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# **Chronological and Biological Ageing in Coronary Artery Disease**

**Submitted in fulfilment of the requirements of the  
Degree of Doctor of Philosophy**

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MPH (Distinction), MBChB**

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Institute of Health and Wellbeing**

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## RELATED PUBLICATIONS

Johnman, C., Mackay, D.F., Oldroyd, K.G., and Pell, J.P. (2013) *Quality of life following percutaneous coronary interventions in octogenarians: a systematic review.* Heart, 99 (11). pp. 779-784. ISSN 1355-6037 (doi:[10.1136/heartjnl-2012-303353](https://doi.org/10.1136/heartjnl-2012-303353))

Johnman, C., Pell, J.P., Mackay, D.F., Behan, M., Slack, R., Oldroyd, K., and Berry, C. (2012) *Clinical outcomes following radial versus femoral artery access in primary or rescue percutaneous coronary intervention in Scotland: retrospective cohort study of 4534 patients.* Heart, 98 (7). pp. 552-557. ISSN 1355-6037 (doi:[10.1136/heartjnl-2011-301032](https://doi.org/10.1136/heartjnl-2011-301032))

Johnman, C., Oldroyd, K.G., and Pell, J.P. (2011) *Elective percutaneous coronary intervention in the elderly patient.* Aging Health, 7 (2). pp. 271-281. ISSN 1745-509X (doi:[10.2217/AHE.11.13](https://doi.org/10.2217/AHE.11.13))

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## **AUTHOR DECLARATION**

The research contained within this thesis was undertaken during my time as a Clinical Lecturer at the University of Glasgow Institute of Health and Wellbeing

The secondary data used in this thesis was extracted from the Scottish Cardiac Revascularisation by Rachel Slack. However; all statistical analyses using the extract were performed by me.

Leucocytes telomere length analysis was undertaken by the Shiels lab; however, all statistical analyses using the data were performed by me.

I can confirm that this thesis, in its entirety, is my own original work.

# SUMMARY

## Background

The elderly account for an increasing proportion of the population and have a high prevalence of coronary artery disease (CAD). Therefore, elderly patients represent an increasing proportion of those presenting for investigation and treatment of CAD. Management of CAD is undertaken to relieve the signs and symptoms of myocardial ischaemia, making quality of life (QoL) a critical consideration in clinical decision making. CAD is associated with both chronological and biological ageing processes. However, conflicting evidence exists as to whether leucocyte telomere length (LTL) is an appropriate biomarker of ageing in CAD.

## Methods

The thesis comprised four complementary studies. Firstly, secondary data analysis of the Scottish Coronary Revascularisation Register was used to undertake two retrospective cohort studies of patients attending for coronary angiography and percutaneous coronary revascularization. The aim was to compare case mix and outcomes of elderly versus younger patients. A prospective cohort study of 437 patients was then undertaken to assess QoL before, and three months after, PCI and to compare QoL changes in elderly versus younger patients. Finally a cross sectional study was used to investigate the association between LTL (T/S ratio - relative ratio of repeat to single copy number) measured using qPCR and CAD (presence and severity) in 1,846 patients attending a regional cardiovascular centre for coronary angiography.

## Results

The number and proportion of elderly patients undergoing coronary angiography increased from 669 (8.7%) in 2001 to 1,945 (16.8%) in 2010. Among the elderly ( $\geq 75$  years old), symptoms were more severe and disease more extensive compared to patients aged  $<75$  years. Peri-procedural complications were infrequent irrespective of age: 2.0% of elderly

patients suffered complications, compared with 1.6% of young patients ( $p<0.001$ ). Thirty-day MACCE were more common in elderly compared with younger patients (2.0% vs 1.6%,  $p<0.001$ ). Elderly patients with evidence of stenosis were less likely to proceed to revascularisation (adjusted OR 0.68, 95% CI 0.65–0.71,  $p<0.001$ ) within one year of angiography, irrespective of disease severity.

There was an increase in the number and percentage of PCIs undertaken in elderly patients, from 196 (8.7%) in 2000 to 752 (13.9%) in 2007. Compared with younger patients, the elderly were more likely to have multivessel disease, multiple comorbidity, and a history of myocardial infarction or coronary artery bypass grafting ( $\chi^2$  tests, all  $P<0.001$ ). Compared with younger patients, the elderly having PCI were more likely to have multivessel disease, multiple comorbidity, and a history of myocardial infarction or coronary artery bypass grafting ( $\chi^2$  tests, all  $p<0.001$ ). The elderly had a higher risk of MACE within 30 days of PCI (4.5% versus 2.7%,  $\chi^2$  test  $p<0.001$ )

Following PCI, mean QoL improved in both elderly and younger patients. Elderly participants had higher baseline mental component score (MCS) but lower physical component score (PCS). After adjusting for baseline differences, QoL (both physical and mental components) in elderly patients improved as much as younger patients, following PCI (SF-12<sub>v2</sub> MCS 50.0(SD 10.4) to 53.0(SD 11.9) vs 46.7(SD 11.1) to 49.7(SD 11.1),  $p=0.652$ ; and SF-12<sub>v2</sub> PCS 37.6(SD 10.1) to 41.9(SD 10.1) vs 39.7(SD 10.0) to 45.6(SD 10.8),  $p=0.373$ ).

An inverse relationship was found between LTL (T/S ratio) and age. No statistically significant difference was found in mean T/S ratio between those with and without CAD (0.87(SD 0.21) vs 0.89(SD 0.21),  $p=0.091$ ), even after adjusting for baseline characteristics. In addition, there was no statistically significant difference in relative T/S length by severity of disease in those found to have stenosis on cardiac angiography: 0.875 (SD 0.211) vs 0.875 (SD 0.212) vs 0.860 (SD 0.203) vs 0.867 (SD 0.200),  $p=0.670$

## Conclusions

This thesis has demonstrated that, in Scotland, elderly patients account for an increasing number and proportion of diagnostic coronary angiograms and PCIs. However, the threshold for investigation and subsequent intervention appears to be higher among the elderly, even after adjusting for co-morbidities. While elderly patients have a higher risk of early complications than younger patients, their absolute risk is, nonetheless, low. This suggests that coronary angiography and PCI are safe procedures to perform in the elderly. Following PCI, the QoL of elderly patients improves at least as much as in younger patients.

A recognized risk factor for CAD is chronological age, and there is increasing interest in whether biological age contributes to the development and progression of disease and can explain socioeconomic inequalities in health. However, the current thesis found no association between LTL and either the occurrence or severity of CAD, or its severity on cross-sectional study. While LTL is considered a useful biomarker of ageing, these findings suggest that LTL may not be as useful in CAD.

Although findings suggest that coronary angiography and PCI are safe procedures in the elderly, results of this thesis suggest an age-based inequality in access to coronary artery investigation and intervention that is not explained by differences in demographic trends, levels of need, potential risk or potential benefit. These findings have significant implications for the delivery of cardiovascular clinical services to an increasing elderly population. Further investigation should be undertaken upstream of these studies, on patients referred for investigation rather than just those receiving it to determine the extent to which there are inequalities in referral threshold as well as procedure threshold. Further research is also required to identify those elderly patients who would most benefit from earlier investigation and management. There is also a need for longitudinal studies to assess the usefulness of LTL as a biomarker of ageing in CAD and to investigate whether LTL is associated with adverse outcomes in patients diagnosed with CAD.

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## GLOSSARY OF ABBREVIATIONS AND ACRONYMS

ACS	Acute coronary syndrome
AGE	Advanced glycation end products
AMI	Acute myocardial infarction
BMI	Body mass index
CABG	Coronary artery bypass grafting
CAD	Coronary artery disease
CABG	Coronary artery bypass grafting
CCS	Canadian Cardiovascular society classification
CDKN2A	Cyclin-dependent kinase inhibitor 2A (Gene, mRNA, and protein)
CoV	Coefficient of variance
CHD	Coronary heart disease
CVD	Cardiovascular disease
DNA	Deoxyribonucleic acid
ECG	Electrocardiogram
FFR	Fractional flow reserve
GROS	General Register Office for Scotland
IDDM	Insulin dependent diabetes mellitus
IVUS	Intra-vascular ultrasound
ISD	Information services division
Lab	Laboratory
LMS	Left main stem artery
LTL	Leucocyte telomere length
MACE	Major adverse cardiac (or cardiovascular events) events
MTR	Mitochondrion, telomere and ribosome biogenesis
MVD	Multivessel disease
NIDDM	Non insulin dependent diabetes mellitus
NHS	National Health Service
NO	Nitric oxide
NSTEMI	Non-ST elevation myocardial infarction
PCI	Percutaneous coronary intervention
PCR	Polymerase chain reaction
PTP	Pre-test probability
QALY	Quality adjusted life years
Q-FISH	Quantitative fluorescence in situ hybridization
QoL	Quality of life

ROS	Reactive oxygen species
RT-PCR	Real time polymerase chain reaction
SIMD	Scottish Index of multiple deprivation
SMR 01	Scottish Morbidity Record 01
STEMI	ST Elevation myocardial infarction
SYNTAX	Synergy between PCI with TAXUS drug-eluting stent and Cardiac Surgery
T/S ratio	Telomere to single copy gene ratio

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# 1. INTRODUCTION

## 1.1. *Overview of thesis*

This thesis comprises of four complimentary studies that investigate the association between chronological and biological ageing in coronary artery disease (CAD). The elderly (when defined in terms of chronological age (time since birth) – see below for further discussion on chronological age cut-offs for defining elderly) account for an increasing proportion of the population and have a high prevalence of CAD.<sup>1</sup> Therefore, elderly patients also represent an increasing proportion of those presenting for investigation and treatment of CAD.<sup>2</sup> Early diagnosis and interventions in CAD are effective at alleviating signs and symptoms of CAD in both elderly and younger patients.<sup>3</sup> However, there is evidence that elderly patients are subject to inequalities in relation to both access to investigations and interventions for CAD, and in participating in clinical trials in this context.<sup>4</sup> This inequality may be partially explained by concerns over adverse outcomes because the morbidity and mortality associated with invasive CAD investigations and interventions are known to be strongly associated with chronological age.<sup>5</sup> In addition, elderly patients have been found to have more advanced disease than younger patients when presenting for treatment and investigation of CAD.<sup>6</sup> It is unclear whether later presentation explains the increased risk of adverse outcomes, or whether it is associated with multiple comorbidity. There is, therefore, a need to investigate factors in elderly patients relating to outcomes of investigations and interventions of CAD.

The first two studies in this thesis are secondary data analyses which were undertaken to examine whether elderly patients undergo coronary angiography at a more advanced stage of disease, whether they are less likely to proceed to revascularisation if CAD is confirmed (see Chapter 4.1), and whether any inequalities in management appear justified by a high risk of peri-procedural complications following percutaneous coronary intervention (PCI) elderly patients (see Chapter 4.2).

In addition, inequalities may be associated with perceived lack of benefits to elderly patients following PCI. However, the management of CAD by PCI is primarily undertaken to relieve the

signs and symptoms of myocardial ischaemia, rather than survival.<sup>7</sup> This makes quality of life (QoL) a critical consideration for clinical decision making at all ages. For elderly patients, an especially important clinical issue is weighing any increased risk of adverse outcomes in the elderly against improvements in QoL. While Study 2 aims to establish if elderly patients do indeed have higher risk of adverse outcomes following PCI, Study 3, assesses the impact of PCI on QoL in elderly compared with younger patients. This is a prospective cohort study of 437 patients that assesses QoL before, and three months after, PCI. The main aim was to compare QoL changes in elderly versus younger patients (ReQoL: REvacularisation and Quality of Life – Chapter 4.3). Together, the findings of these two studies will contribute to clinical decision-making in relation to interventions for CAD in the elderly.

The first three studies in this thesis define age as chronological i.e. the number of years a patient has lived. However, there is currently much interest in whether biological ageing, rather than chronological ageing, may provide a better measurement of age related risks.<sup>8</sup> Biological ageing involves variable, structural and functional changes that take place at the cellular, tissue and organ level; ultimately affecting the overall performance of the body.<sup>9</sup> It is thought to vary between individuals of the same chronological age and increase susceptibility to ill health and disease. If so, biomarkers of biological ageing, rather than chronological age, may provide a more effective basis for clinical decision-making in relation to investigation and treatment in patients with CAD. A number of biomarkers of ageing have been explored, including telomere length.<sup>10</sup> However, conflicting evidence exists as to whether leucocyte telomere length (LTL) is an appropriate biomarker of ageing in CAD.<sup>11</sup> The final study in this thesis aims to provide preliminary evidence as to the potential utility of relating telomere length to presence and severity of CAD, as a precursor to investigating whether it would be more useful than chronological age in predicting clinical outcomes. Therefore, a cross sectional study design was used to investigate the association between LTL (gene ratio -relative ratio of repeat to single copy number) measured using qPCR (quantitative polymerase chain reaction) and CAD (its presence and severity) in 1,846 patients attending a regional cardiovascular centre for coronary angiography (see Chapter 4.4).

Overall thesis objectives, by individual study:

Secondary data analysis using the Scottish Cardiac Revascularisation Register: diagnostic coronary angiography (Study 1)

- to explore time trends in numbers and proportion of elderly patients attending for diagnostic angiography
- to assess the baseline characteristics of patients attending for diagnostic coronary angiography
- to compare baseline case-mix between elderly and younger patients
- to compare clinical outcomes (revascularisation rates and adverse outcomes) between elderly and younger patients attending for diagnostic angiography after adjusting for baseline characteristics

Secondary data analysis using the Scottish Cardiac Revascularisation Register: PCI procedures (Study 2)

- to explore time trends in numbers and proportion of elderly patients attending for PCI
- to assess the baseline characteristics of patients attending for PCI
- to compare baseline case-mix between elderly and younger patients
- to compare clinical outcomes between elderly and younger patients attending for PCI after adjusting for baseline characteristics

ReQoL (Study 3)

- to assess the baseline QoL in patients undertaking PCI using generic and disease specific tools;
- to assess QoL at 3 months post PCI using generic and disease specific tools;
- to compare differences between baseline and 3 months QoL
- to compare differences and changes in QoL between elderly and younger patients
- to use routine hospital data to assess baseline characteristics (such as demographics, comorbidity etc.) and severity of CAD

## Biological ageing and CAD (Study 4)

- to use routine hospital data to assess baseline characteristics (such as demographics, comorbidity etc.) and severity of CAD in a cohort of patients attending for diagnostic angiography
- to undertake LTL analyses in a cohort of patients attending for diagnostic angiography
- to compare LTL between those with and without CAD

The research evidence and theoretical underpinnings in relation to the four studies are discussed in the introduction and the literature review. For example, the introduction discusses general concepts around ageing (including population ageing, biological and chronological ageing), CAD and, QoL. The literature review comprises four main sections, which critically analyse the literature relating to: CAD in elderly patients, PCI in elderly patients, QoL in elderly patients undergoing PCI, and the use of LTL in CAD. After presentation of each of the four studies, the discussion provides a consideration of the results in the context of current literature. The final chapter discusses the overall conclusions and recommendations for future research in this area. As the population ages, the recommendations from this thesis are likely to be of increasing importance for researchers and clinical decision makers.

## **1.2. Ageing**

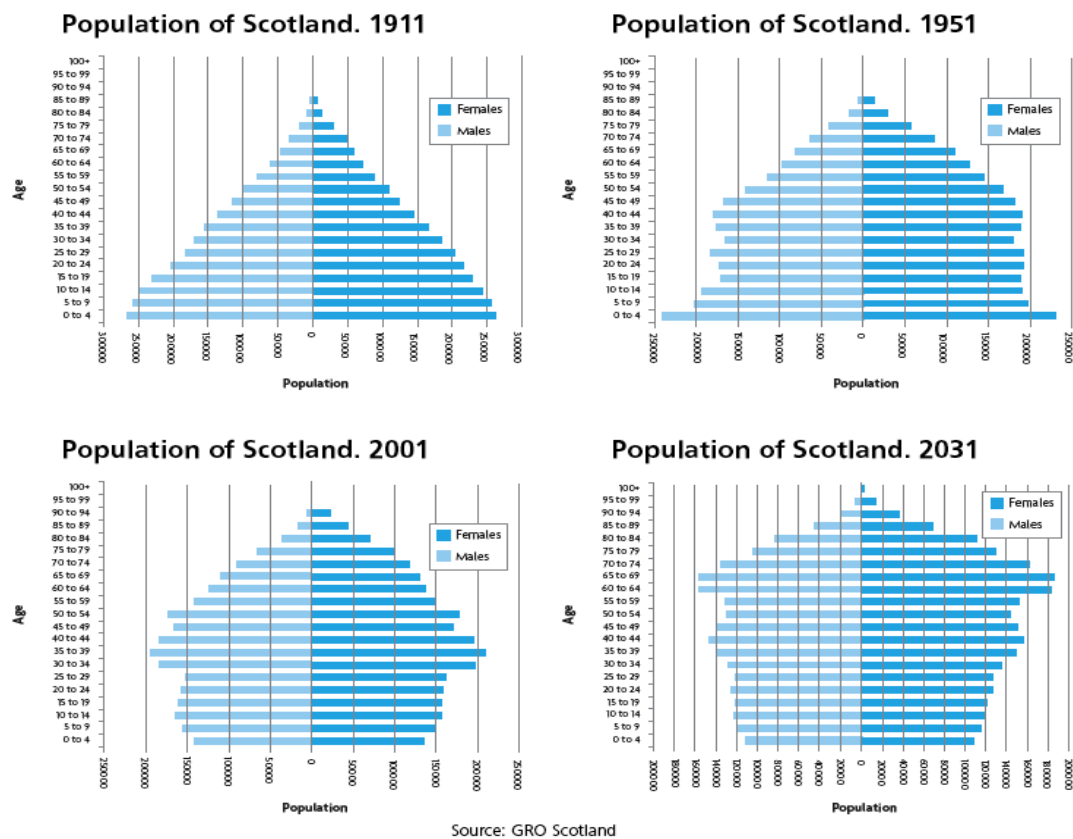
### 1.2.1. Population ageing

Population ageing is the process through which older individuals make up an increasing proportion of the overall population.<sup>1</sup> It can also be considered as an increase in the proportion of people over a particular age (often 65 years of age) in a population,<sup>12</sup> although, an increase in median population age has also been used.<sup>13</sup> Underlying population ageing is the demographic transition from high birth and death rates to low birth and death rates, causing demographic change over time. This results in reduced fertility rates and longer life expectancy, on average, at any age.<sup>14</sup> Driving lower death rates and longer life expectancy is the epidemiological transition:

a move from deaths predominantly caused by infectious diseases to chronic/degenerative diseases. Reductions in deaths from infectious diseases occurred mainly as a result of advances in public health, such as: sanitation; better childhood nutrition; education; and health care improvements (such as antibiotics or vaccinations). As infectious diseases disproportionately affect younger age groups, reductions in deaths from these resulted in people living long enough to develop chronic/degenerative diseases (shift in age at death). In addition, improvements in health care and preventive interventions meant that people were living longer with morbidity of chronic diseases (for example, chronic angina after surviving a heart attack). Together the demographic transition and the epidemiological transition make the “classical” (or “Western”) demographic transition model.<sup>15</sup>

Latest global population projections suggest that population ageing is a feature of all higher income countries and many low-middle income countries. This global trend is likely to continue, with the number of people aged 80 years or over, projected to increase from 69 Million in 2000 to 379 Million by 2050. The proportion of the population aged 80 years or over is also estimated to increase by almost fourfold over the next 50 years, reaching 4.1% in 2050 (estimated as ~1% currently).<sup>16</sup> The 80 years or over age group is the fastest growing of the older population (At the global level, the average annual growth rate of persons aged 80 years or over (3.8 per cent) and is currently twice as high as the growth rate of the population over 60 years of age (1.9 per cent), suggesting that demographic ageing of the older population is also occurring. In addition, global population projections suggest that the median age of the world’s population will increase from 27 years (based on 2000 estimates) to 36 years by the year 2050.<sup>17</sup> These projections assume that baseline estimates of the world’s population are correct (estimates rather than actual numbers are used for many low income countries with less accurate records) and calculations are based on continuation of past trends in fertility and mortality.

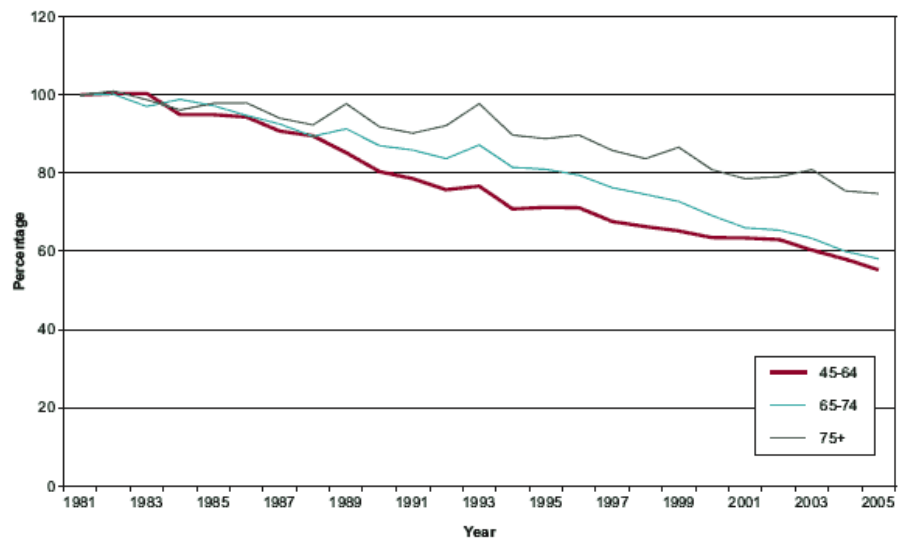
Scotland, like other post-industrial high-income countries, has undergone a similar population change over the last century (Figure 1). The wide based, narrow apex population pyramid of 1911 has been replaced with the narrow based blunt apex of 2001. The 2031 projection shows how the post-war and 1960s baby-boomers are likely to contribute to the ageing population with the pyramid becoming skinnier and more top heavy by 2031.



