it is possible to model coronary arterial blood flow using post-mortem imaging data sets and derive information regarding pressure, velocity and wall sheer stress within the artery. It also demonstrates a novel concept of improving the resolution of luminal anatomy to improve accuracy of the model by co-registering an OCT image with PMCTA. Finally, the derived pressure, velocity and WSS calculations seem to follow a predictable pattern. However, there are several pre-requisites for CFD modelling. Firstly, and most importantly, there must be good quality imaging data. Advances in PMCTA are therefore crucial so more can be learnt about post-mortem artefacts, which can then be subsequently considered when developing the surface mesh from the imaging dataset. Secondly, as discussed before, it remains crucial to investigate what degree of pressurisation is achieved in PMCTA and how comparable are the vessel calibre on PMCTA to clinical CTCA.

There will be major concern about how any physiological assessment can be performed after death, where physiology has ceased and how these CFD data can be validated. Therefore, it is crucial to have clarity about the question being investigated. The objective is not to predict what the flow and pressure parameters are at death, rather what they were in life based on the anatomy of the individual's coronary artery and by using the same boundary conditions as used in the clinical CT-FFR. The intent is to understand how any coronary stenosis, if present, impacted blood flow during life and hence contributed to death, rather than simply manifesting after death. Therefore, if the coronary anatomy can be defined as accurately as possible by PMCTA, CFD modelling should be able to provide

objective data on the significance of the stenosis in life. It is evident from clinical studies that CFD modelling has good correlation to the gold standard of FFR results and significantly improves the diagnostic accuracy of standard CTCA, which only considers anatomical lesion severity.

Finally, to validate all such findings, studies have to be designed to compare the assessment of stenosis using the most objective autopsy techniques, quantitative or semi-quantitative assessment of PMCTA and use of CFD modelling derived parameters and look for accuracy, objectivity and reproducibility. Knowledge and data from clinical FFR-CT studies looking at prognostic implications of FFT-CT values could prove helpful. It is more likely that by incorporating such objective functional assessment values, a new paradigm can be developed similar to the one suggested by MJ Davies [44]. But unlike some subjective assumptions of that approach, this new paradigm will be scientifically more robust and consistent with current clinical understanding of the impact of coronary artery disease. Such an approach would revolutionise and might re-instate the role of autopsy as one of the key learning tools in medicine which currently has lost its place yet continues to be practiced in large numbers with very limited benefit to science and society.

Therefore, there is genuine potential for CFD techniques to finally be able to assess functional severity of coronary stenosis at post-mortem. While much more technical and CFD expertise are required, most of that expertise already exists in the clinical context and research should be focussed on translation of these expertise in the autopsy environment. Thus, no other options other than CFD seem to have the potential to overcome one of the fundamental limitations of post-mortem assessment of coronary stenosis. If it can be developed and validated, the approach will introduce an objective and reproducible method at autopsy taking account of the functional significance of lesions. It will also allow pathologists and clinicians to derive significantly valuable knowledge and further improve the understanding of coronary artery disease, which despite many advancements, is still the leading cause of death in older adults around the world.

8.4 Suggested area for new research

8.4.1 Further modification of the repressurisation techniques

In this study, physiological pressure could not be achieved within the coronary arteries as measured by the pressure wire. Therefore, further research is required to modify the technique by using colloids instead of isotonic saline and/or distal embolization of the microvascular bed to achieve this goal.

Similarly, methods to measure intracoronary pressure during PMCTA need to be developed, possibly by using custom made catheters and use of fluoroscopy to insert a pressure wire in the coronary artery during PMCTA. Depending on the findings, use of colloid or distal embolization might be incorporated to achieve a physiological pressure.

8.4.2 Comparing PMCTA with OCT for assessment of stenosis severity

Once repressurisation at physiological level can be achieved in both settings, OCT pullbacks and PMCTA can be compared for assessment of stenosis severity. This would be the ideal and most accurate way to assess the accuracy of PMCTA unlike histology where the vessel must go through processing and distortion, even if pressure fixation is used.

8.4.3 Development of functional assessment techniques

Finally, the ultimate aim should be to develop methods for functional assessment of coronary stenosis using CFD modelling techniques. Combined with CT based plaque analysis, this might become a new and more robust gold standard in post-mortem assessment of coronary artery stenosis.