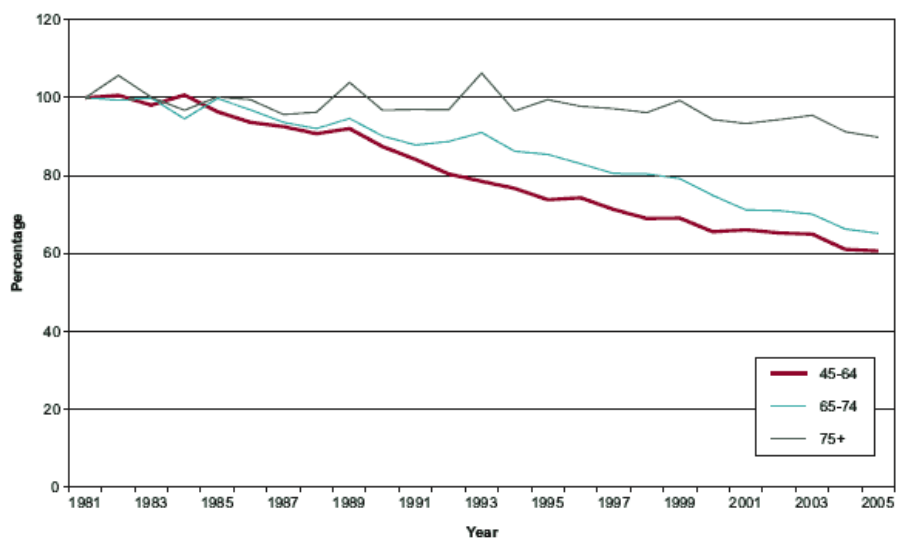
**Figure 1. Scotland's changing population structure 1911 projected until 2031**

Mortality rates for males and females have continued to decline since the 1960's, although this has been less pronounced in women  $\geq 75$  years of age (Figure 2).

a)

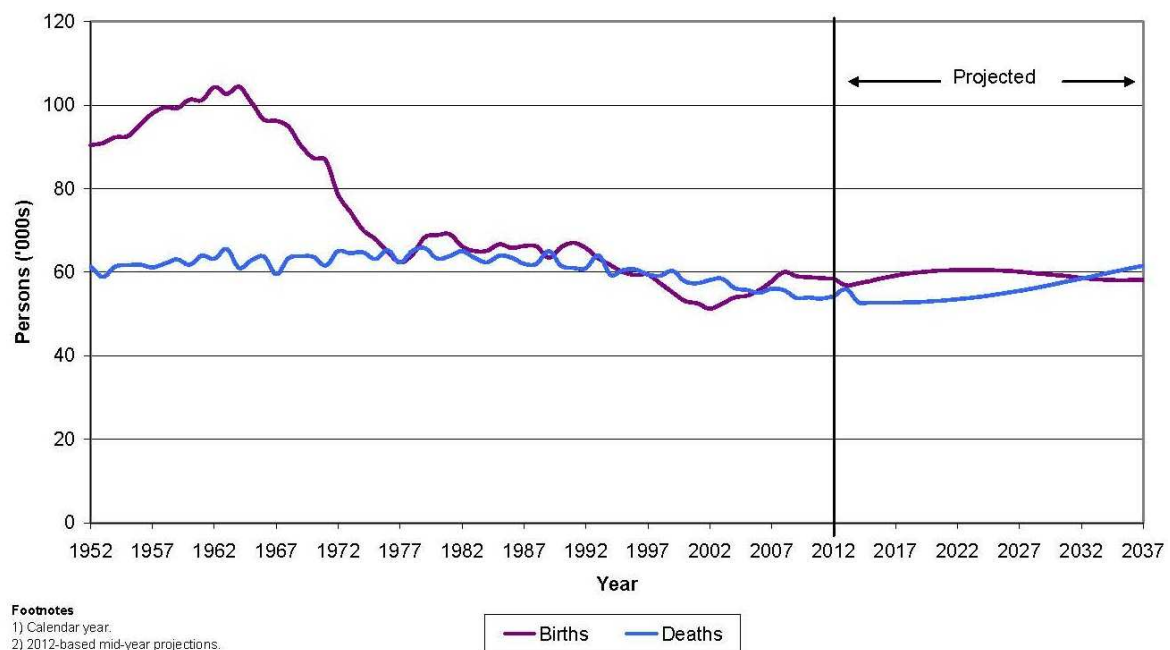


b)



**Figure 2. Age specific mortality rates as a proportion of the 1981 rate, males (a) and females (b) 1981-2005**

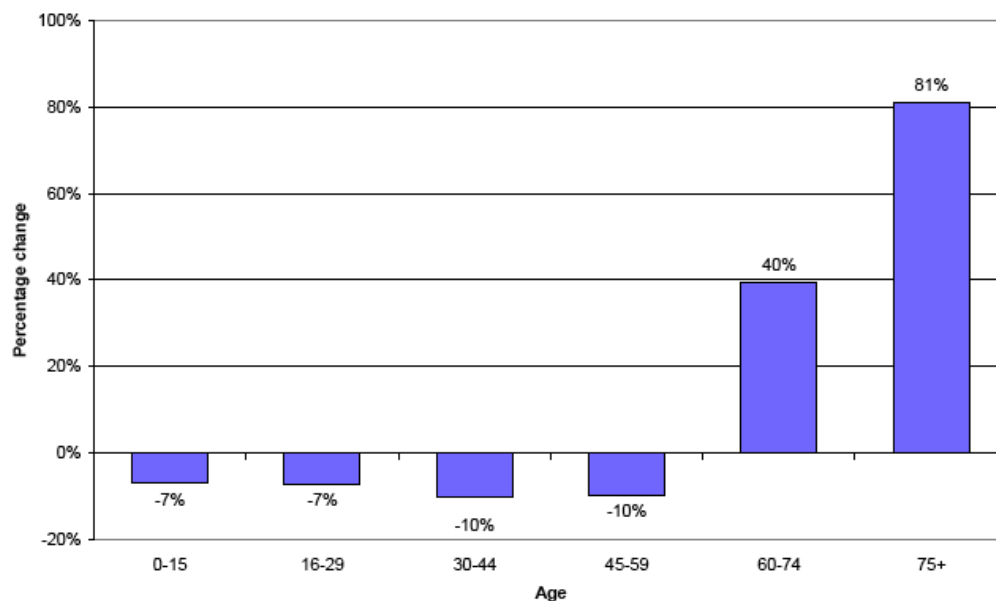
Based on the General Register Office for Scotland (GROS) population projections, deaths are projected to continue to decline until at least 2016 (Figure 3), after which slight increases may occur, as a result of increasing prevalence of type 2 diabetes, obesity and alcohol-related liver disease.



**Figure 3. Births and deaths, actual and projected, Scotland, 1951-2033 (ISD)**

The number of Scots aged 75 years or more is projected to increase by 86% from 418,000 in 2012 to 779,000 in 2037 (Figure 4).<sup>18</sup> With the proportion of the population in Scotland aged 75 years or over projected to almost double from 7.9% to 13.8%. These projections, by GROS, are based on census data and are trend based. They assume that trends in fertility, migration, and deaths will continue. However, these assumptions mean that the estimates are less reliable in

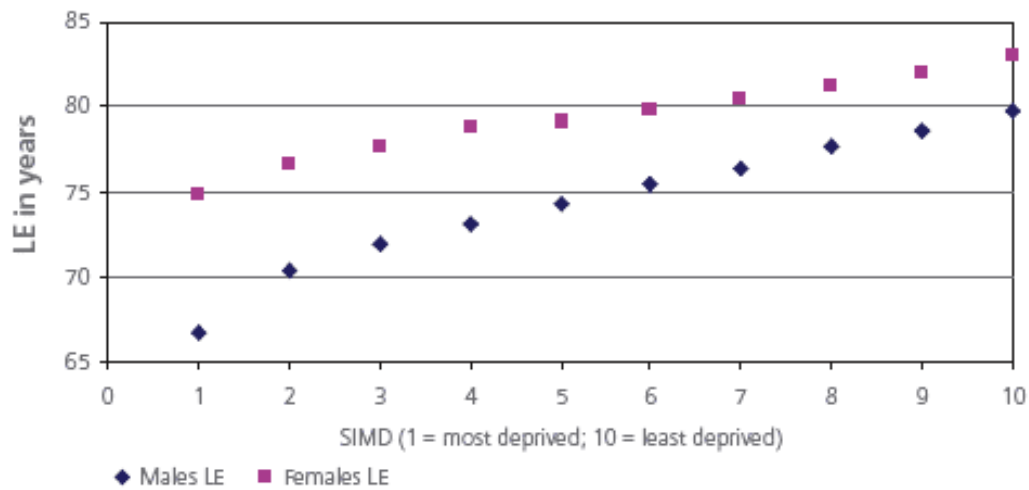
periods of changes, for example, the change in volume of migrants from the EU A8 accession countries to Scotland was not picked up by earlier population projections.<sup>18</sup>



**Figure 4. Projected percentage change in Scotland's population by age group, 2006-2031.**

(<http://www.gro-scotland.gov.uk/files1/stats/projected-population-of-scotland-2012-based/j896103.htm>)

These projections suggest that population ageing will continue to be an increasingly important issue both globally and in Scotland. However, it should be noted that in Scotland ~1:10 people die before they reach 65 years of age with marked inequalities in life-expectancy by socioeconomic deprivation (Figure 5).<sup>9</sup>



**Figure 5. Life expectancy (LE) in Scotland by Scottish Index of Multiple Deprivation (SIMD), 2012.**

The potential impacts of population ageing include: an increased dependency ratio (the ratio of individuals aged 0-14 years and over 65 years of age to those in the economically active age group (16-64 years of age));<sup>20</sup> a rise in the prevalence of degenerative disorders such as arthritis, chronic obstructive pulmonary disease, and cardiovascular disease (CVD); expansion of morbidity (rather than compression) - an increase in the number of years spent living with chronic disease morbidity; increased health and social care costs; increase in prevalence of multiple morbidity and increasingly complex morbidity; and an increased number of older carers who may themselves have degenerative disorders.<sup>21</sup>

However, Spijker *et al.*<sup>22</sup> suggest that relative to remaining life expectancy, populations appear to be growing “younger.” For example, a 50 year old today is considered younger than a 50 year old would have been 40 years ago because they are expected to live for a much longer time. In addition, the number of people working after state pension age is rising. Therefore the dependency ratio may not be as high, in the future, as initially predicted. Also, improvements as a result of early years interventions (as there is growing evidence to suggest that age-related chronic diseases have their origin in experiences in early years),<sup>23</sup> health care (including activities like screening), lifestyle/behavioural modifications (e.g. smoking cessation; and other prevention activities may well off-set some of this impact and result in healthy life expectancy

(average number of years a person is expected to live to in a healthy state) increasing. Interest is growing in investigating the factors which influence healthy life expectancy and healthy ageing,

While there is no universal definition of elderly, or older persons, most high income countries consider it to be 65 years of age<sup>24,25</sup> because this age is often associated with eligibility for statutory and occupational retirement pensions. However, the WHO acknowledges<sup>24</sup> that such an arbitrary age cut-off is not necessarily subject to: the social constructions of what it means to be elderly, to geographical differences in life-expectancy, or the context in which the term is being used e.g. in cardiac patients. The terms “elderly” and “older persons/people” are often used interchangeably in the biomedical literature. There is much debate as to the appropriateness of using the term elderly, as it is thought to be associated with negative and stereotypical images.<sup>26</sup> In response, some of the biomedical science literature has universally adopted the term “older persons” as the standard.<sup>27</sup> This is less so in the cardiology literature where elderly patients tends to be the term used more often to describe chronologically older patients<sup>28,29</sup> For the purposes of this thesis, the term older people has been used when discussing population ageing and ageing processes; however, the term elderly patient has been used in relation to patients with CAD, or attending for investigations of CAD. The choice of chronological age cut-offs for elderly patients is also much debated in the cardiology literature with  $\geq 75$  years or  $\geq 80$  years being commonly used in PCI research.<sup>30,31</sup> It is also subject to the age at which patients present with CAD and, as discussed, variation in life expectancy of a given area (e.g. Scotland’s life expectancy is one of the lowest in Western Europe) has an impact on this. For the purposes of this thesis, elderly patients are defined as  $\geq 75$  years of age for secondary data analyses (Chapter 4.1 and 4.2), and  $\geq 70$  years for ReQoL (following issues with recruitment and a re-analysis of the sample size calculation – see Chapter 3.2). The systematic review of QoL following PCI in elderly patients focuses on octogenarians to allow comparisons to be made with the systematic review of clinical outcomes, which only included octogenarians. In addition, using a more focused population in the search strategy excluded those studies which may have presented some less elderly focused age related sub-group data (for example using “old” or “old\*” in the search strategy included studies which used  $>50$  years of age in sub-group analysis), thus allowing easier identification of studies on elderly patients.<sup>32</sup>

### 1.2.2. Theories of ageing

As discussed, ageing can simply refer to the passage of time since birth (chronological age) or to the physical or biological processes of growing old. Physical ageing is thought to be a complex biological process that may not correspond to the chronological ageing of individuals. In addition, the rate of biological ageing may vary between individuals in response to internal (genetics) and external factors. There are a number of different definitions of the ageing process. Rose (1991), in his book on the evolution of ageing, described it as “a persistent decline in the age-specific fitness components of an organism due to internal physiological degeneration”.<sup>33</sup> It has also been defined as a progressive functional decline, or a gradual deterioration of physical functioning with age.<sup>134</sup> Also as “the intrinsic, inevitable, and irreversible age-related processes of loss of viability and increase in vulnerability”.<sup>35</sup>

Strehler (1959)<sup>36</sup> characterised ageing by 4 main features:-

- It is destructive – compromising functionality
- It is progressive and irreversible
- It is intrinsic i.e. determined by internal rather than external factors
- It is universal, i.e. all individuals of the same species display a largely uniform ageing pattern, with all living beings displaying the ageing phenomenon.

Of note is that while average life expectancy has increased significantly over the last century, maximal longevity (maximal age achieved by any individual) has, shown little or modest evidence of increasing.<sup>37</sup> However, ONS estimates that there has been a 5-fold increase in the number of centenarians. These may go on to live longer than ever before, suggesting that in the future maximal longevity may also increase.<sup>19</sup>

Human ageing is associated with multiple psychological changes and pathological consequences which affect every system, tissue and organ. In ageing, virtually all physiological functions lose efficiency. The effects include:

- appearance: gradual loss of height, muscle and bone mass, and decreased skin elasticity,
- sensory functioning: reductions in vision acuity and hearing capacity,
- cognition: memory and cognitive impairment,
- reduced capacity to maintain homeostasis,
- deterioration of the force and elasticity of skeletal muscle,
- increased susceptibility to stress such as external temperature, infections, hyperglycaemia, and trauma.

There is no universally accepted theory of the ageing process; instead, there are numerous theories which have attempted to explain the mechanisms involved. More than 300 of these “theories of ageing”<sup>39</sup> exist although they are not necessarily mutually exclusive. Generally, they can be grouped into 3 main mechanisms:

- Evolutionary: ageing is part of life’s programming because the old need to remove themselves to make room for the next fertile generation, thus sustaining evolutionary turnover.
- Network: that multiple mechanisms of ageing work in parallel and that ageing results from the interactions between cells, organs and systems.
- Causative Mechanisms: ageing can be explained at the cellular level e.g. accumulation of cellular waste products, causing cellular senescence (when cells cease to divide with resultant impairment of function).

The free radical theory of ageing is one of the most prominent causative mechanism theories and was first proposed by Harman in the 1950’s.<sup>40</sup> This theory proposes that accumulation of free radicals derived from oxygen is responsible for the decline in biological functions associated with ageing. Reactive oxygen species (ROS) within cells are primarily generated by the mitochondrial respiratory chain, mainly due to electron leakage. Thus, ROS are viewed as being involved in cellular regulation by acting as Redox signals. Over time, the antioxidant systems are

unable to counteract all of the ROS (such as superoxide ( $\text{O}_2^-$ ); hydrogen peroxide ( $\text{H}_2\text{O}_2$ ); and peroxynitrite ( $\text{OONO}^-$ )).<sup>41</sup>

These free radicals capture electrons from another molecule, which in turn become unstable and reacts with further molecules. This results in oxidative damage to lipids, DNA, and proteins in the cells and therefore causes damage to the tissue. This process is called oxidative stress. There is experimental evidence in in-vitro and animal models to support this theory. In animals, enhanced oxidative damage by ROS has been shown to lead to shorter lifespans<sup>42,43</sup> while antioxidants have been shown to extend the lifespan in *Drosophila*.<sup>44</sup> However, recent experiments show that, in mice and worms, increases in ROS actually correlate with normal and even longer life span, with the harmful effects of ROS seen as a result of compromised signalling, rather than direct oxidative damage to sensitive targets.<sup>45</sup> The precise relationship between ROS induced damage, mitochondrial damage and ageing is unknown.

### 1.2.3. Healthy ageing

The prevalence of long-term illness and self-reported ill health increases with chronological age,<sup>26</sup> meaning that gains over time in healthy life expectancy and disability-free life expectancy are much smaller than the increase in overall life expectancy. Associated with this is a decrease in the proportion of life spent in “favourable health states”.<sup>46</sup> The proportion of people reporting that they have a limiting longstanding illness is higher in the older population, with 37% of 65-74 year olds and 48% of those over 75 years reporting that they have such an illness.<sup>46</sup> The Scottish Health Survey reported that 13% of people aged 70 years and over have both a long-term illness and disability compared to only 2% of 30-39 year olds.<sup>47</sup> Older people were more likely to report that poor health affects their activities of daily life, for example: 27% of men aged 75 years and over reported that “ill health limited their ability to perform moderate activities 'a lot' compared with only 6% of men aged 18-24 years”.<sup>47</sup> However, many people in older age groups still consider themselves to be in good health, even if they have a long-term illness which restricts their daily lives.<sup>46</sup>

Given that the most important risk factor for chronic diseases which limit health in later life is age itself, we need to better understand the biological process of ageing and how this relates to ageing on an individual and population level.

Healthy (or successful) ageing can be defined as “optimising opportunities for good health, so that older people can take an active part in society and enjoy an independent and high quality of life”.<sup>48</sup> There are three main components to this: low probability of disease; high cognitive and physical functioning; and active engagement in life.

Recently, recognition has been given to the concept of a life course approach to healthy ageing where internal and external (physical and social) environments have been found to affect the ageing trajectory.<sup>49</sup> Critical periods such as early life or later life experiences; accumulation of exposures; and chain of risk (the sequence of linked exposures) throughout life appear to affect the ageing process. Observational and experimental evidence increasingly supports a relationship between growth and development in early years (a critical period) and health in later years.<sup>23</sup>

#### 1.2.4. Frailty

Frailty has been defined as a syndrome of decreased reserve and resistance to stressors causing vulnerability to adverse outcomes (based on the French word *frêle* meaning of little resistance). When exposed to such stressors, frail patients are at increased risk of decompensation, adverse events, procedural complications, prolonged recovery, functional decline, disability, and mortality. Others have suggested that frailty is: “an age-related, biological vulnerability to stressors and decreased physiological reserves”;<sup>50</sup> or that it is more useful to define frailty as “clusters of vulnerabilities, weaknesses, instabilities and limitations, with shared causes”.<sup>51,52</sup>

Many such definitions of frailty have commonality to that of ageing and, similar to biological age, there appears to be wide variations in frailty between individuals of a similar chronological age. Frailty, with its impairment in cognitive and physical function with resultant loss of resilience and reserve, may be seen as a consequence of biological ageing; indeed frailty measurements have been used as a clinical tool for the assessment of biological ageing.

Studies have shown that frailty carries an increased risk of mortality and morbidity associated with CVD and heart failure. In addition, it is associated with increased risk of adverse outcome following acute coronary syndrome (ACS) and PCI. Clinically, frailty is becoming increasingly important, given the ageing population and increasing complexity of care for older patients. Innovations in the investigation and management of CAD, for example, have resulted in interventions, such as PCI, being able to be used in elderly and frail patients. The current ESC guidelines on the management of stable CAD recommends that “frailty should be well assessed eventually by means of current available indices”<sup>3</sup> However, it is unclear what is meant by “well assessed eventually”.

Upward of 20 frailty tools have been developed to measure frailty.<sup>53</sup> Owing to a lack of consensus agreement, there is variability among studies and confusion on which tool to use. For example, van Iersel *et al.*<sup>54</sup> reported, in a sample of 125 elderly people, a prevalence of frailty ranging from 33% to 88% depending on the criteria used. Most tools focus on 1 or more of the 5 core domains that define the frailty phenotype: slowness, weakness, low physical activity, exhaustion, and shrinking (unintentional weight loss). Slowness was measured by a comfortable-pace gait speed test, weakness by a maximal handgrip strength test (using a dynamometer), and other domains by questionnaire or more specialized instruments. These domains may be considered individually or combined into a variety of scales.

The Fried scale<sup>55</sup> encompasses slowness, weakness, low physical activity, exhaustion, and shrinking, with at least three of the five 5 criteria requiring to be satisfied for a diagnosis of frailty. It was developed from measurements in the Cardiovascular Health Study (CHS) and the Women’s Health and Aging Studies (WHAS).<sup>56</sup> It consists of a total score based on:

1. Shrinking: >10lbs unintentional weight loss in the last year or  $\geq 5\%$  of body weight in prior year by direct measurement of weight
2. Reduced grip strength (tested using a hand held dynamometer): as being in the lowest 20%, adjusted for gender and BMI

3. Self-reported exhaustion
4. Slowness (measured using time to walk 15 feet adjusted for gender and height): as being in the lowest 20%, adjusted for age and gender
5. Low physical activity level – using kilocalories expended per week.

A prevalence of 7% in the CHS was found in 4,317 community dwelling adults aged 65 years and over, 30% in the sub-group aged 80 years and over, and 28% in the WHAS moderately to severely disabled population of 1,002 community dwelling women aged 65 years and over. Fried *et al.*<sup>55</sup> concluded that frailty was “not synonymous with either co-morbidity or disability but co-morbidity is an etiologic risk factor for, and disability is an outcome of, frailty.”<sup>55</sup>

The Fried Scale is the most frequently cited frailty scale and has been demonstrated to predict mortality and disability in large cohorts of community-dwelling elderly people and patients with CVD<sup>55</sup>. Whether cognition and mood should be considered as additional domains of frailty scale or as modulating factors (such as having a role in the transition from frailty to overt disability) remains an area of discussion.

The Short Physical Performance Battery (SPPB)<sup>57</sup> encompasses slowness, weakness, and balance. This is measured by a series of three timed physical performance tests (gait speed, chair rises, and tandem balance), each is scored 0 to 4 and frailty is defined as a total score >5 out of 12.

In contrast to these multi-item frailty scales, five metre gait speed and, to a lesser extent, handgrip strength, have been advocated as single-item measures of frailty.<sup>58</sup> The gait speed test has been shown to have high inter-rater reliability (intraclass coefficient 0.88 to 0.96) and test-retest reliability (intraclass coefficient 0.86 to 0.91).<sup>59</sup> It has been found to be responsive to change, with meaningful improvements in gait speed (estimated at 0.05 to 0.2 m/s<sup>59</sup>) predicting positive outcomes on a population level,<sup>60</sup> but not necessarily an individual patient level.<sup>61</sup> The walking distance used in research studies varies between three and ten metres, although the distance appears to have little effect on measured speed.<sup>62</sup> A large number of CAD registries have adopted the five metre distance as it is thought to be a balance between allowing patients to

achieve a steady walking speed without developing angina symptoms.

The Fried scale and the SPPB tools are used to reflect the clinical phenotype of frailty. However, an alternative approach reflects the accumulation of deficits.<sup>63</sup> Deficits encompass an assortment of up to 70 symptoms, signs, comorbidities, disabilities, and frailty traits, which are counted and summed. One version is the Frailty index developed by Mitnitski *et al.*<sup>64</sup> They propose that a Frailty index, based on 20 deficits, can be used as a proxy measure of ageing and mortality as it better reflects physical ageing than chronological ageing, with higher scores being associated with a greater risk of adverse outcomes. However, the International Academy on Nutrition and Aging Frailty Task Force<sup>65</sup> favoured the clinical phenotype approach stating: “comorbidities and disabilities should be disentangled from frailty”.

Disabilities, broadly defined as difficulty or dependency in carrying out activities of daily living (ADL), are often used interchangeably with frailty. However, disability is more accurately considered as an adverse outcome associated with frailty (for example, a frail patient becoming more disabled after an acute myocardial infarction (AMI)). Certain scales may be effective at screening for frailty, whereas others may be required to focus on specific and potentially treatable domains. There is justifiable reason to consider using different scales, more or less challenging versions of such scales, or different cut-offs depending on the population being studied. In addition, heterogeneity amongst patients suggests that the use of a fixed cut-off for frailty may not be appropriate.

A study by Purser *et al.*,<sup>66</sup> was one of the first to prospectively measure frailty, using 3 different frailty tools, to assess mortality outcomes in elderly patients with CVD. In this study, 309 elderly patients with multivessel disease (MVD) admitted to a coronary care unit, found that the prevalence of frailty varied considerably depending on the tool used: 27% using the Fried scale, 50% using gait speed < 0.65 m/s, and 63% using the Rockwood frailty scale. While increases in 6-month mortality was associated with each frailty tool and composite scores (developed by the authors using all the scales), only gait speed reached statistical significance (odds ratio (OR) 3.8; 95% confidence interval (CI) 1.1-13.1). However, the CIs were wide and no statistical significance was presented. This study was influential in highlighting to clinicians the

importance of frailty, in addition to traditional risk factors, for predicting clinical outcomes of CVD.

In a study of 629 elderly patients who underwent PCI at the Mayo Clinic, 21% were found to be frail, based on administration of the Fried scale before discharge (unintended weight loss (>10 lb in the preceding year), exhaustion, physical activity, time required to walk 15 feet, and grip strength by Jamar handgrip dynamometer). Patients who were frail had a significantly increased 3-year mortality: 28% vs. 6%; OR: 2.45; 95% CI 1.33,4.53.<sup>67</sup> However, those who did not survive until discharge were excluded from the study and only 41% of the eligible participants consented; therefore, there is the potential for selection bias as a main limitation of this study. QoL was also measured in this study but was not found to be associated with an increased hazard for death following PCI. Similarly, cachexia/frailty was found to be the most powerful predictor of 18-month mortality (hazard ratio (HR) 14.0 ) in a study of 111 patients undergoing PCI for unprotected left main disease in the Kaiser Permanente database.<sup>68</sup> All participants were considered ineligible for CABG, only 7(13%) of patients were considered cachexil/frail, and definition of cachexil/frail was based on case note review.

Gharacholou *et al.*<sup>69</sup> further showed that, despite a similar severity of angina between frail and non-frail patients aged  $\geq 65$  years and older, those who were frail had lower physical functioning and QoL. Frailty was found to have a greater impact on QoL than severity of CAD or comorbidity. Frail patients were more likely to have MVD and/ or left main stem (LMS) involvement, after adjusting for age and gender (p values <0.05 across the groups). Ekerstad *et al.*<sup>70</sup> explored the relationship between frailty and comorbidities in patients with non ST elevation myocardial infarction and showed that 79% of frail patients had at least one severe comorbidity. Frailty was also found to be independently associated with 1-year mortality after adjusting for cardiovascular risk and comorbid conditions (HR 4.3, 95% CI 2.4-7.8). Frailty was found to be better at predicting mortality than the presence of comorbidity alone. When the comorbidity burden was stratified by severity, moderate to severe frailty was found to be more predictive of mortality. Frailty is associated with less aggressive management compared with non-frail counterparts. Frail patients were less likely to receive angiotensin-converting enzyme inhibitors, and beta-blockers, less likely to be admitted to a coronary care unit, and less likely to

be referred for PCI or CABG.

### 1.2.5. Biological ageing and biomarkers of ageing

In contrast to chronological ageing which is easily defined and measured, biological ageing involves variable structural and functional changes that take place at the cellular, tissue and organ level; ultimately affecting the overall performance of the body.<sup>9</sup> It is thought to vary between individuals of the same chronological age and increase susceptibility to ill health and disease. Biological ageing has also been described as a “steady decrease in physiological ability to meet demands that occur with increasing chronological ageing”.<sup>71</sup> Biological ageing is thought to be due to failure of intracellular and immune system repair mechanisms with resultant cellular damage.<sup>72</sup> The lack of a universal definition of biological age makes consistent measurement, reporting, and comparison of results difficult.

Biological age (“miles on the clock”)<sup>8</sup> appears to be a better reflection than chronological ageing of inter-individual variation in rates and expressions of growth and ageing, given the heterogeneity of life span and healthy life span. There have been numerous studies which have used physical health and various biomarkers to assess biological age,<sup>73,74</sup> with particular interest in models which can be applied to the clinical setting.<sup>75</sup> Biomarkers include biochemical assays, genes and physiological characteristics that can indicate the presence, or severity, of an existing disease, physiological abnormality (e.g. lens opacity) or psychological condition (or increased risk of their future development). Biomarkers of ageing aim to better predict functional capacity than chronological age by correlating more closely with “biological age”. In particular, they aim to identify individuals who are at greater risk of age-associated disease or disability, or who develop it at a younger chronological age.

Baker and Sprott (1988)<sup>76</sup> suggested the following criteria for biomarkers of biological ageing:

1. *The rate of change of the biomarker must, at least in mathematical terms, reflect some measurable parameter which can be predicted at a later chronological age.*

2. *The biomarker should reflect some basic biological process of ageing and certainly not predisposition toward a disease state or some error in metabolism.*
3. *The biomarker should have high reproducibility in cross species comparisons of functional or physiological age versus chronological age, particularly within the same classes and certainly within the same families of species.*
4. *The biomarker should change independently with chronological age and reflect physiological (functional) age.*
5. *Assessment of the biomarker should be non-lethal in animal systems and should cause minimal trauma in humans.*
6. *The biomarker should be reproducible and measurable during a relatively short time interval compared to the life span of the animal.*

Subsequently, a simpler set of criteria for ageing biomarkers was proposed by Miller in Butler *et al.* (2004):<sup>77</sup>

1. *The biomarker should predict the outcome of a wide range of age-sensitive tests in multiple physiological and behavioural domains, in an age-coherent way, and do so better than chronological age.*
2. *It should predict remaining longevity at an age when 90% of the population is still alive, and do so for most of the specific illnesses that afflict the species under study.*
3. *Its measurement should not alter life expectancy or the outcome of subsequent tests of other age-sensitive tests.*

The second criterion suggests that biomarkers of ageing will also be biomarkers of age-related diseases rather than merely measures of degenerative changes. This is contentious because ageing is thought by others to be a distinct process with specific pathways.<sup>78</sup>

The American Federation for Aging Research has proposed the following criteria (reviewed by Johnson, 2006):<sup>79</sup>

1. The biomarker must predict the rate of ageing. In other words, it should tell exactly

where a person is in their total life span. It must be a better predictor of life span than chronological age.

2. It must monitor a basic process that underlies the ageing process, and not the effects of disease
3. It must be able to be tested repeatedly without harming the person, for example, a blood test or imaging technique.
4. It must be something that acts in both humans and laboratory animals, such as mice, so that it can be tested in animals before being tested on humans.

However, even if a potential biomarker could be validated in model organisms, it is still unclear whether it would apply equally well to humans.

Physiological biomarkers of ageing, suggested in the literature, include perceived age, systolic blood pressure, lipid concentrations, body mass index, cognitive function, and lens opacity.<sup>10,74,80</sup> In addition, at the clinical level, geriatric assessments<sup>81</sup> and measurement of frailty<sup>82,83</sup> have also been suggested as complex biomarkers of ageing. Biological markers that measure inflammation, oxidative stress, protein glycation, cellular senescence and hormonal deregulation have all been explored.<sup>10</sup>

- Inflammatory markers: The immune response in humans is designed as a defence system to protect and respond to environmental exposures such as infectious agents. It does this via an innate (natural) response and an acquired (adaptive response). Innate immunity involves cellular (for example, macrophages and T-cells which secrete interleukin 6 (IL6)) and non-cellular (C-reactive protein (CRP) and complement cascade components). Therefore, peripheral blood markers of inflammation include IL-6 and CRP. Although these are non-specific, abnormal values are thought to indicate clinical or subclinical levels of chronic inflammation that contribute to, or are part of, the ageing process. In addition, senescent cells have been found to secrete pro-inflammatory cytokines (such as interleukins).<sup>72</sup> For example, those with elevated IL-6 were significantly more likely to develop disabilities of mobility and activities of daily living over a four-year period, even after adjusting for multiple potential confounders.<sup>83</sup> In a

similar cohort, CRP has been shown to be an independent risk factor associated with a 2-fold risk of death in a cohort of higher functioning elderly patients.<sup>84</sup>

- Oxidative stress: In keeping with the free radical theory of ageing,<sup>40</sup> a number of molecules can be produced as a result of the oxidative stress - ROS (Reactive oxygen species). ROS are constantly being produced in cells and play a role in various signalling pathways and immune response.<sup>85</sup> ROS are removed from cells by various antioxidant defence mechanisms. The imbalance between oxidative metabolism and antioxidant defence is called oxidative stress. Cumulative cellular damage by ROS throughout the life span has been proposed as an important factor in ageing. A number of potential biomarkers of oxidative stress have been developed. For example, 8-hydroxy-deoxyguanosine (8-OHdG) is formed when guanine (a DNA nucleotide) is damaged by oxidative damage.<sup>86</sup> This is excreted in urine and is known to be increased by smoking.<sup>87</sup> Levels have been found to be inversely associated with life span in animal studies.<sup>88</sup>

Senescent cells are associated with a high level of intracellular ROS and accumulation of oxidative damage to DNA and proteins.<sup>73</sup> Telomere shortening (DNA caps at the end of chromosomes – see Chapter 2.4 for further discussion on telomeres) is considered to be one of the major cause of replicative cellular senescence and is increased as a result of mild oxidative stress.<sup>46</sup> Senescence can be by-passed by the introduction of telomerase into human cells.<sup>89</sup>

The amino acid homocysteine is derived from dietary methionine. High levels of methionine can be found in protein dense food such as eggs, sesame seeds, Brazil nuts, fish and meat. Restricting methionine consumption has been shown to increase lifespans in some animals.<sup>89</sup> Homocysteine is thought to increase production of ROS and the cell's ability to protect itself from the damage caused by ROS.

DNA methylation: This involves the addition of a methyl group to cytosine or adenine (DNA nucleotides). The pattern of methylation controls protein binding to target sites on DNA, affecting changes in gene expression and in chromatin organization. For example, it can silence genes responsible for differentiation, or those predisposing to cancer. Research involving saliva samples from 34 pairs of identical male twins aged 21

to 55 years, identified 88 sites on the DNA where DNA methylation was strongly correlated with chronological age.<sup>90</sup> Global DNA methylation levels have been found to be correlated with frailty status in middle/advanced-aged subjects but not with chronological age. A seven-year follow-up study also revealed that worsening in frailty status was associated with a significant decrease in global DNA methylation levels<sup>91</sup>

- Advanced glycation end products (AGEs): These are proteins or lipids that are non-enzymatically glycosylated and oxidized after contact with sugars. AGEs can be produced in cells or absorbed through the diet (foods high in protein and fat such as meat, cheese or egg yolks and food which has been cooked at high temperature, for example, by frying or barbecuing). Within cells, complex ROS-dependent reactions occur which lead to the formation of AGEs. Cell activation by these AGEs leads to the generation of more ROS with resultant oxidative damage. Accumulation of the AGE carboxy-methyl lysine (CML) has been associated with chronological age and age related diseases such as cataracts.<sup>92</sup> CML has been shown to be associated with diabetes disease progression where it has been linked with increased risk of developing retinopathy, nephropathy and cardiac disease.<sup>93</sup>
- Cellular senescence and telomeres: Cellular senescence was first described in 1961 when Hayflick noted that cell proliferation in a cell culture of human fibroblasts gradually declined over time.<sup>94</sup> Eventually, all cells in the culture lost the ability to divide, although they stayed viable for a long time afterwards (in a state of growth arrest described as cellular senescence). He proposed that this resembled the ageing process within systems and organisms, as a whole.  
Cellular senescence in primary somatic cells is accompanied by telomere attrition. Telomeres are nucleoprotein complexes that cap and protect the ends of chromosomes. The DNA component of telomeres consists of repeats of TTAAAGG that shorten as a function of replication in most human somatic cells.<sup>95</sup> Therefore, telomere length is an indicator of a cell's replicative age. Accelerated telomere attrition has been shown to be a feature of many pathologies including cancer, vascular dementia, diabetes, and cardiovascular disease.<sup>96</sup>

Cellular senescence is also accompanied by the expression, or appearance of, markers such as senescence-associated  $\beta$ -galactosidase and CDKN2A (whose conjugate protein is termed P16<sup>ink4</sup>). It appears to act as a tumour suppressor and maintains cells in a state of growth arrest in premature senescence.<sup>97</sup> CDKN2A has been associated with cellular senescence in cell and animal studies. Increasing levels of CDKN2A transcriptional expression (CDKN2A mRNA levels) have been found to occur with increasing chronological age, both in solid organs and peripheral blood leucocytes (PBL). Shiels suggests that CDKN2A can better fulfil Baker and Spott's criteria than other proposed biomarkers of ageing.<sup>98</sup>

Lamb and Shierls<sup>78</sup> also suggest that all these markers operate within the functional framework of the MTR (Mitochondrion, Telomere, and Ribosome biogenesis) trinity (of ageing), which suggests that ageing is associated with interactions between cellular damage responses and any associated fuel utilisation and energy production – the level of damage accrued and the level of energy expenditure required to facilitate repair, dictates whether a cell will live (functioning or senescent) or die. The more cells die or become senescent, correlates directly with organ function and biological age.

- Fetuin-A: This is a serum protein which is secreted by the liver and has multiple diverse effects throughout the body. It has been shown to act as an inhibitor of vascular calcification, by preventing spontaneous mineral precipitation, and it may be capable of attenuating inflammatory processes.<sup>99,100</sup> Despite this, it has been shown to induce insulin resistance in muscle and fat and has been associated with clinical and sub-clinical CVD and type 2 diabetes.<sup>101</sup> However, it has also been associated with a decreased risk of impaired cognitive functioning and was found to be lower in patients with Alzheimer's disease.<sup>102</sup> Maxwell *et al.* suggest that decreased fetuin-A and telomere attrition indicate accelerated biological ageing.<sup>103</sup>
- Sirtuins(1-7) are a family of proteins found in all living things, whose function is linked to cellular metabolism in numerous tissues, including liver, muscle, adipose tissue, heart, and endothelium. Accumulating evidence has indicated that sirtuins are not only important energy status sensors but also protect cells against metabolic stresses. In addition, they have been implicated in the preservation of genomic stability. A number

of studies have shown an association with ageing and they coordinate a wide range of cellular responses that are frequently dysregulated during ageing.<sup>104,105,106</sup> For example, Sirtuin 2 assists in the repair of DNA and regulates genes that undergo altered expression with age in yeast;<sup>104</sup> overexpression of Sirtuin 6 in transgenic mice showed an increased lifespan of about 15% in males;<sup>105</sup> and Sirtuin 1 is thought to behave in humans like yeast Sirtuin 2 by regulating mitochondrion biogenesis.<sup>84</sup> Sirtuins are thought to be the mediators of response in the MTR and appear to regulate health-span in mammals.<sup>107</sup>

While there is increasing and evolving evidence for potential biomarkers of ageing, many researchers suggest that no single or simple combination of biological or genetic markers is likely to provide a useful clinical estimate of (biological) aging.”<sup>108,109</sup> This assumes that biological ageing is the consequence of the deterioration of more than one system. This assumption leads to the conclusion that a “panel” of biomarkers that reflects the condition of an array of critical systems may be needed in order to assess the biological age of any organism. At least two important points can be gleaned from this observation. Firstly, the complexity of ageing and the ageing processes means that the search for biomarkers is likely to continue unless a single or very small number of biological clocks (or pacemakers - marker(s) which change with biological ageing) actually exist. Secondly, and probably even more important, biomarkers may change with chronological age. Biomarkers that are predictive of longevity in early life may not be predictive in late life, and vice versa. Rather than selecting a single panel of potential biomarkers to measure in early life and then repeating the same panel over the lifespan, it may be necessary to measure a very large battery of biomarkers at successive points over the lifespan and treating each as a separate, predictive measure.

#### 1.2.6. Telomere biology and structure

Given their association with chronological ageing and role in cell senescence, telomeres are thought to be important in assessing biological ageing. As discussed, telomeres are specialised

DNA-protein complexes that cap the ends of chromosomes in eukaryotes (organisms whose cells contain a nucleus and other structures (organelles) enclosed within membranes). The basic structure is that of a nucleoprotein complex consisting of telomeric DNA and a number of bound proteins which are vital for the regulation of the telomere length and the functioning of the telomere. The DNA component is a repetitive stretch of TTAGGG (made up of the nucleic acid sequence: thymine, thymine, adenine, guanine, guanine, and guanine) which is non-coding (not translated into proteins). Telomeres safeguard gene integrity during mitosis (cellular division) by preventing DNA degradation at the chromosome terminus because the DNA polymerase complex is incapable of replicating all the way to the end of the chromosome. Without telomeres this would result in loss of vital genetic information which is required to sustain cellular activity. Telomere shortening occurs in almost all mitotic tissues, including peripheral blood leucocytes. Only germ cells, stem cells, and activated leucocytes maintain their telomere length through telomerase enzyme activity (a reverse transcriptase that adds DNA repeats in the telomere region), are excluded from the process of telomere shortening. Mice engineered to lack telomerase prematurely age and their ageing can be reversed by reactivating the telomerase.<sup>110</sup> Gradual loss of telomeric DNA in dividing cells can result in: cellular senescence (because the telomere length has reached a critical level); apoptosis (cellular death); or neoplastic transformation, such as occurs in cancer.

The relationship between ageing and telomere length is still not fully understood; despite being first recognised over 20 years ago. Cook and Smith first linked telomeres to the ageing process<sup>111</sup> and suggested a framework to explain the telomere attrition process and the Hayflick limit (the number of times a normal human cell will divide until division stops). Since then numerous studies have established the inverse relationship between telomere length and chronological ageing.<sup>112,113,114,115</sup> A systematic review found that the association between telomere length in peripheral blood leucocytes and age was a consistent observation in the literature.<sup>116</sup>

Olovnikov was the first to suggest that this is a potential mechanism for a biological clock determining cellular activity.<sup>117</sup> Further research by Vaziri and Allsopp *et al.*<sup>118</sup> demonstrated that telomere length could act as a “mitotic clock”. Telomere length fulfils several, but not all, of the criteria for a biomarker of human ageing: it decreases progressively with chronological age;

varies considerably between individuals and registers the life-cycle of proliferative cells.<sup>119</sup> There is much debate as to whether or not it is a useful biomarker of ageing.<sup>120</sup>

Some studies have shown that shortened telomere length is associated with increased mortality<sup>121</sup> while others have failed to show an association<sup>85</sup> – this is thought to be related to methodological issues or multiple confounders in epidemiological studies.<sup>97,120</sup> Cawthorn *et al*<sup>121</sup> studied unrelated residents of Utah, USA aged 60-97 years who donated blood between 1982 and 1986, and for whom follow up death certification was available. They used the relative ratio of telomere repeat copy number to single gene copy number (called T/S ratio) in participant samples compared with a single reference DNA sample. This is a commonly used method of reporting telomere length (see measurement of telomere length section 1.1.5).

#### 1.2.7. Measurement of telomere length

The most commonly used approach in clinical and epidemiological studies is to measure telomere length in leucocyte DNA from peripheral blood leucocytes. This is mainly because peripheral blood leucocytes are relatively easily obtained from participants and contain an excellent source of telomeric DNA in their nuclei. There is some evidence to suggest LTL reflects alterations in telomere length in somatic cells such as artery endothelial cells.<sup>123</sup> Recently, Daniali and Aviv *et al*.<sup>124</sup> measured telomere lengths in leucocytes, skeletal muscle, skin, and subcutaneous fat in 87 adults aged 19-77 years of age. They found strong correlation between age-dependent telomere length shortening in all four tissues; although, telomeres were found to be longest in muscle and shortest in leucocytes. Differences between the tissues showed no significant change throughout the age range. They conclude that the differences between tissues are likely to be established during early life. However, this was a cross-sectional study rather than longitudinal measurement of telomere length in the same individuals. Further research is likely to be required to confirm this correlation.

LTL also appears to have important functional consequences that may contribute directly to the pathogenesis of CVD. As the leucocyte cells become critically shortened and therefore senescent, they secrete pro-inflammatory cytokines.<sup>72,125</sup> LTL can be measured as a one-off or serial measurements can be used to quantify change over time.

Analysis and quantification of the terminal fragments of DNA in PBLs can be undertaken using:

- Southern Blot – where isolated DNA is cut using a specific restriction enzyme, transferred to a gel membrane and separated by electrophoresis according to size. After transfer to a membrane, a labelled probe is added which allows detection of DNA sequences. It is very accurate and reproducible. However, it is time consuming, relatively insensitive for very short telomere lengths, and requires a large amount of DNA.
- Quantitative fluorescence *in situ* hybridization (Q-FISH) – this technique uses synthetic DNA mimic probes called peptide nucleic acid to hybridise denatured repeat sequences of target chromosomal DNA using fluorescent microscopy. Cells or tissue embedded in paraffin, rather than extracted DNA, are used. It allows single telomere or cell analysis and therefore comparisons between cells can be undertaken. However, as it requires cells to be arrested in the metaphasic phase of mitosis, it is unable to measure telomere length in senescent cells.
- Quantitative real-time polymerase chain reaction (RT-PCR) – this is based on the quantitative PCR method first reported by Cawthon in 2002.<sup>126</sup> It amplifies and simultaneously quantifies a targeted DNA sequence compared with that of a single copy gene. Therefore, it generates a ratio between telomere and the single copy amplification termed T/S ratio. However, some studies report differences in mean base pairs (pb) by previous correlations between PCR and Southern blot. While similar to quantitative PCR, its key feature is that the amplified DNA is detected as the reaction progresses in “real time”. The higher possible throughput and less labour intensive nature of this method have led to its widespread use as the method of choice in the majority of studies. While concerns exist over reproducibility and variability, if performed with the required precision and controls, accurate comparisons with Southern blot are achievable.

As each method employs different laboratory-based tools and methodologies, comparisons between studies have often been difficult. Gardner *et al.* in their meta-analysis of telomere length and gender presented their findings stratified by measurement methodology.<sup>127</sup>

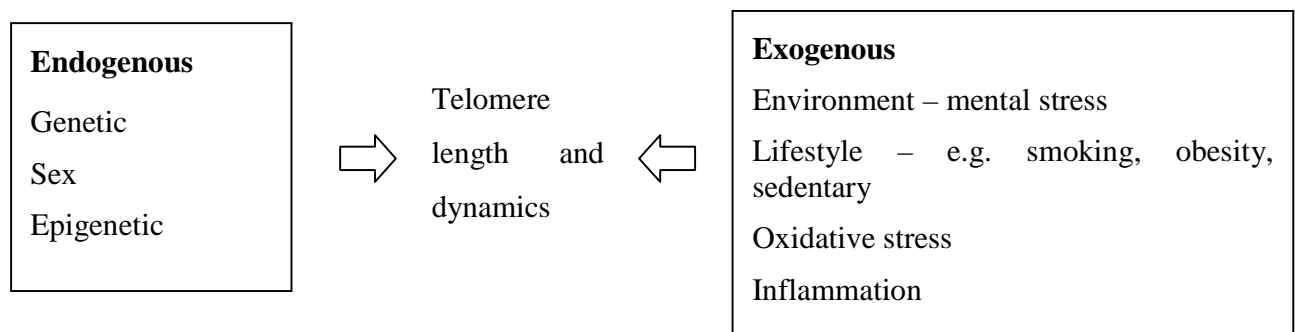
Southern Blotting is considered by many as the ‘gold-standard’ assay; however, it requires large amounts of intact genomic DNA; is time-consuming; costly; and requires a degree of expertise to undertake the analyses.<sup>128</sup> These are less critical for qPCR; however, residual PCR inhibitors may still be present in samples even after DNA purification which could contribute to increased variability in qPCR.<sup>97,127</sup>

Variance is used to describe the level of variability within a population independently of the absolute values of the observations. If absolute values are similar, populations can be compared using their standard deviations. But if they differ markedly, or are of different variables, then you need to use a standardized measure - such as the coefficient of variation. The coefficient of variation (CoV) for a sample is the standard deviation of the observations divided by the mean. The most common use of CoV is to assess the precision of a technique. It is also used as a measure of variability when the standard deviation is proportional to the mean, and as a means to compare variability of measurements made in different units.

Gardner *et al.*,<sup>127</sup> in their systematic review and meta-analysis of telomere length by gender, found that the magnitude of the differences between males and females varied by measurement method. The summary estimates of effect (using a random effects model) showed longer telomeres in women only in the studies which used the Southern Blot method. The authors suggested that “smaller measurement error in the Southern blot method as compared with RT-PCR might explain greater consistency of findings”. The inter-assay coefficient of variance was reported as 1.4% to 12% for the studies using Southern Blot methods and 1.7% to 11.1% for those using RT-PCR. Aviv *et al.*<sup>129</sup> suggest that the inter-assay CoV measurement for qPCR is 6.45%, while that of Southern Blots is 1.74%. CoV of qPCR has been reported to be as large as 27%, which is thought to be so high that drawing any conclusions from such data is “fraught with error”.<sup>130</sup> However, more recently quantitative Real-Time PCR (RT-PCR) has been reported as <1% laboratories including the Shiels lab at the University of Glasgow.<sup>8</sup>

### 1.2.8. Determinants of telomere length

Telomere dynamics appear to be complex and strongly influenced by endogenous and exogenous factors. While telomere length appears to be highly heritable and genetically determined,<sup>137</sup> it can also be affected by many factors including telomerase activity, rate of cell division, and degree of oxidative stress which, in turn, is likely to be affected by a number of factors which are also considered as social determinants of health, such as: genetics, socioeconomic deprivation, environmental factors and life style factors.<sup>8,115</sup>



**Figure 6: Schematic representation of factors affecting telomere length and attrition.**

#### **Genetic factors**

Short telomere lengths have been observed in humans with genetic syndromes that are associated with accelerated ageing such as: Werner's syndrome; congenital dyskeratosis; and progeria (consistent with a significant genetic component).

Heritability of human telomere length has also been demonstrated in several studies<sup>131,132</sup> where it appears to partially explain inter-individual variation,<sup>133</sup> with the majority of the variance in telomere length between close relatives being accounted for by relatedness.<sup>133</sup> In 1994, Slagboom *et al.*<sup>97</sup> studied 123 human mono- and di-zygotic twin pairs, and calculated 78% heritability in telomere length. Monozygotic twins were found to have very similar mean telomere lengths, whereas dizygotic twins showed significant differences. In 2008, Brouillette *et al.*<sup>135</sup> found a significant positive correlation in mean telomere length between parents and their offspring in 45 pairs where the parent had coronary heart disease (CHD) and 59 control pairs

( $r=0.37$ ,  $p=0.002$ ). However, there were significant limitations. Only 67% of parents had telomere analysis; “parent” could be either mother or father and the age range was limited.

Some studies have suggested that telomere length is predominantly inherited paternally rather than maternally.<sup>131,136</sup> Barrett and Richardson<sup>137</sup> suggested that the differences between the sexes is due to deleterious recessive alleles on the X chromosome which have no compensatory alleles on the Y chromosome. However, a recent study by Broer *et al.*<sup>133</sup> examined father–offspring and mother–offspring correlations in telomere length across four different study populations. The resultant sample size was threefold larger than all previous individual published studies. Their results, contrary to previous findings, showed that the mother–offspring telomere length correlation was substantially larger than the father–offspring correlation ( $P=0.007$ ). This may suggest an X–linked component to inheritance. De Meyer *et al.*<sup>138</sup> also found, in keeping with a number of other studies, a positive correlation between the age of fathers (but not mothers) and telomere length in their children. This suggests that vertical transmission is important. It could indicate that telomere length is not fully re-set after fertilisation and that if paternal telomere length is short this is inherited by the offspring. This is a scenario similar to that seen in animals created by cloning, in particular when the donor nucleus is harvested from an older animal e.g. in Dolly the sheep.<sup>139</sup> However, under normal reproductive mechanisms germ cells are the source of parental DNA, which retain the ability to maintain TL using telomerase. This may mean that genomic imprinting (an epigenetic phenomenon) may be a significant mechanism; however the exact mechanism is unknown.