9 Chapter 9: Conclusion

Invasive autopsy remains an important tool for understanding disease pathology in many instances but the number of hospital autopsies with detailed clinico-pathological correlation has plummeted over the past few decades. However, the number of medico-legal autopsies remains high despite many valid criticisms including its limited value to science, education and the society as a whole. Moreover, the attitude of various groups in the society towards invasive autopsy has always been sceptical. Therefore, there is an urgent need to find a robust and modern alternative which can address these concerns.

Given the prevalence and complexity of coronary artery disease, any new approach to autopsy should try to overcome the limitations associated with conventional approaches in the assessment of coronary artery disease.

This research project set out to investigate some of the current assumptions using modern techniques including imaging based autopsy. It can be concluded that the findings have highlighted the following points:

- Assessment of stenosis severity in de-pressurised post-mortem vessels is a major limitation of the current approach
- Repressurisation has significant potential benefit and implications
- The accuracy of PMCTA in identifying significant coronary stenosis attributable to death is still modest
- PMCTA coupled with CFD techniques for functional assessment of coronary stenosis might lead to a major paradigm shift in post-mortem assessment of coronary artery disease but further research is needed.

Therefore, in light of the findings of this study, further research should be considered as the next step in developing modern and less invasive autopsy which will be more accurate, objective and acceptable with significant educational and scientific value.

Appendix A: List of materials and equipment

Name and Description	**Manufacturer**
6 Fr. Coronary guide catheter	Various brands
0.9% Normal Saline for infusion	Generic
Intravenous infusion set	Generic
Y connector with haemostatic valve	Generic
Clinical pressure transducer system	Generic
No 1.0 or 2.0 Silk suture	Any brand
Dissection set (Scalpel, scissors, forceps)	Generic
Plastic rig for suspension of ex-vivo hearts	Custom made
0.038" Hydrophilic coronary guide wire	Various brands
Finecross™ microcatheter	Terumo inc., Japan
PressureWire™ CERTUS™ (clinically used)	©Abbott, Maidenhead, UK
RadiAnalyzer™ pressure wire system	©Abbott, Maidenhead, UK
ILUMIEN™ Optical coherence tomography system	©Abbott, Maidenhead, UK
DRAGONFLY™ OCT imaging catheter (clinically used)	©Abbott, Maidenhead, UK
ACCOSSON Clinical Sphygmomanometer	AC Cossor & Son (Surgical) Ltd ©, UK
OSIRIX MD DICOM Image analysis software	Pixmeo SARL, Bernex, Switzerland
QAngio CT RE and QCU-CMS OCT image analysis software	*Medis Medical Imaging Systems, 2316 XG Leiden, The Netherlands*

Appendix B: The research team and roles

The research team:

Dr. Kazi Asif Adnan	Interventional Cardiology trainee; Research fellow and Lead researcher for all the work in this thesis
Miss Claire Robinson	Forensic Radiographer; PhD student and co-researcher
Dr. Mike Biggs	Forensic Pathologist
Mr. Shiju Joseph	Mechanical engineer with experience in computational modelling of fluid, part of the research team
Dr. Juke Dijastra & Dr. Alex Borsen	Department of imaging, University of Leiden, Netherlands. Collaborators for image processing

Role of the author of this thesis in different elements of the study

Chapter 3: (PMCTA)

The PMCTA scans were performed by the Forensic Pathology and Radiology team at University of Leicester. Study design and data analysis was performed independently by the researcher.

Chapter 4-6:

The practical experiments were designed, performed and raw data collected jointly with the co-researcher. The researcher was the lead in use of all clinical cardiology concepts and devices. All practical aspects of wiring a vessel, translating and modifying clinical approach in using pressure wire and performing OCT were led and performed by the researcher with help from the co-researcher. Automated measurements of the cadaveric coronary OCT images were performed by collaborators at University of Leiden using their proprietary software. The pathologist performed cannulation of the carotid artery for PMCTA and the scans were performed by the co-researcher with help and support from the researcher.

The concept of all the chapters, analysis and conclusion were drawn independently.

Chapter 7: CFD

The author of this thesis conceptualised the work. PMCTA scans were performed by the forensic research group along with the researcher and co-researcher. The geometric model from CT and OCT were developed by collaborators at University of Leiden. Dr Shiju Joseph at the University of Leicester performed the CFD analysis in discussion with the author to decide on boundary conditions and developed the CFD output images together. All conclusion drawn independently by the author.

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