## **Epigenetics**

Epigenetics can be defined as the study of heritable changes in gene expression that are not due to changes in the DNA sequence. Epigenetics was first suggested by Waddington who stated that: “between genotype and phenotype, and connecting them to each other, there lies a whole complex of developmental processes” – he called this complex the epigenotype.<sup>140</sup> Epigenetic changes (such as DNA methylation) appear to switch genes on or off and can determine which proteins are transcribed by different cells. Epigenetic silencing is one way to turn genes off, and it can contribute to differential expression. Silencing might also explain, in part, why genetic twins are not phenotypically identical. In addition, epigenetics is important for X-chromosome

inactivation in female mammals, which is necessary so that females do not have twice the number of X-chromosome gene products as males.<sup>141</sup> Thus, there is evidence of significant turning off of genes via epigenetic change. Recent experimental data point to epigenetics as a fundamental process in telomere length and dynamics.

Within cells, there are three systems that can interact with each other to silence genes: DNA methylation (a chemical process which adds methyl to DNA); histone modifications (histones are proteins which can influence how chromatin is arranged, which in turn, can determine whether the associated chromosomal DNA will be transcribed); and RNA-associated silencing (where RNA interference affects gene expression). These are thought to be important in telomere attrition.

Telomere length has been shown to be longest at birth, shortens rapidly until around 5 years of age, remains relatively stable until early adulthood, followed by a more gradual reduction thereafter.<sup>114</sup> This pattern suggests that early life factors may well be an important determinant of adult telomere length. While telomerase activity is likely to be high in the rapidly dividing foetal cells, little is known about telomere length changes in-utero. Growing evidence suggests that antenatal stress and impaired intrauterine growth could lead to accelerated telomere attrition, and thus shortened telomeres at birth, which may in turn result in shorter telomeres in adulthood.<sup>105</sup> Therefore, a one-off measurement of telomere length could represent the combined effects of both telomere length at birth and subsequent rate of attrition experienced by the individual. This is in keeping with the life-course approach to ageing suggested by Kuh and others.<sup>23</sup> Barker demonstrated a link between dysfunctional growth in early life and onset of atherosclerosis later on in life. The Barker Hypothesis proposes that events leading to restricted growth in-utero may programme the foetus for increased risk of adult CVD and other long term conditions with resultant premature death.<sup>142</sup> This restricted growth is thought to increase ROS and oxidative stress. Several studies in animals have reported that intrauterine adversity is associated with shorter telomeres in cells of different tissues. Entringer *et al.* measured telomere length in 94 healthy adult subjects, 45 of who were offspring of mothers who had experienced severe stress during the pregnancy.<sup>143</sup> After adjusting for available potential confounders, stress during pregnancy was associated with shorter telomere lengths in adult life. However, further

research is required to investigate this.

## **Sex**

While female sex is associated with a higher life expectancy and longer life span in a number of different populations, inconsistencies exist in the literature regarding longer telomere length and gender. Some studies have found LTL to be longer in women than men.<sup>144,145,146</sup> while others found no difference;<sup>146</sup> or even the reverse<sup>147</sup> to be the case. A recent meta-analysis<sup>127</sup> has been conducted in accordance with PRISMA guidelines. It included data from 36,230 participants (40 datasets from 32 separate studies) and showed that, on average, females had longer telomeres than males (age-standardised difference in telomere length of: 0.09, 95% CI: 0.015, 0.16). There was no evidence that this association varied by age group (defined as above or below the median age of 55.6 years); however, significant heterogeneity between studies was detected. Only two of the included studies assessed gender differences in LTL at birth.<sup>148,149</sup> Okuda *et al.*<sup>148</sup> found no difference between the genders at birth, while Aubert *et al.*<sup>149</sup> found that female newborns had longer telomere lengths than males. These contradictory studies led the authors to state that it was “not clear if gender dependant differences are present at birth” and therefore it is unclear whether or not differences in telomere length between adult men and women occur at birth, or are due to increased attrition throughout the lifespan. Okuda *et al.*<sup>148</sup> suggest that the lack of evidence for differences at birth indicates that factors which contribute to telomere maintenance, or accelerate attrition, are likely to accumulate throughout an individual’s lifetime.

There is evidence from studies of adults to support this. The Bogalusa Heart Study (participants aged 19-37 years) found no difference in telomere length between men and women, while the Family Heart Study with its older participants (30-93 years) did.<sup>150,151,152</sup> The Bogalusa Heart study and Bakaert *et al.*<sup>152</sup> found that telomere attrition was slower in women compared with men. Bekaert *et al.* used a cross-sectional study design with 2509 participants to estimate yearly telomere attrition rate, while the Bogalusa Heart Study used a longitudinal study design. Njajou *et al.*,<sup>153</sup> demonstrated that men have shorter overall TL and faster rates of telomere attrition over the 2.5 years they studied. However, in all of the studies, the association was not found to

be strong.

Several hypotheses have been suggested to account for sex difference later in life. These include the effects of oestrogen exposure on telomere length. Lin *et al.*,<sup>156</sup> reported that increased endogenous oestrogen exposure was associated with greater LTL, suggesting oestrogens may decelerate cellular ageing. Exogenous hormone replacement of oestrogen and progesterone have also been associated with greater LTL in post-menopausal women.<sup>157</sup> In a molecular context, an oestrogen-responsive element is present in telomerase transcriptase and therefore oestrogen may up-regulate telomerase activity with a concomitant increase in telomere length.<sup>158</sup> As discussed, telomere length is sensitive to oxidative stress and women have been found to produce less ROS than men;<sup>144</sup> this may also be mediated by oestrogen.

### ***Lifestyle***

The literature suggests that telomere length is associated with similar lifestyle factors to those associated with CVD. For example, smoking has been shown to be associated with accelerated telomere attrition in a number of studies<sup>159,160</sup>. Valdes *et al.*<sup>159</sup> observed that telomeres shortened linearly with age by 27 bp (base pairs) per year in a study of 1,122 white women aged 18 to 76 years. Age-adjusted telomere length was found to be ~5 bp shorter for every pack-year smoked, with 40 pack-years of smoking corresponding to 7.4 years of age-related shortening in telomere length. However, while this study adjusted for age, other confounders may be responsible for the association. Morla *et al.*<sup>160</sup> observed a dose-response relationship between cumulative lifetime exposure to tobacco smoking and shorter LTL in a case-control study of 26 never-smokers, 24 smokers with normal lung function and 26 smokers with moderate-to-severe airflow obstruction (forced expiratory flow in one second  $48 \pm 4\%$  predicted). Both of these studies are cross-sectional in nature, making causal inferences difficult.

It is thought that smoking acts by amplifying tissue inflammation through oxidative stress,<sup>161</sup> therefore augmenting the link between ageing and CVD. The mechanism underlying this has been studied at a cellular level, using cultured endothelial cells isolated from the internal mammary artery of smoking and non-smoking patients with CAD. They suggest that cellular senescence appeared to be independent of telomere length, but was strongly related to oxidative

damage and markers of inflammation.<sup>162</sup> Despite this, the exact mechanism is unclear.

Weischer *et al.* investigated whether lifestyle factors were associated with telomere length change in 4,576 healthy individuals from the general population.<sup>163</sup> Individuals had relative LTL measured twice with a 10-year interval, and were then followed for a further 10 years after the second measurement. They found that LTL shortening was associated with smoking cross-sectionally but not over the 10 years of follow-up. However, these findings may be subject to survivor bias. In addition, the findings could be partly explained by regression towards the mean, ie. the combined effect of biological and analytical variability will push the highest levels to a lower level on retesting and vice versa for those with the lowest levels.

Obesity has been associated with increased oxidative stress and DNA damage.<sup>164</sup> Although the precise mechanisms linking obesity to age-related disorders remain largely unknown, it has been suggested that biological ageing driven by telomere shortening plays a central role.<sup>165</sup> In cross-sectional epidemiological studies, shorter LTL has been associated with body mass index (BMI), waist-to-hip ratio (WHR) and visceral fat.<sup>166</sup> Also in an intervention trial of 521 subjects, reduction in adiposity indices corresponding to a Mediterranean diet intervention was accompanied by increased LTL,<sup>167</sup> attributed to telomere maintenance.<sup>168</sup> Weight loss induced by calorie-restricted diets was also found to be associated with increased telomere length in rectal mucosa of obese men.<sup>169</sup> However, these findings, in the literature, were primarily derived from relatively small subgroups of patients or research subjects, and results were inconsistent across study populations.<sup>170,171,172,173</sup>

It has been suggested that biological ageing reflected by shortened telomere length is also associated with diabetes.<sup>174,175,176</sup> Proposed pathophysiological mechanisms include a reduction in insulin secreting beta-cell mass in the pancreas, impaired insulin secretion and adipocyte insulin resistance elicited by cellular senescence. Studies on South Asian populations have shown shorter LTL in individuals with either non-insulin dependent diabetes mellitus (NIDDM) or impaired glucose tolerance compared to healthy controls.<sup>174,175,176</sup> Harte *et al*<sup>174</sup> compared LTL in South Asians with (n=142) and without (n=76) Type 2 diabetes (T2DM). This case-control study found an association between LTL and Type 2 diabetes in men only (only 56 cases and 43 controls). They also found that reduced LTL observed in T2DM South Asian males was

inversely associated with total cholesterol and triglycerides. However, no association was found between LTL and age, BMI, or waist circumference, which maybe as a result of the small numbers included in the study Adaikalakoteswari *et al.*<sup>175</sup> also found an association between LTL and type 2 diabetes in south Asians. While this study did show an association between LTL and age, it included only 40 cases and 40 controls. Jeanclos *et al.* demonstrated similar findings in a case-control study (54 with type 1 diabetes, 74 with type 2 diabetes, and 106 healthy controls) for insulin dependent diabetes mellitus (IDDM) but not for NIDDM, in Caucasian men.<sup>176</sup> However, LTL was not associated with duration of type 1 diabetes. While diverse in aetiology, there is a strong genetic susceptibility to type 1 diabetes, which may also affect telomere length and attrition. The studies by Harte *et al.*,<sup>174</sup> Adaikalakoteswari *et al.*,<sup>175</sup> and Jeanclos *et al.*<sup>176</sup> are cross-sectional in nature, precluding causal inferences. However, Zhao *et al.*<sup>177</sup> used the Strong Heart Family Study to investigate the association of leukocyte telomere length at baseline with future risk of diabetes over an average follow-up period of over 5 years. They found that individuals in the lowest quartile of leukocyte telomere length were at almost twice the risk of developing diabetes compared with those with longer telomeres. Notably, they highlight a nonlinear association between telomere length and diabetes risk in which the increased risk is largely confined to those with the shortest telomere length – consistent with the hypothesis that there is a critical limit of telomere length that induces cellular senescence. A key strength of the study is the prospective design, which should minimize the possibility of reverse causality. There are, however, some important limitations, particularly given the long latency that can precede diabetes diagnosis. Even with a 5.5-year follow-up period, many of those who went on to develop type 2 diabetes were likely to be experiencing subclinical metabolic changes at baseline.

In the Helsinki Businessman Study, there was a dose-dependent, linear, inverse relationship between LTL and alcohol consumption in 622 men followed up over 38 years to a mean of 78 years of age<sup>178</sup> Alcohol intake was assessed by self-report at the beginning of the study and LTL was assessed in old age, thus restricting the study to survivors, and did not contain data on changes in LTL with time. In addition, data on type of alcohol and pattern of use was limited. While the statistical adjustments were applied for measured potential confounders, the authors acknowledge that they could not exclude residual confounders such as genetic and

environmental factors. Again the mechanism of telomere shortening associated with alcohol intake is currently unknown; however, possible explanations include alcohol-induced oxidative stress and inflammation.

Ornish *et al.*<sup>178</sup> undertook a small pilot study which showed for the first time that improvements in diet, exercise, stress management and social support may result in longer LTL. The study included 35 participants who all had biopsy-proven low-risk prostate cancer and had chosen to undergo active surveillance rather than conventional treatment. The intervention group (n=10) underwent a lifestyle intervention. This was the first controlled (but non-randomised) trial to show that a lifestyle intervention may lengthen LTL over time (the difference remained significant at 5 years). It also demonstrated a dose response correlation between degree of lifestyle change and increase in LTL. However, the generalizability of the study findings is limited because all participants had prostate cancer. In addition, debate exists as to whether this apparent lengthening of telomere length may be a result of changes in peripheral blood stem cells or shifting patterns of leucocyte sub-populations rather than being an actual increase.

### ***Environment***

Hoxha *et al.*<sup>179</sup> evaluated telomere length in the leucocytes derived from 57 office workers and 77 traffic police officers exposed to traffic pollution. Exposure to pollution was assessed by levels of toluene and benzene measured using a personal passive sampler over 1 work shift. After adjusting for available confounders, the within each age-band telomere length was shorter in traffic police officers than office workers. However, the non-random selection of participants and potential residual confounders are limitations of this study.

A number of studies have examined the association between area, socio-economic status, deprivation and LTL.<sup>8,180</sup> Robertson and Shiels *et al.*<sup>180</sup> found that the rate of age-related telomere attrition was significantly associated with low relative income, housing tenure and poor diet. Notably, telomere length was positively associated with LDL and total cholesterol levels, but inversely correlated to circulating IL-6. However, no such association was found with area-based measures. However, this meta-analysis of 29 study populations found weak evidence for an association between SES (when measured by education) and biological ageing (as measured

by telomere length), although there was a lack of consistent findings when different SES measures investigated.<sup>180</sup> This meta-analysis was conducted following the robust PRISMA guidelines and different SES measures were included in the same analysis (social class, income, and employment status), which allowed each of the meta-analyses to be maximized in terms of size. In addition, a wide range of sensitivity analyses were conducted to investigate the effect of different aspects of study heterogeneity on the findings. While the studies were limited to English language only, there was little evidence of publication bias based on the funnel plots and rank correlation results.

### **1.3. Coronary artery disease**

#### **1.3.1. Definition**

CVD are diseases relating to the heart, blood vessels, or the circulatory system. Historically, they comprised of various diseases including: heart failure, CHD, stroke – ischaemic and haemorrhagic, arrhythmias, and peripheral artery disease. The International Classification of Disease (ICD) is the accepted standard to classify diseases and other health problems; recorded on many types of health and vital records including death certificates and health records (ICD 10 is the current version in use). In this classification, CVD also includes: rheumatic heart disease; valvular heart disease; disorders of the venous and lymphatic system; hypertension; pulmonary artery disease; peri-, myo- and endo-cardial disorders; blood vessel aneurysms; and intracranial haemorrhages.

CHD, ischaemic heart disease (IHD), and CAD are often used interchangeably; however, CAD usually refers more specifically to atherosclerotic involvement of the coronary arteries. In contrast, IHD most commonly refers to the presentation of clinical symptoms and CHD often includes other causes of inadequate blood flow to muscles, such as valvular heart disease or pulmonary hypertension. For the purposes of this thesis, CAD will be used to denote atherosclerosis of the coronary arteries.

Atherosclerosis is a complex process affecting the coronary artery vessels walls. The endothelial cells that line arteries provide a semi-permeable barrier between the blood stream and the artery

wall. Their main function is to regulate the exchange of fluid, nutrients, gases, and waste products between the blood and tissues. Endothelial cells also regulate constriction and relaxation of vessels by releasing vasodilatory molecules (e.g., nitric oxide (NO) and prostacyclin (PGI<sub>2</sub>)) and vasoconstrictive molecules (e.g., endothelin and angiotensin-II). They provide a unique surface that normally allows the cellular elements of blood to flow without adhering to the vessel lining.

Atherosclerosis is a disease process which is sometimes triggered by quite subtle physical or chemical insults to the endothelial cell layer of arteries. The "Response to Injury Theory" now has widespread acceptance in the literature.<sup>181</sup> This theory suggests that the earliest event in atherogenesis is injury to the endothelium, which can be triggered by any number of insults, either alone or in combination. These include:

- Physical injury or stress as a result of direct trauma or hypertension
- Turbulent blood flow, for example, where arteries branch
- Circulation of reactive oxygen species (oxidative stress)
- Inflammation
- Hyperlipidemia (high blood concentrations of Low Density Lipoprotein (LDL) or Very Low Density Lipoprotein (VLDL))
- Chronically elevated blood glucose concentrations
- Homocysteinaemia, in which an inherited metabolic defect leads to very high levels of the homocysteine, a metabolite of methionine, high concentrations of which are toxic to the endothelium

In response to these insults, perturbation occurs where the endothelial cells secrete cytokines which then trigger and maintain an inflammatory response. The endothelial cells begin to produce cell surface adhesion molecules, causing monocytes and T-lymphocytes (specific types of leucocytes which play a central role in cell-mediated immunity) to adhere to the endothelium

and then migrate beneath it, by squeezing between the endothelial cells. Circulating monocytes and T-lymphocytes are attracted to the sites of injury by the cytokines.

The endothelial cells also change shape, causing the tight junctions between endothelial cells to loosen, increasing the permeability to fluid, lipids, and leucocytes. Lipoprotein particles, and especially LDL, enter the artery wall and undergo oxidation. Oxidation of LDL in the artery wall occurs as a result of its exposure to nitric oxide, macrophages, and some enzymes such as lipoxygenase. Once they have migrated into the intima layer, monocytes differentiate into macrophages and begin to take up oxidized LDL that has entered the intima. Macrophages retain the lipid they take up, and as they become more lipid-laden, they are referred to as "foam cells." Eventually, the foam cells will undergo apoptosis and die, but the lipid will remain and accumulate in the intima.

Fatty streaks are the first signs of atherosclerosis that are visible without magnification. A fatty streak consists of lipid-containing foam cells in the artery wall just beneath the endothelium. It appears as a yellow discoloration in the artery's inner surface and occurs in the aorta and coronary arteries of most people by age 20. Over time, these fatty streaks can evolve into atherosclerotic plaques or they can remain stable or even regress.<sup>182</sup>

Slowly growing plaques expand gradually due to the accumulation of lipid in foam cells and the migration and proliferation of smooth muscle cells. These plaques tend to stabilize and are not prone to rupture. The so-called fibrin cap on the lesion matures. These plaques can build up and harden causing narrowing with reduction of blood flow to the heart. This can cause symptomatic (angina) or asymptomatic ischaemia.

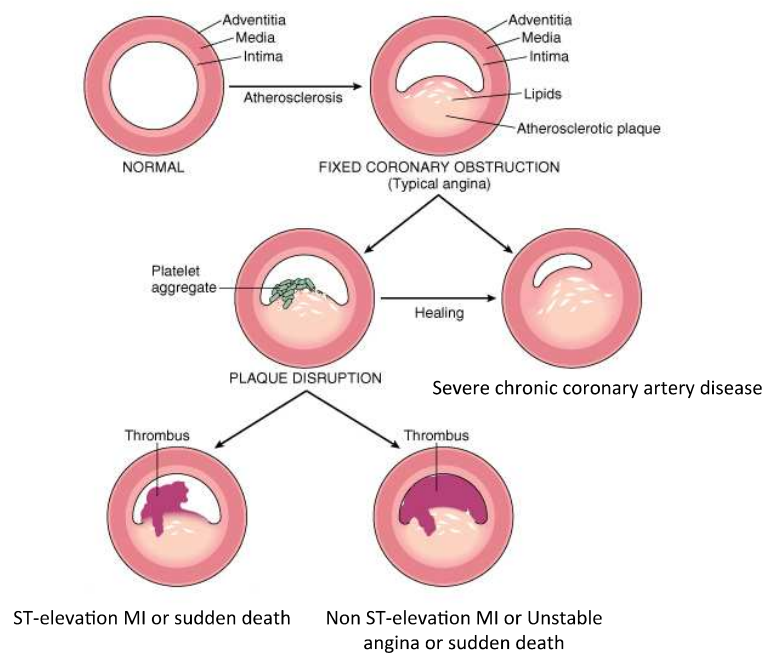
In contrast, other plaques grow more rapidly as a result of more rapid lipid deposition. These have thin fibrin caps that are prone to rupture. Once a plaque ruptures, it can trigger an acute thrombosis (clot) by activating platelets and the clotting cascade. This blood clot can cause partial or complete blockage. This results in an ACS.<sup>13,183</sup>

ACS is a spectrum of events which include:-

- STEMI (ST elevation MI) – elevation of the ST segment is seen on the electrocardiogram (ECG) – this is often referred to as an acute myocardial infarction (AMI) or heart attack

and usually involves complete blockage of the artery. Cardiac enzymes are raised. (Cardiac enzymes are proteins produced by the damaged heart muscle and released into the blood stream).

- NSTEMI (Non-ST elevation MI) – is where there is an AMI but ST elevation does not occur on the ECG. This is usually associated with partial rather than complete blockage of the culprit artery. Cardiac enzyme blood tests are also raised in NSTEMI (although often not as high as in STEMI), indicating that damage is occurring to heart muscle.
- Unstable angina – this is where there is partial blockage of the artery but the severity is insufficient to cause release of cardiac enzymes. However, this is also thought to be caused by coronary artery spasm where there may be partial and temporary blockage of arteries.



**Figure 7. Coronary artery disease morphology**

Stable CAD is characterised by episodes of reversible ischaemia or hypoxia (transient imbalance between blood oxygen supply and myocardial demand) usually induced by exercise, emotions or other stressors, although it can occur spontaneously. Traditionally the underlying mechanisms are thought to be plaque related obstruction of coronary arteries. However, more recently, focal or diffuse spasm of normal or plaque diseased arteries, microvascular dysfunction and left ventricular dysfunction have been shown to also cause symptoms of CAD.<sup>3</sup> These mechanisms are thought to be able to act singly or in combination.

Stable CAD and ACS are not considered to be distinct entities, rather they are part of a continuum; although stable CAD lesions less commonly show erosion or rupture of the endothelial layer.<sup>3</sup>

### 1.3.2. Epidemiology of coronary artery disease

CVD is the leading cause of death worldwide; the World Health Organisation (WHO) estimates that globally 17.3 million people die from CVD each year.<sup>184</sup> Of these deaths, an estimated 7.3 million were due to CAD. CVD is projected to remain the leading cause of death with the number of people who die from heart disease and strokes increasing to 23.3 million by 2030. WHO statistics are often the most complete, comparable, or often the only data available on a global scale. However, the quality of the data collected varies substantially across countries. Many high income countries have highly evolved health surveillance and death certification record systems. However, in many countries—especially in low and middle income countries—health statistics are often based on surveillance that does not cover all areas of the country, is incomplete in the areas it does cover, or is collected by undertrained staff who may not accurately report the pertinent data.

In the United Kingdom (UK), there are around 180,000 deaths recorded each year from CVD, with CAD being the most common cause (45%). Mortality rates for CAD have been falling in the UK since the early 1970s; although this has fallen more slowly in younger age groups, with CAD being the leading cause of premature mortality. Despite these declines, mortality rates

from CVD and CAD in the UK remain amongst the highest in Western Europe. Declining mortality rates have been attributed to reductions in major risk factors, advances in treatments (such as improved management of AMI resulting in a reduced case fatality rate), and to secondary prevention.<sup>185</sup>

Within the UK, mortality rates from CAD are highest in Scotland and the North of England and lowest in the South of England. For example, the premature mortality rate from CAD for men living in Scotland is 65% higher than in the South West of England and 112% higher for women.<sup>186,187</sup> Within Scotland itself there are also geographic variations in mortality rate declines.<sup>142</sup> Reductions have been slower in the most deprived areas of Scotland than elsewhere, meaning that relative inequality has increased slightly over the long-term while the absolute inequality gap has narrowed. However, there are signs that relative inequality has begun to stabilise in recent years.<sup>2</sup>

Incidence of AMI has also been declining, with the most recent Scottish estimates (based on hospital and death certification) suggesting around a 25% decrease from 2002 to 2010. However, the absolute number of people who have had an AMI has actually increased since the 1960s. This is because incidence of AMI increases with age and Scotland has an increasingly ageing population. Age-sex standardised incidence rates for AMI decreased by 22.3% between 2003/04 and 2007/08 but increased by 12.9% between 2007/08 and 2010/11. This increase is thought to be due to the introduction of more sensitive tests for diagnosis (such as cardiac enzymes), meaning that more cases are being diagnosed with an AMI.

Data on CAD morbidity are harder to collect. In Scotland the incidence of CAD has been tracked using the Information and Statistics Division (ISD) linked data set (based on national hospital discharge records). Incidence of CHD has decreased over the past decade, with the standardised incidence rate falling by 27.3% from 361.7 per 100,000 in 2003/04 to 262.8 in 2012/13. Between 1994 and 2003 incidence fell by 14% in men (from 585 to 501 per 100,000) and by 19% in women (449 to 366 per 100,000). Incidence rates for CHD also show regional and socioeconomic variations within Scotland. For example, the age-sex standardised rates were 238.0 per 100,000 in 2012/13 in the NHS Lothian Health Board area compared with 286.9 per

100,000 in the more deprived Greater Glasgow and Clyde Health Board area. Although the premature death rate has fallen across all social groups for both men and women in the UK, the decline has been greater in people in higher socio-economic groups.<sup>186</sup> The incidence of CAD increases sharply with age. In Scotland, the standardised incidence rate for the under 75 age group in 2012/13 was 197.5 per 100,000 compared with 1,829.2 in the 75 and over group.

Data on prevalence of CAD in Scotland can be obtained from a number of sources including: the Scottish Health Survey, and ISD (which uses inpatient hospital discharge and day case procedure data). The self-reported prevalence of CAD was found to be 7.3% (8.2% of men and 5.7% of women) in the 2012 Scottish Health Survey, with this showing little change since the 2003 survey. However, this rose to 30.7% in men and 21.2% of women, for those aged 75 years and over. ISD estimates the prevalence of CAD in Scotland to be 3.3% (4.2% in men and 2.5% of women) and that 16% of the Scottish population aged 75 years and over is living with CAD. Similar to incidence, ISD found marked socioeconomic variations in prevalence, for example, in some more deprived areas around 25% of men aged 75 years and over are living with CAD.

### 1.3.3. Symptoms of coronary artery disease

The main symptom of CAD is chest pain. It is often described as feeling like a dull, heavy, or tight pain. It can also spread to the left arm, neck, jaw, or back. However, the presentation of CAD chest pain is variable. The traditional classification of chest pain is:

Typical angina	Meets all three of the following characteristics: <ul style="list-style-type: none"><li>• Sub-sternal chest discomfort of characteristic quality and duration</li><li>• Provoked by exertion of emotional stress</li><li>• Relieved by rest and/or nitrates within minutes</li></ul>
Atypical angina (probable)	Meets two of these characteristics
Non-anginal chest pain	Lacks or meets only one or none of these characteristics.

The Canadian Cardiovascular Society (CCS) classification is widely used as a grading system for severity of stable angina.<sup>188</sup> It quantifies the threshold at which symptoms occur in relation to physical activities. The Rose Angina Questionnaire is similar and can also be used for this purpose, although it is usually used as a screening tool for CAD in epidemiological studies.<sup>189</sup>

CCS classification	Description
Class I	Ordinary activity does not cause angina such as walking and climbing stairs. Angina with strenuous or rapid or prolonged exertion at work or recreation.
Class II	Slight limitation of ordinary activity. Angina on walking or climbing stairs rapidly, walking or stair climbing after meals, or in cold, wind or under emotional stress, or only during the first few hours after waking. Walking more than two blocks* on the level and climbing more than one flight of ordinary stairs at a normal pace and in normal conditions.
Class III	Marked limitation of ordinary physical activity. Angina on walking one to two blocks on the level or one flight of a stairs in normal conditions and at a normal pace.
Class IV	Inability to carry on any physical activity without discomfort' – angina syndrome may be present at rest'.

\*Equivalent to 100-200m

Sometimes CAD chest pain can be difficult to distinguish from other causes of chest pain (such as oesophageal pain) and therefore further investigation may be indicated. The latest European Society of Cardiology (ESC)<sup>3</sup> guidelines on the management of stable CAD have recommended applying a well-validated prediction rule (to determine risk of CAD) containing the five determinants:

- male  $\geq 55$  years or female  $\geq 65$  years);
- known vascular disease;
- patient assumes pain is of cardiac origin;
- pain is worse during exercise; and
- pain is not reproducible by palpation.

According to the guideline, using one point for each determinant can be used to rule-out CAD at a specificity of 81% (for  $\leq 2$  points) and a sensitivity of 87% (for 3–5 points). However, they emphasise that this rule should be used in the context of other clinical information, such as the presence of cough or stinging pain (making CAD more unlikely). In contrast, clinical features such as radiation of pain into the left arm, known heart failure and diabetes mellitus make CAD more likely. Some people with angina also become breathless when they exert themselves.

#### 1.3.4. Investigation of coronary artery disease

After considering general health, co-morbidities and quality of life (QoL), non-invasive testing for CAD includes:

- Resting electrocardiogram (ECG) to look for evidence of ischaemia (such as ST/T changes) which may show other abnormalities, such as conduction defects which may contribute to symptoms. A normal resting ECG is not uncommon in stable CAD and can occur irrespective of severity of angina. All patients with suspected CAD have ECGs

undertaken prior to more advanced or invasive investigations. In addition, ECGs are sometimes carried out in routine clinical situations (for example prior to surgery). If the ECG changes are consistent with CAD, irrespective of symptoms, this may prompt a diagnosis of CAD (for example, if the ECG is consistent with previous AMI) and/or further investigation.

- ECG exercise testing is useful in patients with suspected CAD and an intermediate risk based on the pre-test probability (PTP) (see below for more discussion on PTP). ECG monitoring is undertaken while the patient is exercising using a treadmill or bicycle to look for evidence of ischaemia – either during or in the recovery phase. Positive finds can also lead to more advanced or invasive investigations.
- A chest X-ray is useful in patients where there is suspicion of a pulmonary cause and to assess if the heart appears enlarged - indicative of cardiomyopathy or heart failure.
- Echocardiography at rest is generally undertaken to assess cardiac structure and function. The diagnostic yield of resting echocardiography is mainly in sub-groups with murmurs, previous MI or symptoms/signs of heart failure. However, as left ventricular function is now an important risk predictor, the current ESC guidelines recommend that it is performed in all patients presenting with symptoms suggestive of angina. Therefore patients attending for invasive investigations in the future will have had an echocardiography. This allows an assessment of the thickness of the heart muscle to look for hypertrophy. Measurement of the left ventricular ejection fraction (volumetric fraction of blood pumped out of the left ventricle with each contraction of the muscle) can also be undertaken using echocardiography. This is used to assess left ventricular function.

- In ACS, biomarkers of myocardial injury, such as troponin, should be measured.

Troponin can be raised in patients with stable CAD; however, the levels are usually below the cut-off for being defined as elevated and the level provides no additional diagnostic or prognostic benefit in stable CAD. Therefore, it is usually reserved for patients presenting as an emergency with suspected AMI.

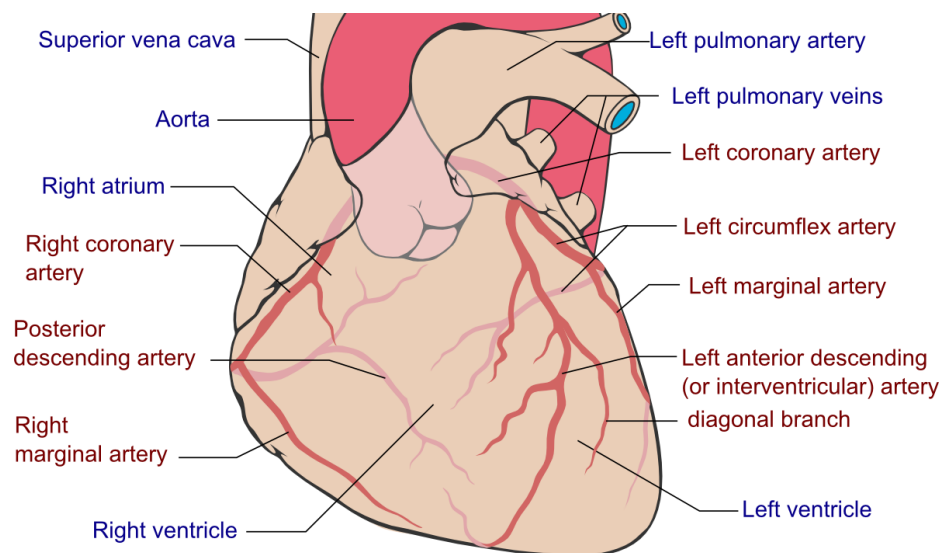
### **Invasive coronary angiography**

Coronary angiography is an invasive diagnostic procedure which is generally considered as the “gold standard” in diagnosing CAD.<sup>190</sup> It is used to visualise the coronary arteries. It involves the injection of an iodine based contrast into the coronary arteries. The contrast absorbs X-rays, making the blood vessel lumen radio opaque, and producing a sharp contrast with the surrounding cardiac tissue. An image intensifier converts the X-ray shadow into a visible light displayed on fluoroscopic monitors or a flat panel detector can be used to produce a direct digital image signal from the original visible light fluorescence.

After introduction of local anaesthesia, percutaneous access to a large peripheral artery (such as the femoral, radial or brachial artery) is achieved by puncturing the skin. A sheath is then inserted into the artery. Heparin (an anticoagulant used to inhibit thrombus formation during the procedure), nitrates (a vasodilator) or other adjuvant drugs may be inserted into the blood stream via this sheath. Through this, a small catheter is fed, via a guide wire, to the coronary ostia of the aorta, where the coronary arteries originate. Contrast can then be injected into coronary arteries and X-ray films are taken rapidly (Cine images) to show the vessels filling with blood and the sites of any stenosis (narrowing of the arteries) can be seen.

The heart is supplied by the right coronary artery and left coronary artery. The left coronary artery is wider. The first part, termed the left main stem (LMS) is around 1cm in length. It then divides into the left anterior descending artery and the circumflex artery. The left coronary artery and its branches are most commonly found to be stenosed in CAD. In around 70% of people, the right coronary artery supplies the posterior descending coronary artery. This is described as being right-dominant, while around 20% are left dominant (where the left coronary artery

supplies the posterior descending artery), and around 10% have co-dominant supply from both the right and the left coronary arteries. Three-vessel disease (involving 3 arteries) with proximal stenosis, significant stenosis of the LMS and proximal anterior descending artery lesions, have been associated with higher mortality rates.<sup>191</sup>



**Figure 8. Anatomy of the coronary arteries**

The severity and manifestation of CAD generally depends on the degree of stenosis and on the type and number of arteries affected. However, paradoxically, severe stenosis can result in no, or mild symptoms, and conversely severe symptoms can occur in patients with mild stenosis.

Currently, the ESC defines significant stenosis as  $\geq 50\%$  in the LMS (or  $<90\%$  in two angiographic views when there is no evidence of ischaemia) or  $\geq 70\%$  in one or several of the major coronary arteries (although  $\geq 50\%$  has been used in the past and is currently used in other areas of the world). Single vessel disease (SVD) is defined as a significant stenosis in one of the

major coronary arteries, other than the LMS. Multivessel disease generally refers to significant stenosis in two or more vessels. Involvement of the LMS is often classified as multivessel disease or as “MVD with LMS” involvement to distinguish it from MVD without significant LMS stenosis. The term triple vessel disease is also used to describe MVD where there is involvement of the right coronary artery, left descending, and left circumflex.

However, classification of angiographic appearance of the coronary arteries has evolved over time based on evidence from clinical trials. One such classification is the SYNTAX scoring.<sup>149</sup> The SYNTAX score is an angiographic grading tool to determine the complexity of CAD. The SYNTAX score was developed by Serruys and colleagues at the Thoraxcenter, Erasmus Medical Center in The Netherlands and was published in 2005.<sup>192</sup> The SYNTAX score was derived from pre-existing classifications, including the American Heart Association (AHA) classification; the Leaman score; the American College of Cardiology (ACC)/AHA lesion classification system; the total occlusion classification system; the Duke and International Classification for Patient Safety (ICPS) classification system for bifurcation lesions; and a consensus opinion from among the world’s experts. The coronary artery tree is divided into 16 segments according to the AHA classification (Figure 8). Each lesion is given a score of 1 or 2 based on the presence of disease and this score is then weighted based on a chart, with values ranging from 3.5 for the proximal left anterior descending artery to 5.0 for left main, and 0.5 for smaller branches. The SYNTAX score is the sum of the points assigned to each individual lesion identified in the coronary tree with >50% diameter narrowing in vessels >1.5mm diameter. The ESC currently recommends that the SYNTAX score is derived and used for diagnostic and management decision making. For example, it recommends that a patient with a SYNTAX score  $\geq 33$  with LMS plus 2 or 3 other vessel involvement, undergo CABG (as long as they have a low risk of surgical complications) rather than PCI. However, decision making is also influenced by a number of factors including: patient preference, assessment of potential risks from surgery, clinical factors, and clinical team decisions.

As with any invasive procedure, coronary angiography is associated with a number of potential complications such as: access site complications (bleeding, pseudoaneurysms, fistulas, or infection); renal failure resulting from contrast medium damage; stroke; AMI; cardiac arrhythmias; aortic aneurysms; and damage to the coronary vessels (perforation, embolism, or

dissection). However, the risk of any complication during coronary angiography is said to be 1%-2%, with the risk of death, AMI or stroke around 0.1% - 0.2%<sup>193</sup>

The methods used to perform coronary angiography have improved substantially since its introduction, with, for example, vascular closure devices (medical devices used at access sites to achieve haemostasis). This is especially true for radial access rather than via the traditional femoral artery route. Since its introduction by Lucien at the Montreal Heart Institute, radial access has been increasingly used, particularly in the UK (with recent BCIS audit suggesting that 65.3% of procedures are now undertaken using radial access). It requires less contrast medium<sup>151</sup> and carries a lower overall risk of complications, fewer access site complications,<sup>194,195,196</sup> fewer bleeding episodes and a reduced likelihood of renal damage. Because there is better collateral circulation, there is less chance that an access site complication will result in limb-threatening damage. Collateral circulation can be assessed prior to coronary angiography, by measuring the time for the hand's circulation (colour) to return to normal following compression of the radial artery (known as Allan's test). Despite the radial artery having a smaller diameter than the femoral artery, the success rate (defined as successful balloon dilatation with or without stent placement and <30% residual stenosis) associated with radial access is well over 90%.<sup>152</sup> Radial access, with its lower complication rate and the possibility for early ambulation, has facilitated the use of day case and rapid discharge protocols for patients who have had a successful PCI and are at low risk of complications (following the Amsterdam criteria for same day discharge).<sup>197</sup>

Guidelines for investigating CAD have evolved based on emerging evidence and innovations in non-invasive techniques (see below). Currently a two-stage approach is recommended based on clinical assessment of risk of CAD followed by assessment of the likelihood of benefiting from invasive management for those found to be at increased risk of CAD. Risk is assessed using the PTP. The PTP is influenced by the background prevalence of CAD in the population and the risk factors and clinical features of the individual, including age, gender and nature of the symptoms. Points are assigned to the presence of these factors, enabling patients to be classified into four categories based on their likelihood of having CAD: low-likelihood (PTP < 15%), low-intermediate likelihood (PTP 15-65%), high-intermediate likelihood (PTP 65-85%), and high-likelihood (PTP > 85%). Low-likelihood patients are not recommended for further investigation. High likelihood patients (defined as a PTP >85%) are assumed to have CAD without need for

further non-invasive testing. Those with an intermediate risk should be referred for further investigations, such as non-invasive testing or coronary angiography (depending on the availability of non-invasive testing and severity of symptoms).

Coronary angiography has a sensitivity of 95-99%, and specificity of only 64-83%<sup>3</sup> and is associated with a risk of a major complication (albeit low). Therefore it is recommended to be used in those who would most benefit from revascularisation, for example, high PTP, where it is used to assess the severity of CAD to inform future management options (such as, PCI or coronary artery bypass grafting (CABG)); in patients where the findings of non-invasive testing are inconclusive or in those where optimal medical management fails to control symptoms (also in some professions for regulatory reasons). However, severity of clinical symptoms, the patient's risk of adverse events from coronary angiography, and patient preferences, are also taken into consideration in determining investigation of CAD. In addition, while coronary angiography is an important invasive investigation to assess the presence and severity of coronary artery stenosis, current understanding of angina demonstrates that clinical symptoms can be more severe than suggested by the degree of stenosis and that angina can be caused by other mechanisms (such as microvascular dysfunction).

Additional imaging procedures, such as intra-vascular ultrasound (IVUS) and fractional flow reserve (FFR), may be performed along with coronary angiography in some cases to obtain detailed images of the walls of the blood vessels. Both of these imaging procedures are currently only available in specialized hospitals and research centres. With IVUS, a miniature sound-probe (transducer) is positioned on the tip of a coronary catheter. High-frequency sound waves produce detailed images of the inside walls of the arteries. Therefore, IVUS produces a more accurate picture of the location and extent of plaque. With FFR, vasodilator medication is used to perform a very high quality stress test over a short segment of the artery.

Prior to recent guidelines, coronary angiography was considered a first-line investigation among patients at high risk of CAD.<sup>193</sup> All men and women older than 70 years with typical or atypical symptoms were considered to be at "high risk". Therefore, in the past, elderly patients were more likely to have undergone coronary angiography. In spite of being at higher risk, previous studies have suggested that elderly patients may receive less aggressive investigation and

management of CAD.<sup>199,200</sup> Having a higher threshold for investigation among the elderly results in diagnostic coronary angiography being delayed until the disease and symptoms are so severe that they are less amenable to PCI.

The literature suggests that elderly patients may be at increased risk of complications following coronary angiography<sup>3,201</sup> However, elderly patients have historically been underrepresented in large epidemiological and clinical studies.<sup>202</sup>

### **Non-invasive investigations**

Non-invasive imaging includes multi-detector computed tomography, cardiac magnetic resonance imaging, or myocardial perfusion scintigraphy (such as, single-photon emission computed tomography or positron emission tomography). These use contrast or isotopes injected into a peripheral vein (intravenous injections) to visualise the coronary arteries. Therefore, unlike coronary angiography, which requires artery access for a catheter to inject contrast directly into the coronary arteries, these non-invasive imaging techniques are not associated with access site bleeding or coronary artery damage. Availability of these tests varies across the UK. Stress testing can also be undertaken in conjunction with non-invasive imaging and can provide an assessment of coronary artery function. Sensitivities and specificities for these techniques vary depending on the test. Further discussion on non-invasive imaging techniques is outwith the scope of this thesis.

#### **1.3.5. Management of coronary artery disease**

The management of CAD has improved significantly over the last few decades resulting in improved life expectancy and survival.<sup>3</sup> It can be managed using either medical therapy or revascularisation. As discussed, revascularisation can be achieved via PCI or CABG. In addition to these, lifestyle (and environmental) modification should always be addressed e.g. diet, exercise, smoking, exposure to second hand smoking. Medical therapy includes statins to maximise blood lipids, ACE inhibitors to manage blood pressure and left ventricular function,

anti-anginals (e.g. nitrates) for symptomatic relief, and antiplatelets (where indicated) which are used to prevent the formation of blood clots.

### **Percutaneous coronary intervention**

PCI is a non-surgical procedure used to open narrowed coronary arteries to improve blood flow to the heart. PCI is also known as balloon angioplasty, or percutaneous transluminal coronary angioplasty (PTCA). PCI can be performed as an immediate follow-on procedure if significant stenoses are identified during coronary angiography or it may be performed as a separate procedure. PCI can be used to treat patients with stable CAD or patients presenting with AMI. In relation to the latter, it can be used as the first-line treatment (primary PCI) or following failure of anti-thrombotic medication (rescue PCI). PCI encompasses a number of techniques including: balloon angioplasty (sometimes described as “Plain old Balloon Angiography” (POBA)), insertion of a coronary stent, rotablation, a cutting balloon or a combination of these.

Balloon angiography: during balloon angiography a small balloon at the tip of the catheter is inflated within the stenosed lesion, compressing the fatty plaque and dilating the vessel lumen and increasing blood flow to the heart. This procedure is sometimes complicated by vessel rupture, aneurysm formation, and acute occlusion. As discussed, angiographic appearance (for example, which vessels are involved, SYNTAX score), patient preference, clinical factors, and clinical team decisions, all influence whether patients have a PCI undertaken.

**Balloon angioplasty with stenting:** Increasingly, balloon angioplasty is often used in combination with insertion of a coronary stent in order to reduce the risk of requiring repeat revascularisation. A stent is a small, metal mesh tube that acts as a scaffold to provide support inside the coronary artery. Coronary stents can be either bare metal or drug-eluting. A balloon catheter, placed over a guide wire, is used to insert the stent into the narrowed artery. Once in place, the balloon is inflated and the stent expands to the size of the artery and holds it open. The

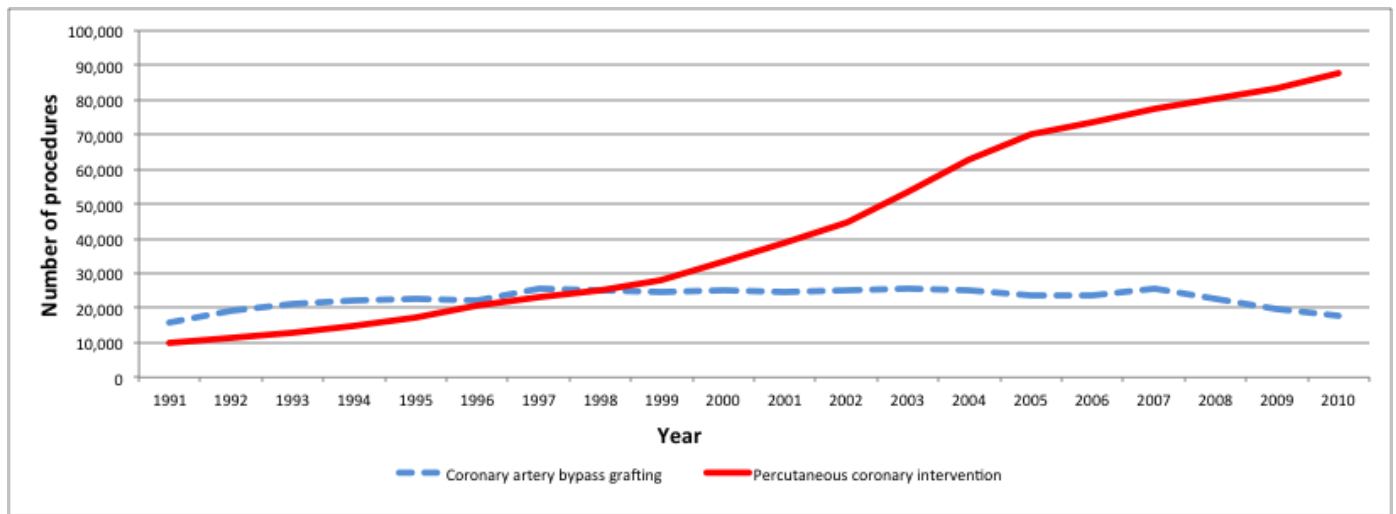
balloon is deflated and removed, and the stent stays in place permanently. Over a period of several weeks, the artery heals around the stent.

**Drug-eluting stents:** Drug-eluting stents contain a medication that is actively released at the stent implantation site and reduces the risk of stent thrombosis as a result of delayed development of endothelial layer cover. Concern was raised in 2006 regarding the safety of drug-eluting stents due to the risk of late stent thrombosis causing AMI. In 2007, the Food and Drug Administration stated that drug eluting stents, when used according to approved indications, are safe and effective.<sup>203</sup> However, longer post PCI antiplatelet use is advised with drug eluting compared with bare metal stents to reduce the risk of late thrombosis. The majority of stents deployed during PCI procedures in the UK are now drug eluting.<sup>3</sup>

**Rotablation:** In rotablation, also known as percutaneous transluminal rotational atherectomy, a special catheter, with an acorn-shaped, diamond-coated tip, is guided to the point of narrowing in the coronary artery. The tip spins at high speed and grinds away the plaque on the artery walls. This process is repeated as needed to remove the blockage and improve blood flow. The microscopic particles enter the bloodstream and are filtered out by the liver and spleen.

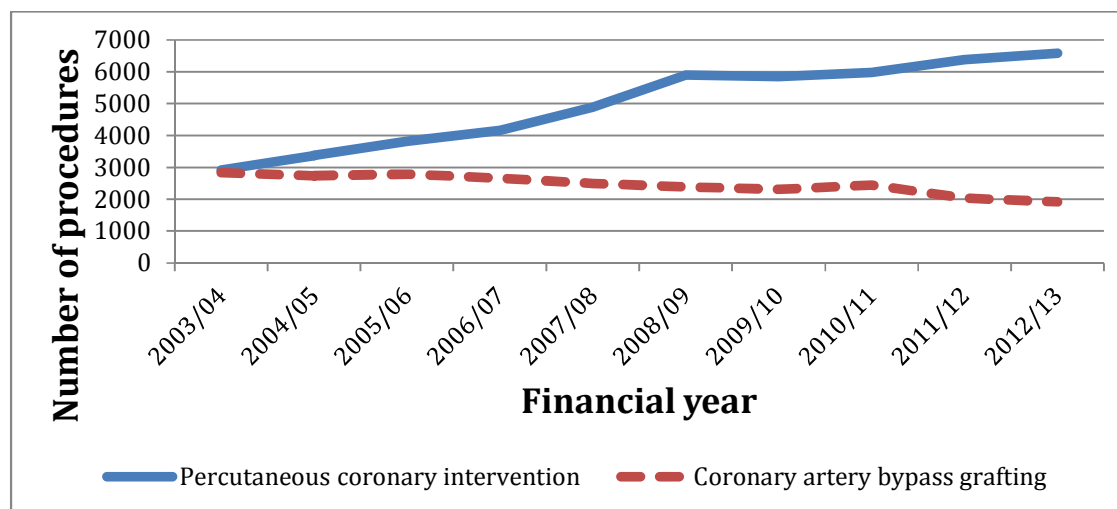
**Cutting balloon:** The cutting balloon catheter has a balloon tip with small blades. When the balloon is inflated, the blades are activated. The small blades score the plaque, then the balloon compresses the fatty matter into the artery wall. This type of balloon may be particularly useful in treating the build up of plaque within a previously placed stent (restenosis).

While medical therapy remains the cornerstone of the management of chronic stable angina, randomized trials have consistently demonstrated greater symptomatic relief from coronary revascularization compared with medical therapy alone.<sup>204,205,206,207</sup> Since the introduction of PCI in the UK in 1991, there has been a major shift in the proportion of patients treated with PCI compared to CABG. The number of CABG operations performed each year rose until 1997 then remained relatively static until falling again after 2007.



**Figure 9 UK Time trends in percutaneous coronary intervention and coronary artery bypass grafting: 1991-2010**

In Scotland, the number of CABG operations decreased from 2,032 in 2008/09 to 1,919 in 2009/10. This follows a slight increase between the years 2000/09 and 2010/11. However, overall there is a general downwards trend in the numbers of CABG operations which, again, is thought to reflect the increased use of PCI. The number of PCIs increased from 6,374 in 2008/09 to 6,583 in 2009/10.



**Figure 10. Scottish time trends for percutaneous coronary intervention and coronary bypass grafting: 2003/04-2012/13**

### **Risks associated with percutaneous coronary intervention**

Similar to coronary angiography, PCI is associated with a number of potential complications such as: access site complications (bleeding, pseudoaneurysms, fistulas, or infection); myocardial infarction; cardiac arrhythmias; aortic aneurysms; and stroke. Damage to the coronary vessels (perforation, embolism, or dissection) is more likely with the balloon inflation or stent deployment of PCI. Risk of renal failure resulting from contrast medium damage is also more likely in PCI as larger amounts of contrast may be utilised to guide balloon inflation, stent deployment, and to assess their effects on lumen patency. Early stent thrombosis is a risk until the stent becomes covered with an endothelial layer. This presents more frequently during the first month, occurs in 1-2% of patients, usually presents as an AMI, and is associated with high mortality. To reduce the risk of stent thrombosis, antiplatelet therapy is given to patients following PCI. Restenosis of the artery lumen is also possible which can may result in a angina symptoms, AMI, sudden death, or need for repeat revascularisation. In patients with three-vessel disease, the evidence suggests that, while there is no over all difference in mortality between management by CABG or PCI, CABG is associated with a lower requirement for repeat

revascularisation. Further discussion on the risks of PCI in relation to elderly patients can be found below in Chapter 2.1.

## **1.4. *Health related quality of life***

The World Health Organization defines health as "a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity."<sup>208</sup> This definition highlights that health is not simply the absence of pathology but is a multidimensional concept that captures several domains of well-being simultaneously. Similarly, health related quality of life (HRQOL) has evolved as a multi-dimensional concept which includes domains related to physical, mental, emotional and social functioning. HRQOL has been studied for at least 40 years with no commonly accepted definition of the concept. Often HRQOL is synonymously referred to as simply quality of life (QoL) in contexts including health research and will be used in this way throughout this thesis.

The Central Union for the Welfare of the Aged defines QoL as "an individual's understanding of his/her life situation with respect to his/her values and cultural context, as well as in relation to his/her goals, expectations and concerns".<sup>209</sup> Material well-being (income, level of housing, availability of services, environment), close relationships (social relationships, social well-being, support, societal involvement), health (physical health, fitness, ability to move, symptoms of illness, ability to work), emotional well-being (emotions, self esteem, spirituality, cognitive functions) and productivity (satisfaction with ability to work, competence, autonomy, meaningful roles) have been viewed as essential dimensions of QoL

QoL is used to imply an individual's experience of his or her health status and health-related well-being. QoL is usually examined in terms of impacts of illness on an individual - as negative deviations from health. However, the concept of QoL is value-laden and attached to social norms defined by healthy individuals, according to whom QoL is always weakened when a person is sick. QoL measurements are usually used to examine the effectiveness of treatments. QoL is thought to be an equivalent to a meme, which is "an idea, behaviour or style that spreads from

person to person within a culture.”<sup>210</sup> A meme acts as a unit for carrying cultural ideas, symbols or practices, which can be transmitted from one mind to another through writing, speech, gestures, rituals or other imitable phenomena. Supporters of the concept regard memes as cultural analogues to genes in that they self-replicate, mutate and respond to selective pressures. This idea of a meme was coined by Dawkins.<sup>211</sup>

QoL tools have been developed to document experience of illness and healthcare and to measure what effects of treatment are deemed to be of greatest importance to patients. They can also act as a proxy for measuring health needs within assessment. In health economics, QoL tools are used to develop QALYs (quality-adjusted life year) for use in cost–utility analyses. QALYs are overall measures of health outcome that weight the life expectancy of a patient with an estimate of their QoL score.<sup>212</sup> QALYs are widely recognised as a useful approach for measuring and comparing the efficiency of different health interventions.

QoL is particularly important in long term conditions (such as CAD) where prevention, rehabilitation and other disease management strategies have resulted in an increase in the number of people living and growing older with these conditions. It is also important for treatments such as PCI which are intended to improve symptoms rather than increase life expectancy. Studies have shown that CAD imposes a substantial burden on QoL<sup>213, 214</sup> though this can be improved significantly by interventions.<sup>215, 216</sup> Consequently there has been a significant growth in the measurement of QoL as a prime indicator of health outcome and therapeutic benefit in patients with CAD.

QoL instruments should be “comprehensive, reliable and valid, easy to score and interpret, and minimise respondent burden in order to quantify the impact and burden of disease”.<sup>217</sup> The real value of QoL instruments in research and in clinical practice is to show that changes are not just statistically significant but are also clinically meaningful. This is particularly important for assessing the impact of an intervention such as revascularisation, which is primarily undertaken to relieve symptoms of CAD.

The instruments selected should measure the health dimensions relevant to that particular patient cohort. For instance, an instrument intended for use in a patient with CAD should take into account the individual's responses to living with the disease, in terms of recreational,

occupational, social, personal, emotional and sexual aspects, as well as the acute and chronic physical consequences of the disease.

There are two types of QoL instruments: ‘generic’ and ‘disease-specific’. Generic tools are designed to address multiple aspects of QoL across a range of different patients or disease groups<sup>218</sup> and include the Short-Form 36-item (SF-36, SF-12, SF-24) health survey<sup>219</sup> and the EuroQoL (EQ-5D)<sup>220</sup>. They assess multiple aspects of patients’ experience without focusing on specific features of a particular disease.

The EQ-5D is a widely used simple and validated general instrument to measure QoL in a standard way. It has been used to assess QoL in a wide range of conditions, treatments and interventions.<sup>221</sup> In addition, it is used extensively in health economics to derive a quality adjusted life year (QALY). The EQ-5D was originally designed to complement other instruments, although, is increasingly used as a “stand alone” measure. While it has a condensed format and assigning values to health states is straight forward, a limiting factor is its restricted ability to discriminate small to moderate differences in health states. In addition, evidence suggests that there is a ceiling effect i.e. patients who start with a higher quality of life than the average patient do not have much room for improvement. This could compromise accurate conclusions in relation to a treatment's effectiveness or lack thereof.<sup>222</sup>

The SF-36 was designed as a generic indicator of health status for use in population surveys and evaluation of the impact of health policy.<sup>223</sup> However, it has also been used in a wide range of clinical research areas - on its own, and in conjunction with, disease specific measures. It measures both physical and mental health components including behavioural functioning and role limitations, such as questions on work, self-care, and mobility. Evidence from studies suggests that the SF-36 has been found to be more sensitive to change than the EQ-5D and has less of a ceiling effect.<sup>224</sup> However, it is much longer for participants to complete and creating a utility score for assessing QALYs is much more complex.

Disease specific instruments measure the multiple aspects of QoL relevant to a specific disease group and, for patients with CAD, include the Seattle Angina Questionnaire (SAQ)<sup>225</sup> and the MacNew questionnaire.<sup>226</sup> Disease-specific instruments are generally considered to be more clinically sensitive and potentially more responsive in detecting change,<sup>227</sup> though each type has

its own particular strengths and weaknesses and there is “established merit in using both”.<sup>217</sup> For example, a generic instrument might ask if someone has problems walking about (such as in the EQ-5D), while a disease specific questionnaire would ask if a person’s disease has limited their walking (such as in the MacNew).

The SAQ is a disease-specific instrument designed to assess the functional status of patients with angina. It comprises 19 questions that quantify five clinically relevant domains: physical limitation, anginal stability, anginal frequency, treatment satisfaction and disease perception/quality of life. It is often used as a QoL instrument because 7 of its 19 items relate to emotional health (psychological/emotional health being important for QoL in patients with a chronic disease such as CAD<sup>229</sup>) and because it has been shown to be demonstrably valid, reproducible, and sensitive to clinical change.<sup>230</sup> In addition, SAQ score has been found to be strong predictor of 1-year mortality and hospitalization for ACS.<sup>231</sup> It is used extensively in research trials to quantify the symptoms, functional limitations, and QoL of patients with stable heart disease in the previous 4 weeks. Use of the SAQ can help healthcare providers identify patients at high risk for morbidity and mortality and can also help identify those patients who may require more aggressive medical therapy or revascularization. However, it is specific to angina symptoms, making comparisons with other heart conditions difficult.

The MacNew Heart Disease Health-Related QoL Questionnaire was designed to evaluate the effect of heart treatments on daily activities and physical, emotional, and social functioning. This self-administered questionnaire generates an overall score from 27 questions that cover physical limitations, emotional and social function, and angina symptoms experienced in the previous 2 weeks. It has been used in multiple clinical studies in patients with different types of heart disease (rather than being specific to CAD). It has also been shown to be valid, reproducible, and sensitive to clinical change and performs well in comparison to the SAQ.<sup>232</sup> In addition, the reading level required to understand the MacNew Questionnaire was found to be on average to be 1 year lower than the SAQ.<sup>233</sup> Also, the MacNew questionnaire is the only disease-specific instrument that contains a question regarding sexual functioning.

#### 1.4.1. Measurement of quality of life in older people

Despite increasing interest, the concept of QoL in older people is still inadequately defined and the measures exploring it have been studied insufficiently among this group. With the existing QoL measurements, the meaningful aspects of QoL for older people may not be measured comprehensively.<sup>234</sup> For example, effects of environment on older people's well-being and functional status, meanings of social contacts, and adaptation to changing situations, are of particular importance and may not be covered by some QoL tools. Research undertaken in lonely older people's group rehabilitation indicated that these issues are significant for an individual's health, well-being and prognosis.<sup>235</sup>

In measuring the QoL of older people, the strengths and weaknesses of the measurement tools should be recognized. QoL measurements tend to involve only few, if any, positive aspects of well-being (e.g. satisfaction with life) where elderly may do better than younger respondents. In addition, groups with special requirements, such as demented persons or frail older people living in institutions, need their own measurements since other people cannot "objectively" define their QoL on the basis of their own values.

WHOQOL-BREF was developed, using international collaborations, by the WHO to be a universal tool.<sup>236</sup> It is thought to be one of the most comprehensive measurement tools and comprises 26 items, which measure the following broad domains: physical health, psychological health, social relationships, and environment. It defines QoL as an individual's understanding of his/her life situation with respect to his/her values and cultural context, as well as in relation to his/her goals, expectations and concerns. In addition to breadth of coverage, it has been showed to be sensitive to change with respect to rehabilitation interventions designed to improve QoL in elderly people. However, it has been criticised for its subjective approach and that few items directly assess functional limitations or disability: suggesting it may not be universally applicable. In addition, there is limited evidence for its use in assessing improvements in QoL following interventions such as PCI. Recently, the WHOQOL-OLD add-on module for the WHOQOL-BREF has been developed which has been designed for use in older adults.<sup>237</sup> While it appears to perform well against other QoL tools such as the SF-36 with less ceiling effect,<sup>238</sup>

completion rates have been an issue and using the module as a “stand alone” has still to be assessed fully.

The 15D measurement tool discriminates very well between older people receiving various levels of health and social care and with different functional status or need of help (for example, community-dwelling, hospital and nursing home patients). It can predict mortality and utilisation of hospital care to a moderate extent. A great benefit is its ability to show change as a result of an intervention. In addition, its ability to quantify QoL in the form of a single number is an advantage compared to several other QoL measurements. However, its limitations – such as the absence of certain aspects of QoL important for older people (for example, sensory abilities, autonomy, attitudes to death and dying) – should be considered when interpreting the results.

The SF-36 and 15D QoL measurement tools appear to explore the QoL and well-being of lonely older people from different perspectives. SF-36 takes better account of an individual’s subjective opinion of his or her health status. It is also better at measuring psychological and social well-being. The disadvantage of SF-36 is that older people may have difficulties in understanding the measurement items and it takes longer to complete than the EQ-5D. In several international studies, completion of SF-36 was found to be low.<sup>239,240</sup> The advantage of using generic tools, like these, is that they allow comparisons to be made between groups, such as comparing elderly and younger patients having PCI. They also allow comparisons with normative data and are used to generate QALYs.

A number of QoL measurement tools have been developed for use in older people. For example, the Older People’s Quality of life (OPQOL) was developed from lay person views (using a constructivist approach), while the WHOQOL-OLD is largely expert led.<sup>241</sup> These are increasing being used in older populations; however, their use in the context of CAD is limited.

P 141 In addition further explanation has been given for the choice of QoL instrument:

**EQ-5D:** This is a widely used simple and validated general instrument to measure QoL in a standard way. This instrument is used in a wide range of conditions, treatments and

interventions. It is also used extensively in health economics to derive a quality adjusted life year (QALY). The EQ-5D descriptive system consists of five dimensions (mobility, self-care, usual activities, pain/discomfort, anxiety/depression) with three levels each (no problem, some problems, extreme problems), thus defining 243 distinct health states. Low scores on the 5 items of EQ-5D reflect a favourable assessment of each component. Responses were used to derive the single index value based on the algorithm provided by EuroQoL. This single index represents the overall health status by applying preference weights to participant responses. The index-based score is typically interpreted along a scale where 1 represents best possible health and 0 represents dead, with some health states being valued as worse than dead ( $<0$ ). In addition, EQ-5D contains a visual analogue scale (VAS). The endpoints of the VAS are labelled 'best imaginable health state' and 'worst imaginable health state', at 100 and 0, respectively. Respondents were asked to indicate how they rated their health by drawing a line from an anchor box to the point on the VAS which best represented their own health that day. Permission to use the EQ-5D was obtained from EuroQol([www.euroqol.org](http://www.euroqol.org)). This widely used QoL tool was chosen because it is simple for researchers to administer, score and interpret. It also imposes minimal burden on participants, as it is a brief, simple measure to understand and to complete (with resultant high completion rates. In addition, the generation of the utility index based health status are useful for both clinical outcomes and economic evaluation.

**SF-12:** This is a commonly used health status questionnaire and is a shortened version of the SF-36. It appears to be more sensitive to differences in health status for people with less morbidity. Like the SF-36, summary scores for physical and mental health status can be derived from the SF-12, referred to as the Physical Component Summary (PCS-12) and the Mental Component Summary (MCS-12), respectively. The summary scores of the SF-12 have also been shown to closely represent the summary scores of the SF-36.<sup>343</sup> The PCS-12 and MCS-12 scores have also been found to be virtually identical to the equivalent SF-36 summary scores in indicating the level of health and are equally sensitive to changes in health status among patients with various conditions. Permission to use the SF-12<sub>v2</sub> was obtained from QualityMetric Incorporated ([www.qualitymetric.com](http://www.qualitymetric.com)). This is the most commonly used generic QoL tool for assessing QoL following PCI in both young and elderly patients (see Chapter 2.3). This was chosen, rather than other instruments that are thought to be more appropriate to use in older people, because the

questionnaire will be used for both elderly and younger patients. In addition, it allows multiple comparisons to be made with existing literature on QoL outcomes following PCI.

**MacNew:** Disease specific QoL was assessed using the MacNew health-related QoL questionnaire. It was designed to assess how heart disease affects daily physical activities and emotional and social functioning. It has been widely used and validated in cardiology patients with AMI, angina and heart failure at the time of designing the ReQoL study. It was chosen because it had not been utilised for assessing QoL following PCI in the UK; therefore, providing an opportunity to address a gap in the literature. It also allows validation of the questionnaire, by comparing it to both the EQ-5D and SF-12, in this population (see Chapter 4.3). In addition, the MacNew questionnaire has been used to assess QoL following PCI outwith the UK e.g. Sipötz *et al.*,<sup>372</sup> providing evidence for its use in this context. It contains 27 items (questions), the responses to which, map to 3 domains: physical limitations, emotional function, and social functioning. Global QoL score and individual domain subscale scores can be calculated. Scores are from 1 (low QoL) to 7 (high QoL). In addition, MacNew has an established the minimal important difference (MID) of  $\geq 0.5$ . Permission to use the MacNew questionnaire was obtained from MacNew ([www.macnew.org](http://www.macnew.org)).

## **2. LITERATURE REVIEW**

### ***2.1. Management of coronary artery disease in elderly patients***

As discussed in Chapter 1.2.5 CAD may be managed using medical therapy or with revascularization techniques such as PCI or CABG. Medical therapy remains the cornerstone of the management of chronic stable angina, but randomized trials have demonstrated greater symptomatic relief from coronary revascularization compared with medical therapy alone.<sup>241,242</sup> However, PCI treatment for stable CAD has shown no evidence of an effect on death or AMI when compared with medical therapy.<sup>3,241</sup> As described in Chapter 1.2.2, CAD incidence and mortality are strongly associated with chronological age, and, due to the ageing population, the elderly account for an increasing proportion of those requiring investigation and management of CAD. In spite of this, the trials have tended to exclude or under-represent elderly patients.<sup>202</sup>

One important study specifically undertaken in elderly patients was the Trial of Invasive versus Medical therapy in Elderly patients (TIME) study in which 305 patients aged over 75 years with stable angina were randomized to either an invasive strategy (n=153) or optimal medical therapy (OMT) (n=148).<sup>243</sup> The patients all had angina, resulting in at least mild limitation of their usual ordinary activity (scoring CCS Class II or worse) that was refractory to two or more types of medication. At baseline, patients had a mean age of 80 years. In the interventional group (CABG or PCI), all patients underwent coronary angiography (dye injected into the heart to assess degree of stenosis), with the clinician then deciding whether or not to proceed to revascularization and which approach to use. At 6 months follow-up, both groups reported improvements in both angina severity and QoL.<sup>243</sup> The improvement was significantly greater in the revascularization group compared with OMT alone. OMT was associated with an increased

risk of major adverse cardiac events (49% vs 19%;  $p<0.0001$ ) and, in particular, with subsequent hospital admission for ACS with or without the need for emergency PCI. At 1 year follow-up,<sup>244</sup> using intention-to-treat analysis, improvements in angina and QoL were maintained in both groups but the differences between them were attenuated and no longer statistically significant. However, major adverse cardiac events continued to be more common in the OMT group (64.2% vs 25.5%,  $p<0.001$ ) and importantly 46% of this group ultimately required revascularisation (most commonly by PCI) due to refractory symptoms.<sup>244</sup> When re-analysed on the basis of actual treatment received, the differences in angina symptoms and QoL between the groups were significant at one year follow-up. This suggests that the negative results using intention to treat reflected the high cross-over from medical therapy to revascularization.<sup>245</sup> Subsequent follow-up over a median of 3.1 years, demonstrated that long-term survival was similar for both groups. Irrespective of which treatment arm patients were assigned to initially, revascularization within the first year was associated with better survival.<sup>246</sup>

A pre-specified sub-group analysis of the COURAGE (Clinical Outcomes Utilizing an Aggressive druG Evaluation) study assessed the proportion of elderly patients (defined in this study as aged 65 and over) who were angina free at 60 months following PCI in combination with OMT group versus OMT alone. This study recruited only 6.4% of the screened population; there was low representation of women (15%) and drug eluting stents were used in only 2.7% of PCIs. Nevertheless, the proportion of elderly patients who were angina free at 60 months was found to be higher following the addition of PCI to OMT (80% vs 73%,  $p=0.01$ ). Despite this, the authors suggest that PCI in addition to OMT “does not reduce clinical events or improve angina relief during long-term follow up” of elderly patients.<sup>247</sup>

## **2.2. Coronary revascularization in the elderly**

A large number of randomized trials have demonstrated comparable survival rates following PCI and CABG for most patient populations.<sup>7,248,249,250</sup> Again, elderly patients have tended to be excluded from these trials. As yet, no randomized controlled trials comparing PCI and CABG have been undertaken in elderly patients. Therefore, the choice of type of revascularisation in the

elderly has to be based on evidence from observational studies and a limited number of non-randomised studies. Whilst observational studies are more likely to include elderly patients than clinical trials, they still suffer from age-dependent bias as elderly patients are less likely to receive evidence-based and guideline-recommended care including coronary angiography and PCI.<sup>251,252,255,256,257</sup> A meta-analysis of these observational studies reported that peri-procedural and long-term outcomes in elderly patients were equivalent for PCI and CABG.<sup>258</sup> Thirty day case fatality was 7.3% (95% CI 6.3% - 8.2%) following PCI, compared with 5.4% (95% CI 4.4% - 6.4%) following CABG. One year survival was 86% (95% CI 83% - 88%) and 87% (95% CI 84% - 91%) respectively, suggesting that comparable survival seen across younger age-groups extends to the elderly. However, the authors acknowledge that differences in survival between PCI and CABG in the elderly could still exist in view of the low level of the evidence available, use of group-level data (rather than meta-regression), and because of differences in baseline case-mix found in the studies.

Over the past decade there has been a major shift in treatment patterns, whereby the number of CABG operations performed each year has decreased whilst PCI procedures have increased in number and now account for an increasing proportion of revascularization procedures.<sup>256</sup> PCI is now the most common form of revascularization in both young and elderly patients in both acute and elective settings.<sup>256,258</sup> Elderly patients tend to present greater technical challenges in relation to PCI due to heavier coronary calcification, tortuous anatomy in both coronary and peripheral arteries and reduced tolerance to bleeding problems.<sup>252</sup> They also tend to have a greater risk profile due to more severe coronary disease and multiple co-morbidities.<sup>5</sup>

### 2.2.1. Outcomes of percutaneous coronary intervention in the elderly

Previous studies have shown that elderly patients have higher in-hospital (death, MI, bleeding) and long term complication rates (mortality and MACCE – major cardiac and cardiovascular complications (or MACE – major cardiac events which excludes non-cardiac cardiovascular events, such as strokes) than younger patients following revascularisation procedures.  
5,32,253,254,255

As discussed, clinical trials tend to exclude elderly patients. However, there are a number of retrospective cohort studies which have examined outcomes in elderly patients undergoing PCI.<sup>5,234,258,259,260,261,262,263,264,265</sup> One of the largest of these studies examined outcomes in 82,140 consecutive PCI cases undertaken in New York State in 2000 and 2001.<sup>5</sup> This multicentre study reported in-hospital mortality and major adverse cardiac events following elective and emergency procedures in three age-groups: <60, 60-80 and >80 years. In-hospital mortality was 1%, 4.1% and 11.5% respectively ( $p<0.05$ ) and in-hospital major adverse cardiac events were 1.6%, 5.2% and 13.1% respectively ( $p<0.05$ ). This study found that, after adjusting for patient co-morbidities and severity of coronary disease, age remained the strongest predictor of in-hospital complications in elective procedures and the second strongest in emergency procedures.

As previously discussed, thirty day and long-term survival rates in elderly patients were examined in a 2003 systematic review, which included only those studies that separately reported baseline characteristics and outcomes for patients aged >80 years and included elective and emergency procedures collectively in the pooled analysis.<sup>32</sup> The pooled estimate suggests that octogenarians were found to have acceptable short and long term outcomes following PCI. A number of the studies included in the review reported in-hospital rather than 30-day outcomes following PCI, possibly resulting in an under-estimate of the pooled risk of adverse events.<sup>32</sup>

A study comparing the safety and efficacy of second-generation coronary stents utilized pooled data from six major clinical trials.<sup>267</sup> This found, that despite more complex coronary lesions, stenting could be performed safely in patients aged over 80 years; with low rates of early and late restenosis. After one year, the incidence of clinically evident restenosis was similar to that in

patients aged under 80 years (11.2 vs 11.9%,  $p=0.78$ ). However, it was observed that elderly patients had higher rates of major bleeding complications (5.0 vs 1.0%;  $p < 0.001$ ), in-hospital mortality (1.3 vs 0.1%;  $p = 0.001$ ) and 1-year mortality (5.7 vs 1.4%;  $p < 0.001$ ). Their increased risk of mortality remained statistically significant after adjustment for higher baseline risks (more complex lesions, comorbid conditions and higher prevalence of multi-vessel disease (MVD) – defined in their study as involvement of 3 vessels or more).

Elderly patients do have an increased risk of procedure-related complications that are associated with increased early mortality. Almost all of the studies have demonstrated increased risk for vascular access complications and associated major bleeding events in elderly patients, with chronological age being the strongest predictor of vascular complications.<sup>268,269,270,271,272,273</sup> Bleeding events and transfusion requirement after PCI have been associated with increased mortality during follow-up.<sup>261</sup> Several studies have also shown increased risk for in-hospital stroke in elderly patients.<sup>267,269</sup> This excess risk of vascular and bleeding complications in the elderly is probably due to a higher prevalence of non-cardiac vascular disease and a greater susceptibility to the risk associated with adjuvant medical therapy.<sup>266,270</sup> In addition, elderly patients have an increased risk of contrast-induced nephropathy following PCI. This is particularly important as elderly patients are more likely to have the complex lesions and tortuous vasculature which require greater amounts of contrast utilization during the procedure. Age related changes in renal function, particularly glomerular filtration rate and tubular function, are also important.<sup>270</sup> Contrast nephropathy has been associated with both early and late mortality.<sup>271</sup>

A multi-centre European registry of 47,407 consecutive patients from 2005 to 2008 included over 8,000 patients aged 75 years and older.<sup>272</sup> PCIs undertaken for ACS and stable angina were examined separately. Patients aged 75 years and older were at significantly greater risk of in-hospital death, especially those presenting with ACS, and age remained an independent risk factor following statistical adjustment for baseline characteristics, including co-morbidities and severity of CAD. This study has the lowest ever reported in-hospital mortality rates (ACS  $\geq 75$  years 5.2% and  $< 75$  years 1.7%; unstable angina  $\geq 75$  years 0.5% and  $< 75$  years 0.2%). The

authors suggest that this low rate is related to advances in PCI, such as adjuvant medical therapies, drug eluting stents and operator experience. However, in a retrospective analysis of observational data it is impossible to rule out residual bias.

### 2.2.2. Reducing the risk of percutaneous coronary revascularization in the elderly

In the last decade, a series of technological and therapeutic developments have reduced in-hospital complications following PCI including adjuvant drug therapies, drug eluting stents and trans-radial access.<sup>229,260,273,274,275</sup> Historically, PCI has been carried out via the femoral artery. Over the last decade, radial access for PCI has become increasingly popular in many countries, with 42.8% of all PCI procedures in the UK now undertaken using this approach.<sup>276</sup> This route is still uncommon in some countries, such as the United States of America, where less than 2% of PCI procedures are undertaken via radial artery access.<sup>277</sup> It has been suggested that the slow adoption of the trans-radial approach in USA may be due to a lack of operator familiarity and limited availability of training.<sup>279</sup>

The major advantages of using a radial, rather than a femoral, approach are reduced access site complications,<sup>248</sup> earlier ambulation of patients,<sup>250</sup> increased patient satisfaction<sup>280</sup> and reduced costs.<sup>278,279</sup> The benefits of early ambulation are particularly important for elderly patients who have a higher baseline risk of venous thromboembolism, healthcare acquired infection and oosteoarthritic problems. The risk of access site complications is reduced using transradial access because the hand has a dual blood supply via the ulnar artery, the radial artery is accessed distal to major nerves and veins and the superficial location of the radial artery makes it easier to achieve hemostasis using local compression. Again, these benefits are particularly important for elderly patients who have a significantly higher baseline risk of artery access complications.<sup>265</sup> Elderly patients are more likely to have heavily calcified coronary lesions<sup>257</sup> and therefore are more likely to require complex PCI strategies such as high-speed rotational atherectomy

(HSRA). The transradial approach for HSRA in has been shown to be feasible, safe and effective<sup>281,282</sup> in a number of small studies which included younger and older patients.

In a meta-analysis<sup>249</sup> of studies comparing radial and femoral routes of access, the former was associated with a 73% reduced risk of major bleeding following elective or emergency PCI (0.05% vs 2.3%, OR 0.27, 95% CI 0.16, 0.45,  $p < 0.005$ ). Using radial access, the length of stay in hospital was shorter, with a weighted mean difference of -0.4 days (95% CI -0.2 to -0.5,  $p < 0.001$ ). However, only two of the randomised controlled trials in the meta-analysis focused on elderly patients.<sup>273,283</sup> In a study by Achenbach *et al.*,<sup>273</sup> 307 patients over the age of 75 years presenting with suspected CAD, or worsening of existing CAD, were randomized to either the transfemoral or transradial approach. Sixty three patients (16%) were excluded because of clinical contraindications to using a transradial approach; including 37 patients who were excluded because of a positive Allen's test using pulse oximetry. Allen's test is used to assess the collateral circulation in the hand, but there is a lack of evidence that it can predict hand ischemia after radial artery occlusion (a possible vascular complication of PCI) and therefore its use may have excluded patients unnecessarily.<sup>284</sup> In the Achenbach *et al.*<sup>273</sup> trial, the data were analysed according to intention-to-treat principles. Among the 152 patients randomized to transradial access, this approach was successful in 91%, with the remaining 9% having to undergo transfemoral access. None of the patients with transradial access suffered major adverse events (defined as death, periprocedural infarction, stroke, vascular access complications that required surgical intervention, blood transfusion or a fall in haemoglobin of more than 3g/dl) compared with five (3.2%) patients with transfemoral access. Only two (1.3%) transradial patients suffered minor bleeding complications, compared with 9 (5.8%) transfemoral patients. A similar sized, but multi-centre, study of octogenarians reported comparable cross-over rates from radial to femoral access (8.9%) as from femoral to radial access (8.1%). Analysis according to intention to treat, again demonstrated a significantly lower risk of vascular complications following radial (1.6%) compared with femoral (6.5%) access ( $p = 0.03$ ).<sup>283</sup>

Jaffe *et al.*<sup>285</sup> assessed the safety and efficacy of the approach in an observational study of 228 consecutive, octogenarian patients undergoing PCI for either chronic stable CAD or ACS. The

initial choice of approach was at the operator's discretion and the transradial approach was chosen in 97 (42.5%) patients. There was a cross-over rate of 11% in the transradial group compared with only 4% in the transfemoral group ( $p=0.03$ ). Procedural success rates were comparable. However, the transradial approach was associated with shorter cannulation and procedure time, reduced use of contrast media, fewer vascular complications (5% versus 20%,  $p<0.001$ ) and shorter time to ambulation ( $5.2 \pm 3.1$  versus  $11.6 \pm 6.3$  hours,  $p<0.001$ ). The mean lengths of stay were 1.7 days for transradial access and 3.1 days for transfemoral access. A single-centre, observational study conducted in China recruited 2,058 consecutive patients undergoing PCI with transradial access.<sup>286</sup> Of these, 719 (35%) were aged 65 years and over. Procedural success rates were high (94.7%). Vascular complications occurred in 4.9% of patients with no statistically significant difference between elderly and younger patients.

### 2.2.3. Antithrombotic therapy in elderly patients undergoing elective percutaneous coronary intervention

The development of newer antithrombotic strategies has markedly reduced cardiovascular mortality and ischemic complications in patients undergoing percutaneous coronary intervention. However elderly patients have age-related changes in haemostasis and drug metabolism, distribution and clearance as well as non-cardiac comorbidities and resultant polypharmacy, all of which are associated with an increased risk of bleeding with antithrombotic therapies.<sup>287</sup>

Current guidelines recommend that dual antiplatelet therapy (DAPT) with aspirin and clopidogrel be used prior to and after percutaneous coronary intervention in all patients, irrespective of age.<sup>287</sup> The duration of DAPT is dependent on the type of stent deployed. Following bare metal stent (BMS) deployment the recommended duration of DAPT is 1 month compared with 6-12 months for drug eluting stents. The extended period of DAPT is necessary because drug eluting stents are associated with delayed or incomplete re-endothelialization which can result in an increased risk of late stent thrombosis. Prolonged DAPT is a concern in elderly patients who, as discussed above, have an increased risk of bleeding. Concurrent warfarin therapy for atrial fibrillation (which is more common with age) is an additional problem.<sup>288</sup> The elderly also have a higher risk of requiring non-cardiac surgery and an increased

risk of falls. All of these factors need to be considered when deciding on the choice of stent during PCI for elderly patients.

Heparin is currently the standard peri-procedural antithrombotic therapy to provide anticoagulation during elective PCI, with GPIIb/IIIa inhibitors being used only for high risk lesions or “bail out” situations (thrombus, slow flow, acute occlusion).<sup>288</sup> GPIIb/IIIa inhibitor use during PCI generally precludes same day discharge. Direct thrombin inhibitors (e.g Bivalirudin) have been used as an alternative to Heparin. Randomised controlled trials (RCTs) comparing Heparin and Bivalirudin have tended to include mainly younger patients or to recruit patients with ACS.<sup>289,290</sup> Meta-analysis shows that Bivalirudin is associated with a significant reduction in major bleeding complications (1.7% vs 3.4%,  $p<0.0001$ ), but no difference in mortality (1.73% vs 1.67%,  $p=0.15$ ).<sup>290</sup> The largest individual study is the ACUTY (Acute Catheterization and Urgent Intervention Triage Strategy)<sup>291</sup> trial which demonstrated an absolute reduction in bleeding events with the use of Bivalirudin instead of Heparin. This was most pronounced in patients aged  $\geq 75$  years, with no significant differences in efficacy between the groups. A large observational study of elective PCI in 2,766 octogenarians found that the use of Bivalirudin was associated with a decreased risk of in-hospital bleeding (HR 0.41, 95% CI 0.23 – 0.73,  $p=0.003$ ) and 6 months MACE (adjusted HR 0.5, 95% CI 0.4 – 0.7,  $p=0.001$ ). Whilst this provides supportive evidence for the use of Bivalirudin, further studies are required to clarify its use in elderly patients in the elective setting – particularly in light of the increased use of the radial approach with its reduced risk of bleeding.<sup>292</sup>

#### 2.2.4. Outpatient percutaneous coronary intervention in the elderly

Increased use of transradial access, with its reduced ambulation time and vascular complications, has facilitated the introduction and expansion of outpatient PCI. Outpatient PCI is an attractive option as it reduces procedural cost, decreases bed utilization, and addresses the demand for increasing numbers of PCI procedures. It has been shown in numerous randomized and observational studies to be both safe<sup>293,294,295,296,297,298,299,300,301,302,303,304,305</sup> and preferred by the

majority of patients.<sup>303</sup> In some centres, outpatient PCI has become the preferred method for managing patients with chronic stable angina who have no medical contraindications (such as comorbid conditions), good angiographic results and an uncomplicated immediate observation period. The Criteria for Overnight Hospital Stay developed by Slagboom *et al.* has been widely used (and modified) to inform decision making<sup>197</sup> However, some operators remain concerned about the safety of outpatient PCI, in light of possible delayed bleeding complications and acute coronary occlusion following discharge. In the UK only 17.4% of all (elective and emergency) PCIs are undertaken on an outpatient basis, but this ranges from 0% to 85% across sites.<sup>248</sup>

Only two studies have compared the outcomes of outpatient PCI in elderly and younger patients.<sup>30,306</sup> The first involved only 117 patients aged 75 or over, attending for transradial PCI between 1998 and 2001. Thirty six (31%) patients reported one or more access site complications during the first 24 hours, including pain, bleeding, numbness, bruising, haematoma, swelling and infection. However, only 3 of these 36 patients consulted their doctor and none had to attend hospital. There were no major access site complications reported in this group. One patient reported chest pain within 24 hours but did not require investigation or intervention, and one further patient had stent thrombosis 37 hours after the procedure. Whilst this retrospective, observational study may suffer from selection bias and minor complications may be subject to reporting bias, secondary data analysis ensured that major complications were identified even in patients who did not respond to the questionnaire. No difference in rates of entry site complications were found between younger and elderly patients in this study.

A more recent study which included 212 patients aged 75 years and older in New Zealand demonstrated that same-day discharge following a 6 hour observation period is possible in the majority of elderly patients (84%).<sup>306</sup> This study did, however, show that elderly patients were more likely to be excluded from consideration for outpatient PCI compared with younger patients (4.5% vs 1.2%,  $p=0.02$ ). Patients were excluded for a number of reasons including inadequate social circumstances, further intervention or testing planned the next day and previous contrast reaction. In those who were put forward for outpatient PCI, it was successfully achieved as often as in younger patients. Suboptimal angiographic results, evidence of periprocedural myocardial ischaemia or infarction, access site complications, late sheath removal and glycoprotein 11b/11a inhibitor infusion were cited as the primary reasons for the failure of

same-day discharge. In this study, only 19 (9%) procedures were undertaken using the transradial approach. All of the elderly patients who had a transradial approach were successfully discharged on the same-day. Despite the low utilization of transradial access, there were no deaths in the 24 hours following discharge and 24 hour readmission rates were very low (0.5%). Since only hospital data were used in this study, the investigators did not have access to information on minor complications in the elderly that were managed without recourse to hospital. Whilst there is a general dearth of literature, it appears that outpatient PCI is feasible and safe in elderly patients who are at low risk of complications and following an uneventful period of post-procedural observation.

In conclusion, the elderly account for an increasing proportion of the population in many countries and the prevalence of CAD is known to increase with age. Therefore elderly patients account for an increasing number and proportion of patients attending for PCI. This trend is likely to continue and should be reflected in the recruitment of more elderly patients into clinical trials to allow for improved evidence-based decision making.

The transradial approach for PCI is associated with fewer vascular complications, reduced bed utilization and reduced time to ambulation. Even in elderly patients, in whom the baseline risk of vascular complications is higher, the transradial approach is safe, effective and associated with less bleeding from the access site. The transradial approach has facilitated the introduction and expansion of outpatient PCI which has been shown in two small studies to be as safe and effective in elderly patients as it is in younger patients. Whilst this area would benefit from further research, outpatient PCI appears to be a realistic option for many elderly patients. Over the next decade the proportion of PCI undertaken on an outpatient basis is likely to increase for both younger and elderly patients, becoming the routine choice for patients without contraindications.

PCI is now a routine treatment for both acute and chronic CAD in both elderly and younger patients. In the elderly, it has been associated with a greater risk of in-hospital, 30-day and long term complications. However the improvements in QoL are at least as significant as those observed in younger patients. Potential improvements in QoL following PCI are likely to become increasingly important in decision making, rather than chronological age per se.

### **2.3. Systematic review of quality of life in elderly patients following percutaneous coronary intervention**

Although in-hospital and late mortality following PCI are important, the effect on symptom relief and QoL are critical considerations and inform patient decision-making. This is particularly pertinent in the elderly population as “the longevity benefits are frequently limited by multiple competing risks and the goals of therapy are often to maintain independent living with reasonable comfort”<sup>29</sup>. There has been a previous systematic review which assessed clinical outcomes following PCI in octogenarians (e.g. death or major adverse cardiac events). This did not assess any impact on QoL;<sup>32</sup> however, it demonstrated that PCI in octogenarians was well tolerated and associated with acceptable short-term and long-term outcomes. However, the authors described the evidence as of ‘low quality’ because of small study sizes or observational nature—despite containing some large well conducted observational studies.

As previously discussed, the TIME study demonstrated an improvement in QoL following revascularization in elderly patients which appeared to be maintained long-term<sup>244</sup> and initially superior to that achieved by OMT alone. There are a number of studies which have shown that PCI produces improvements in QoL measures in the elderly that are equivalent to, or even better than, those observed in younger patients. Spertus and colleagues<sup>307</sup> concluded that age was an independent predictor of QoL benefit while Seto *et al.*<sup>308</sup> concluded that QoL improvements after PCI were not age-dependant. However, no systematic review of the evidence on QoL outcomes in elderly patients undergoing PCI has been undertaken.

A systematic review of the literature was undertaken in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) guidelines (<http://www.prisma-statement.org/>). Systematic reviews are used due to their ability of synthesizing, selecting and appraising all high quality evidence from relevant studies to answer the research question, and to extract the required data for a meta-analysis. Furthermore, systematic reviews are considered to be updatable, accountable and replicable pieces of work which follow distinctive steps.<sup>282</sup> The

PRISMA (Preferred Reporting Items for Systematic reviews and Meta-analysis <http://www.prisma-statement.org/>) checklist was created as an instrument for ensuring transparent and comprehensive reporting of systematic reviews.

The aim of the search was to identify articles reporting QoL outcomes in older patients aged 80 years or older who underwent PCI and, where possible, compare results with younger patients. Eighty years and older was chosen to reflect the findings of the systematic review of clinical outcomes and to allow for easier identification of studies on elderly patients.<sup>32</sup>

### 2.3.1. Search Strategy and selection criteria

Four journal databases were used:

- Ovid Medline 1948 to September week 1 2011,
- Ovid Embase 1996 to 2011 week 36,
- Science Direct, and
- Cochrane Library of Systematic Reviews from 1995 to 2010 inclusive

The following search terms and Boolean connectors were applied.

1	"coronary" OR "heart" OR "cardi*" OR "myocardi*"
2	"stent" OR "PCI" OR "revascularisation" OR "percutaneous coronary" OR "percutaneous intervention" OR "angio*" OR "percutaneous transluminal"
3	"quality of life" OR "psychological adaptation" OR "attitude to health" OR "health status" OR "life change events" OR "EQ-5D" OR "ED5D" OR "SF*36" OR "SF*12" OR "SF*24" OR "DASI" OR "HRQOL" OR "Seattle Angina Questionnaire" OR "RAND 36" OR "DASI"
4	"octogen*" OR "*80 year*"
5	1 AND 2 AND 3 AND 4

The search was limited to studies conducted on humans and those which were available in English and was last run on 15.09.2011. In cases where studies had duplicate publications, the most recent publication was used.

Inclusion criteria:

Only studies which reported QoL and outcomes in cohorts older than 79 years were included.

Exclusion criteria:

- Reviews, editorials, studies of procedures other than PCI and those which reported clinical outcomes only (e.g. death or major adverse cardiac events)
- Non-English language papers
- Papers with non-human subjects

The reference lists of relevant articles (including reviews and editorials) were reviewed to identify additional articles that were potentially relevant. Titles and then abstracts were reviewed to exclude articles that did not satisfy the inclusion criteria.

The full texts of the remaining articles were obtained and reviewed by two people independently (PhD student and supervisor) in detail to determine their eligibility for inclusion. Methodological quality scores were independently assigned and compared. During the review process, all included studies were appraised against a list of specific criteria as suggested by CASP guidelines (<http://www.casp-uk.net/>). The criteria for critical appraisal included these main points;

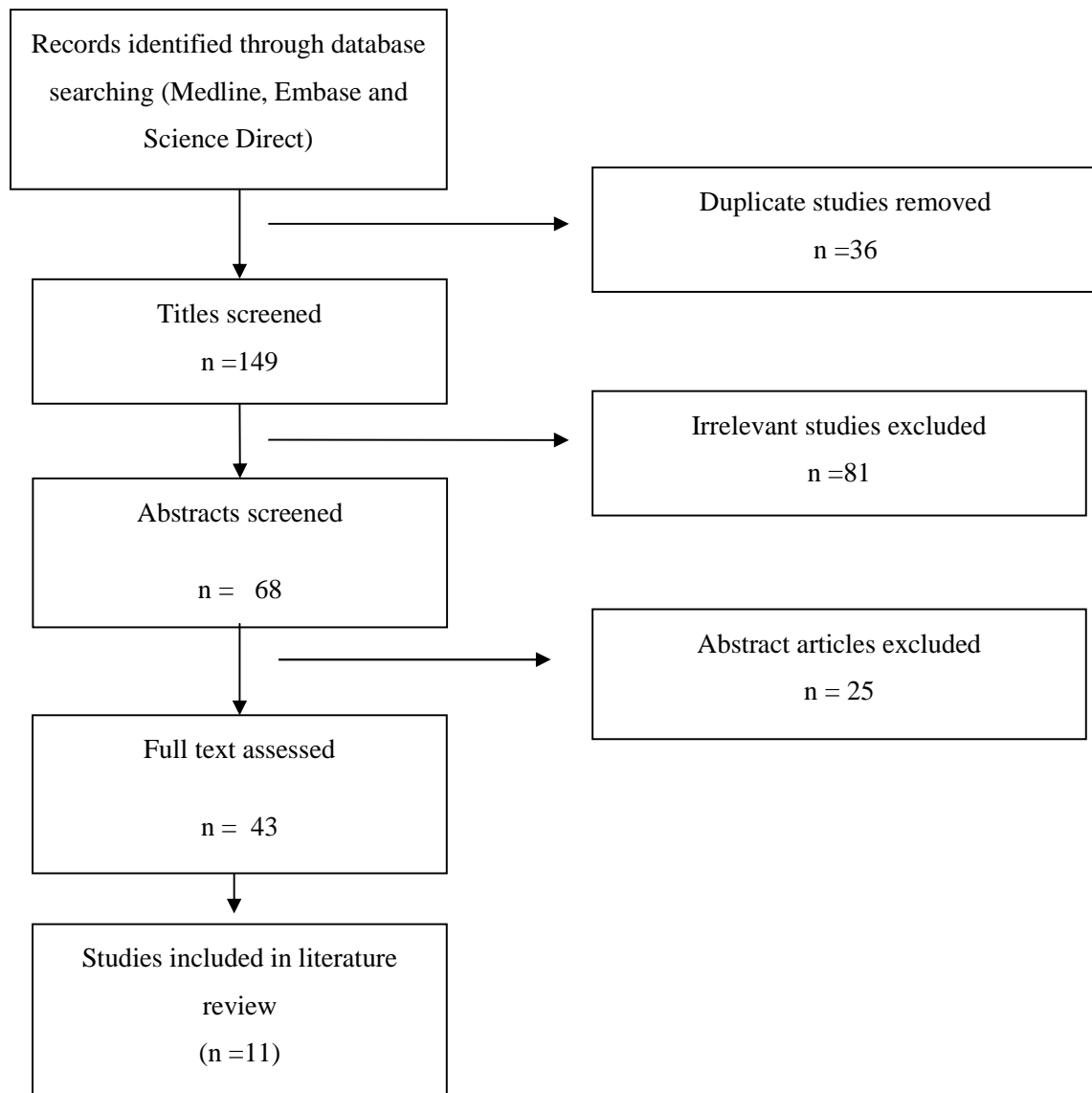
- The presence of clear study questions, which focused on the relationship between PCI and QoL outcomes in elderly patients.
- The usage of valid epidemiological methods in conducting the study.
- The presence of any kind of bias either in the methodology part of study or in the results section.
- The presence of clear description of the sampling method and whether the sample was truly representative of the population from which it was drawn.
- The presence of proper definition of the outcome, such as QoL
- Controlling for confounding factors (e.g. gender)

The data extracted from eligible articles included the publication date, indication for PCI, study setting, study population size and characteristics, intervention details, QoL measurement, length of follow-up, results and measures of statistical significance (Table 1). Corresponding authors were contacted to obtain any missing information and to request sub-group data where appropriate: only 1 such author provided additional information.

Meta-analysis was attempted using the "metan" command in Stata software version 11. The purpose of using this statistical technique is to combine the effect sizes from different eligible studies to obtain a pooled estimates of the overall effect of PCI procedures on QoL in elderly patients. There are two statistical models which are considered as a fundamental for meta-

analysis, the fixed effects models or random effects models. The assumptions used in the two models are different. The fixed effects models assumes that all the studies included in the meta-analysis will have one true effect size and that the sampling error is the underlying cause of the all variations in the observed effects.<sup>309</sup> In contrast, the random effects model assumes that the true effect may vary from study to study. This model assumes that the heterogeneity is attributed to the diversity of the underlying factors in different studies.

Eligible studies for the systematic review were conducted in different years and countries. In addition, they used different QoL tools, with measures applied at different time intervals following PCI. These differences resulted in much variability between the studies. Therefore, the random effect model was used to attempt to calculate the combined effects of PCI on QoL in elderly patients.<sup>309</sup> The  $I^2$  quantifies the effect of heterogeneity and provides a measure of the degree of inconsistency in studies' results.<sup>310</sup> Negative values of  $I^2$  are put equal to zero so that  $I^2$  lies between 0% and 100%. A value of 0% indicates no observed heterogeneity, and larger values show increasing heterogeneity. Ioannidis *et al.* suggests that  $I^2$  values >75% should be considered high.<sup>311</sup>



**Figure 11. PRISMA flow diagram.**

### 2.3.2. Findings

One hundred and thirty-one articles were identified by the electronic search, of which 28 were discarded as duplicates (Figure 11). Title review resulted in the exclusion of a further 61 articles. The abstracts of the remaining 42 were screened and an additional 18 were excluded. The full manuscripts of the remaining 24 were reviewed and 9 were judged eligible for inclusion in the review. The eligible studies were published between 1993 and 2011 (inclusive). They included a total of 671 older patients within the 9 studies, with a mean age of 82.9 (ranging from 82.1 to 83.9 years) across the studies. The oldest patient was reported as 96 years of age. Four studies reported separate results for both older patients and their younger counterparts, with three of them making direct comparisons. Four studies provided before and after comparisons.

Six studies included patients with a mixture of stable and unstable angina; one included only elective PCIs and two had only PCIs undertaken following acute events. Follow up of QoL ranged from five to 40 months following PCI. The SF-36 was the most commonly used tool and was used in three studies, followed by Seattle Angina Questionnaire (SAQ) in three studies. The EQ-5D (EuroQol 5 Domains) and the RAND 36 (a 36-Item Health Survey from RAND Health) were used in only one study each. (One study assessed QoL using two different assessment tools in the same population). Two studies did not use a validated QoL instrument but asked patients to classify their own QoL following PCI as either excellent, good, fair or poor; or score it on a 0 – 10 scale, with no pre-intervention measurement.

**Table 1: Summary of studies: study design, sample, and instruments: quality of life following percutaneous coronary intervention in octogenarians.**

Authors/Country (publication year)	Study Design	Quality Score*	Sample	PCI indication	Mean Age (years) (age range or SD)	Instruments	Intervals of testing and number of patients in each interval
Before and after comparisons							
Agarwal <i>et al.</i> (2009), UK	Prospective cohort Multiple measurements	3	74 patients (50♂) ≥80 yrs	51 ACS 23 Chronic stable	82.5 ± 2.1 (80 to 96)	SF-36 SAQ	Baseline n=74 6 months n=73 12 months n=71
Graham <i>et al.</i> (2006), USA	Prospective cohort/ register of cardiac catheterisation Patients. Compares medical, PCI and CABG	4	137 ≥80 yrs (819 70-79 yrs) (2698 <70 yrs)	13 Stable 43 Unstable 56 AMI 26 Other	82.1	SAQ	1 year n=118 3 years n=94
Kähler <i>et al.</i> (1999), Germany	Prospective cohort with external comparison group	3	34 ≥80 (24♂) yrs (34 <80)	Elective	83± 3 (80-89)	SF-36	Baseline n=34 6 months n=34
Li <i>et al.</i> (2010) China	Prospective cohort	3	23 ≥80 yrs (132 60-79 yrs) (78 <60 yrs)	ACS	**	SF-36	Baseline 6 months
Yan <i>et al.</i> (2012) (conference abs)	Prospective cohort	3	64 ≥80 yrs (476 60-70 yrs) (255 <60 yrs)	Stable & AMI	**	EQ-5D	

Authors/Country (publication year)	Study Design	Quality Score*	Sample	PCI indication	Mean Age (years) (age range or SD)	Instruments	Intervals of testing and number of patients in each interval
QoL assessed post procedure only							
Krumholz (1993), USA	Cross-sectional	1	41 (23♂) ≥80 yrs	AMI	83.9 ± 0.5	Excellent, good, fair or poor	No baseline 12 months
Günel <i>et al.</i> (2008), Netherlands	Cross-sectional QoL compared to general population	2	98 patients (39♂) ≥80 yrs	63 elective 35 acute/stable	82.7 ± 2.9	RAND-36	No baseline 1 year post PCI N=68
Kamiya <i>et al.</i> (2007) Japan	Observational, comparison of PCI, CABG & medical	3	100 PCI patients(63♂) ≥80 yrs	56 emergent 44 elective	83.3 ± 2.8	SAQ	No baseline 39.9 ± 30.1 months n=58
Little <i>et al.</i> (1993), USA	Cross-sectional comparing to Patients <80 yrs	2	118 patients (67♂) ≥80 yrs	102 AMI 16 Other	83 (80-91)	Self ranked on a 0-10 scale	No baseline 18.1 ± 10 months

QoL: Quality of Life; PCI Percutaneous Coronary Intervention; CABG Coronary Artery Bypass Grafting; AMI Acute Myocardial Infarction; ACS Acute Coronary Syndrome; SF-36 Short Form Health Survey; SAQ Seattle Angina Questionnaire; EQ-5D EuroQol 5 Domains; RAND-36 Rand Health's quality assessment tool; WHOQOL-BREF World Health Organisation Quality of Life shortened questionnaire – Maltese version.

\*Quality score 1-5 (5 denoting best score) \*\* missing data.

Krumholz *et al.*<sup>312</sup> in 1993 was one of the first studies to report QoL outcomes following PCI in octogenarians. They compared 41 patients undergoing PCI with 18 undergoing coronary artery bypass grafting and 34 managed medically. They used a simple self-rating system where patients graded their average QoL after discharge as: excellent, good, fair or poor. At one year follow-up, 86% of patients treated by PCI rated their QoL as good or excellent, compared with 89% treated by CABG and 44% of those managed conservatively. No tests of statistical significance were reported but post hoc analyses suggests that the outcome following PCI was comparable to CABG and better than medical management alone. However, five patients who underwent PCI died prior to follow-up, making generalisation of the findings problematic.

A simple self-rating scoring system was also used by Little *et al.* in 1993<sup>31</sup> to compare outcomes of PCI in 118 octogenarians to that of 500 younger patients. There were significant baseline differences between the groups in sex, left ventricular dysfunction, indication for PCI, severity of angina and complexity of PCI. QoL outcomes were measured between 6 and 48 months later in 110 of the 112 octogenarian hospital survivors, but not in the younger patients. Among long-term survivors, QoL was rated as  $8.3 \pm 2.0$  using a 10 point scale, with 95% stating that they felt their QoL had improved following the procedure. No baseline scores were undertaken to assess improvement from baseline.

Four additional studies assessed QoL after PCI without formal baseline comparisons. Günel *et al.*<sup>313</sup> assessed outcomes at one year follow-up using the RAND-36 instrument in 68 of the 75 octogenarians recruited to the study. These patients rated their general health as  $57 \pm 19$  points on a 0-100 scale. The authors stated that “at follow-up the general health was rated as fairly good and better than before PCI,” but provided no data to support this statement. Older patients had fewer symptoms of angina at follow up, assessed using the CCS classification. RAND-36 scores were compared with octogenarians from the Netherlands general population and no differences were demonstrated in the scores for either physical or mental well-being. In a retrospective

study, Kamiya *et al.* used a modified SAQ in 58 PCI patients aged  $\geq 80$  years who survived to follow up ( $39 \pm 20.4$  months).<sup>314</sup> Favourable QoL scores were obtained for the physical and mental domains. Both univariate and multivariate analyses of the predictors of unsatisfactory QoL were undertaken. This showed that left ventricular dysfunction was the only significant factor that influenced QoL scores were also found to be comparable with optimal medical therapy or coronary artery bypass grafting.

Martin *et al.*<sup>315</sup> compared QoL outcomes of minimally invasive direct coronary artery bypass (MIDCAB) and PCI for left anterior descending artery (LAD) revascularisation. The SF-36 was used in 330 patients (172 MIDCAB, 158 PCI). No baseline measurements were undertaken, however follow-up was continued up to 84 months (average follow-up to 38 months). The authors concluded that QoL was better in patients aged  $<80$  years who had undergone MIDCAB compared with PCI, but not in those aged  $\geq 80$  years. The number of patients aged  $\geq 80$  years is not presented. While propensity score matching was used to select the PCI patients, baseline QoL is not included.

Cassar *et al.*<sup>316</sup> used the WHOQOL-BREF (Maltese version) tool to assess QoL in 228 patients following PCI to examine differences between sub-groups. Patients aged  $\geq 40$  years were chosen randomly to receive the questionnaire; a response rate of 64% was achieved. Only 11 patients were aged  $\geq 80$  years and no statistically significant difference was found by age group. Agarwal *et al.*<sup>317</sup> assessed QoL in 74 consecutive octogenarians undergoing PCI using both the SAQ and the SF-36. Baseline measurements were undertaken. Functional status and QoL of life, at both 6 and 12 months, were found to be lower than the age-specific general population norms, but substantially improved compared with baseline measurements. The use of both SAQ and SF-36 enabled the researchers to assess both disease-specific and general health status. The consecutive nature of recruitment resulted in mainly patients with ACS and MVD being included in the study.

Graham *et al.*<sup>318</sup> used the Alberta Provincial Project for Outcomes Assessment in Coronary Heart Disease (APPROACH) register to assess QoL in patients undergoing PCI compared with optimal medical therapy and coronary artery bypass grafting. Revascularisation patients reported

better QoL scores than those treated medically over the full three year follow-up period. Overall response rates for patients aged  $\geq 80$  years was reported as 77.7% at one year. Sixty-nine percent of those responding at one year also completed a SAQ questionnaire at 3 years. Non-responders were much more likely to have received medical therapy alone suggesting possible over-estimation of the QoL benefit in older patients. They were also more likely to have missing data, particularly their exertional capacity scores. No patients  $\geq 80$  years of age who were managed by optical medical therapy subsequently underwent revascularisation.

Kaehler *et al.*<sup>319</sup> compared QoL, measured using the German translation of the SF-36 at 6 months following PCI (in only 34 patients aged 80 years and over) with the same number of younger patients. Immediately following PCI, physical functioning and general health were worse in those  $\geq 80$  years. However, all other SF-36 domains were better in patients aged  $\geq 80$  years at baseline. They found that the benefits of PCI were at least equal, and for some measures were even more pronounced, in the older patients.

More recent studies have also demonstrated greater improvements in QoL among octogenarians than younger patients, following PCI. In 2010, Li *et al.*<sup>320</sup> reported the results of a prospective study of 624 patients who underwent PCI or medical therapy for acute coronary syndrome in a single centre. Older patients reported lower QoL scores at baseline but reported greater improvements in physical health following PCI than younger patients (using the validated SF-36 Chinese version) ( $p < 0.001$ ). Eighty percent of patients were followed-up to six months, which is comparable with other PCI follow-up studies. However, longer-term QoL was not assessed.

An abstract presented orally at the Cardiac Society of Australia and New Zealand conference 2010<sup>321</sup> reported the results for 647 consecutive patients in Hong Kong who were managed by PCI for stable angina or acute coronary syndrome. The EQ-5D was used to assess QoL at baseline and six months following PCI and compared with medical therapy across 3 age groups ( $< 60$ , 60-79 and  $> 80$  years). EQ-5D scores at baseline and at 6 months showed no significant

differences between the age groups, with 86%, 84% and 73% ( $p=0.32$ ) respectively of patients in each age group experiencing improvements in QoL following PCI.

### 2.3.3. Conclusion

This systematic review identified 9 studies which have examined the impact of PCI on QoL among 671 octogenarian patients. The mean age was 82.9 years (ranged from 82.1 to 83.9 years). The evidence, to date, suggests that the QoL for octogenarians does improve following PCI. The benefits are found to be greatest in the first 6 months but may continue for at least 3 years. Older patients improve at least as much as younger patients and appear to gain more in the areas of physical functioning and angina status.

This is the first systematic review to assess QoL after revascularisation by PCI in octogenarians. The results are in keeping with other studies which demonstrate improved QoL in younger and older patients following PCI.<sup>244,322,279,281,308,320</sup> For example, The TIME study (Trial of Invasive versus Medical therapy in Older patients) demonstrated significant improvements in QoL following revascularisation compared with baseline.<sup>244</sup> These QoL improvements in older patients (defined as  $\geq 75$  years rather than as octogenarians), as measured by the SF-36 and DASI, appeared to be maintained at one year follow-up and were found to be initially superior to optimal medical therapy alone. Improvements in QoL in the optimal medical therapy group were thought to be the result of a high cross-over rate (46%) to revascularisation. While this small selective randomised controlled trial may not necessarily be generalisable, it did consider QoL as a primary end point and therefore included it in the power calculation. In addition, CABG and PCI have been reported together as “revascularisation” making it difficult to assess the improvement following PCI alone.

There have been a number of other studies that have shown that PCI produces improvements in QoL measures in older patients that are equivalent to, or even better than, those observed in younger patients. Spertus and colleagues<sup>307</sup> assessed this and concluded that age was an independent predictor of QoL benefit in 1,518 consecutive patients in the United States of America. Seto<sup>281</sup> and colleagues assessed QoL using the SF-36 and angina symptoms with the

SAQ during 1-year follow-up in 295 patients aged over 70 years compared with 1,150 younger patients (maximum age 89 years). At baseline, older patients reported lower scores for physical functioning but similar scores for mental health, when compared with younger patients. At six month follow-up, both older and younger patients reported significant improvements in mental health, physical health and angina status. Similar to the findings of this study, the benefits persisted and, at one year follow-up, 60% of each group reported no angina. The conclusion was that QoL improvements after PCI were not age-dependant.

The studies included in this systematic review were heterogeneous in nature: 4 studies included differences between older patients and their younger counterparts; 6 provided before and after comparisons and 8 included a mixture of stable and unstable patients; 1 included only elective patients and 2 included only PCIs undertaken following acute events. Follow up of QoL following PCI ranged from 6 to 39.9 months. QoL was assessed using 5 different validated QoL tools or by informal self-reported QoL. Studies were from 6 different countries (including 3 from the USA, 2 from the UK, and 2 from China). The quality score of the studies (which included the number of participants in the study) varied from 1-5 (5 denoting the best score possible), with the majority of studies scoring 3 and no studies scoring 5.

As discussed, QoL tools can be generic (e.g. SF-36) or disease specific (e.g. SAQ). Generic instruments address multiple aspects of patients' experience following interventions rather than focusing on specific features of a particular disease. There is much debate in the literature as to which tool is most appropriate to measure improvements following PCI or to assess QoL in older people,<sup>218,241,323</sup> disease specific instruments (e.g. SAQ) are considered more appropriate to assess QoL directly in the presence of CAD.

This systematic review concentrated on octogenarians as representative of much older patients. There are numerous studies in the literature which assess clinical outcomes in octogenarians following PCI.<sup>5,6,32,259</sup> The ageing population and increased life expectancy has resulted in more octogenarians presenting for PCI, suggesting that investigating outcomes in this group is important and hence the choice of age cut-off. Authors of individual studies, which were identified through the search strategy as likely to contain participants >80 years were contacted

with requests for sub-group data. Only one study was able to provide such data – the RITA study.<sup>216</sup> This has not been included in this systematic review as it contained only 1 participant aged  $\geq 80$  years.

As with all systematic reviews, publication bias may be important because negative studies are less likely to be published. Publication bias is particularly important in this field, given that the number of published studies is small. The possibility of confounding and bias is a likely issue within the studies included in this review because only one study reported statistical adjustment for confounding variables, such as baseline QoL and participant characteristics. The studies also used widely different selection criteria, making it difficult to generalise the results. The search strategy excluded non-English language studies, which may have excluded some studies. However, English tends to be the language used in cardiology interventional studies, therefore there is likely only to be a very small number of studies which report in another language. Heterogeneity ( $I_2 > 75\%$ ) amongst the populations and the QoL tools utilised, plus the missing QoL scoring data from most of the studies meant that meta-analysis was inappropriate. This high degree of statistical heterogeneity is in keeping with what is known about differences in the studies in: country of origin, study designs, quality of the studies, sample size, election criteria, range of time of follow-up, and QoL measuring tools.

The benefits of this systematic review are that it used a robust search strategy, which used multiple databases and followed the PRISMA guidelines - making it unlikely that relevant studies were missed. Two independent reviewers were used to assign quality scores to each of the studies.

Overall, this systematic review shows that QoL following PCI in octogenarians improves at least as much as younger patients. Given the small number of studies with only 750 octogenarians, further studies would be useful in determining those octogenarian patients who are likely to derive the greatest benefit from the procedure and to investigate a consensus in the measurement tool utilized to measure QoL following PCI in older patients. This is particularly important as we have an ageing population; this will result in an increase in the number and proportion of octogenarians likely to undergoing PCI in the future.

## **2.4. Review of the literature: telomere length and coronary artery disease**

As discussed, there is emerging evidence of an association between telomere length and attrition with age-related diseases such as CVD. Many such studies explore telomere length as a biomarker of ageing, with the potential to better predict CVD incidence and prognosis than chronological age. Shorter LTLs have also been associated with: CVD risk factors (see Chapter 1.1.4); atherosclerosis;<sup>11</sup> chronic heart failure;<sup>329</sup> peripheral artery disease;<sup>330</sup> degenerative aortic valve stenosis;<sup>331</sup> intima-media thickness of the carotid artery; coronary artery calcification; and CAD, independent of age.

Two main hypotheses exist (these are not necessarily mutually exclusive) to explain this association:

- Telomere attrition, caused by ageing, is accelerated by cardiovascular risk factors and disease, and reflects the overall burden of inflammatory, oxidative, and mechanical stress induced by increased heart rate on the cardiovascular system.<sup>329</sup>
- The Telomere Hypothesis of CVD<sup>330</sup> suggests that shorter inherited telomere length is the primary abnormality and is causally associated with atherosclerosis and CVD.

However, there is no consensus in the literature as whether or not telomere attrition is causally linked to CAD,<sup>331</sup> although there is growing evidence to support an association.

Brouillette *et al.*<sup>332</sup> evaluated whether shorter TL is the primary abnormality in healthy offspring of subjects with, and without, a family history of CAD. Their study design eliminated potential baseline effects: because TL and CVD risk are to an extent inherited, they measured the TL of healthy young adults. Results from this pilot study of 104 subjects indicated shorter telomeres in the offspring of CAD patients, suggesting that shorter inherited telomeres predispose to CAD. This finding supports the Telomere Hypothesis of CVD implying that shorter telomeres are a primary abnormality. This finding has been

partially confirmed in a study that showed shorter LTL in the offspring of patients with ischaemic heart failure than in the offspring of healthy controls, but no difference between the two groups in telomere length in CD34+ mononuclear cells or buccal cells.<sup>333</sup> A small study by Dei Cas *et al.*<sup>334</sup> (82 offspring), the larger Bruneck study (800 offspring),<sup>334</sup> and the European Atherosclerosis Research Study II (EARSII)21<sup>335</sup> (765 offspring) have shown a similar association. However, results were often only borderline significant, and could be prone to confounding effects.

Although experimental evidence in support of the role of telomere length in cardiovascular disease is compelling, there is much less epidemiological evidence. The majority of epidemiological studies have shown an association with age, cardiovascular disease and its risk factors. However, as most studies are cross-sectional, case controls or disease cohorts (rather than prospective cohort studies in those without CAD) there is less evidence to support a causal association. While important, these findings could equally suggest either hypothesis is appropriate - that CAD causes telomere attrition rather than vice versa. Or, alternatively, that both telomere attrition and cardiovascular disease might be caused by common risk factors (smoking, hypertension, high total cholesterol level, obesity, physical inactivity), which contribute to inflammation and oxidative stress.<sup>331</sup>

Several studies have identified an increased risk for AMI and heart disease in subjects with shorter telomeres,<sup>336,337,338,339</sup> although it is unclear to what extent telomere length might have already been affected by subclinical disease status or by confounding variables at baseline.

De Meyer *et al.*<sup>138</sup> assessed the association between atherosclerosis and telomere length in the large Asklepios Study population (2509 subjects). They concluded that LTL is not a substantial underlying determinant of preclinical atherosclerosis. The only marginally significant finding was observed in women, where combined atherosclerotic plaque (carotid and femoral) prevalence was increased in those with shorter telomere length. It has also been shown that LTL is shorter in patients with carotid plaques compared with those without.<sup>315</sup>

While a meta-analysis has been undertaken by Chen and Zeng in 2012<sup>340</sup> exploring the relationship between telomere length and CAD, it has only been presented as a conference abstract in Heart. They used telomere length data from 13 publications consisting of 1985 cases and 3435 controls in a pooled meta-analysis to assess the association between CAD and telomere length. They suggest that telomere length is associated with CAD (WMD=−0.26, 95% CI −0.29 to −0.23). This association between the telomere length and CAD was statistically significant in studies from European-American countries (WMD=−0.25, 95% CI −0.29 to −0.22), and Asian countries (WMD=−0.83, 95% CI −1.21 to −0.45), with the authors stating there was no publication bias (no indication is given to how this was assessed in the abstract). Given the limited evidence and the absence of a full publication of the Chen systematic review and meta-analysis, a literature review was undertaken following systematic review processes to assess the literature on the link between CAD and telomere length in peripheral leucocytes. This was primarily undertaken to provide background and a basis for comparison of results, for the biological ageing in CAD study in Chapter 4.4.

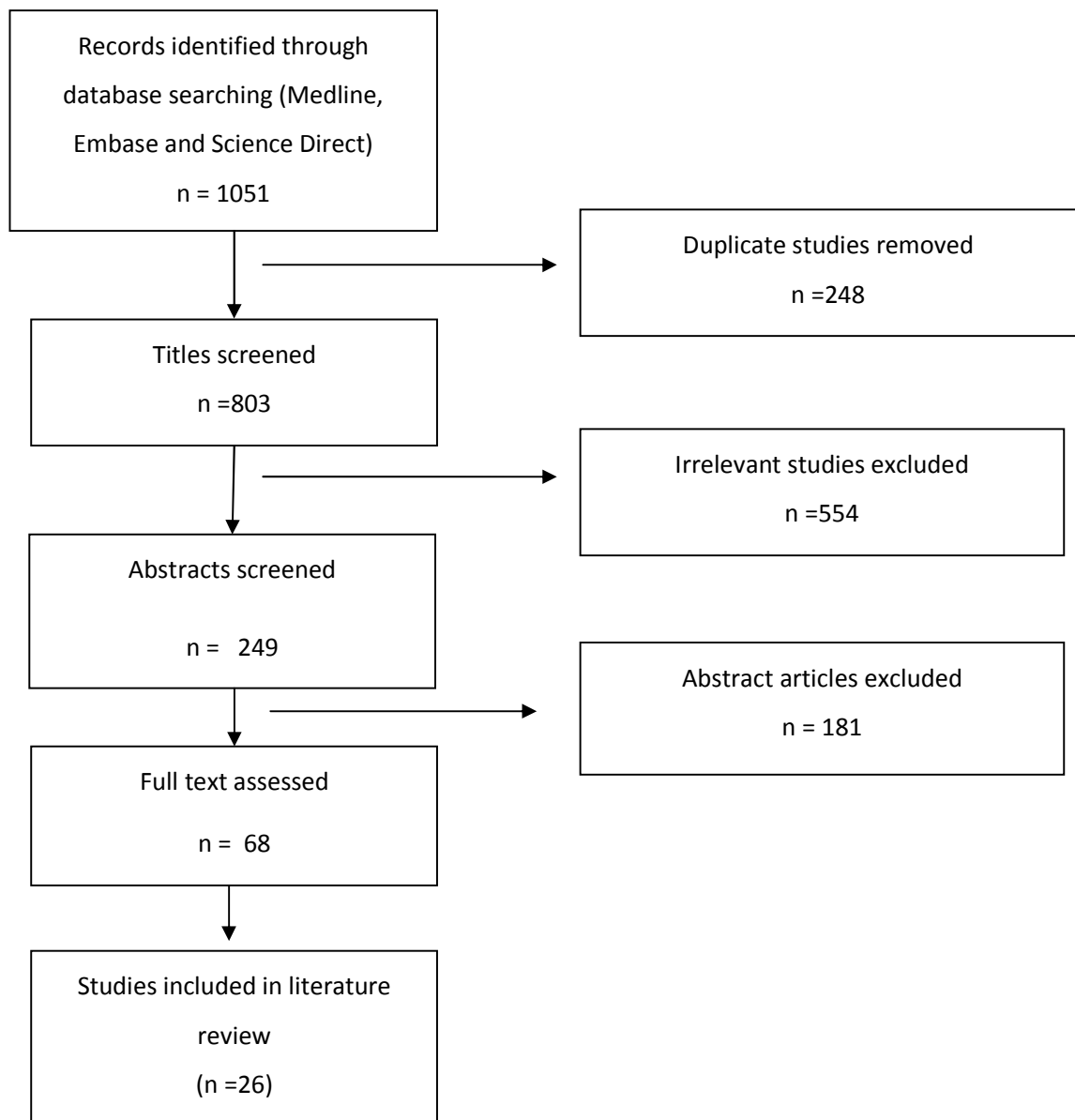
#### 2.4.1. Search strategy and search criteria

Three journal databases were used (Ovid Medline 1948 to September week 1 2013, Ovid Embase 1996 to 2013 week 36, and Science Direct 1996 –present (31.12.2013)) with the following search terms and Boolean connectors applied:

1	“coronary” OR “heart” OR “cardi*” OR “myocardi* OR ”submyocard*” OR “arter*”
2	“disease*” OR “infarct*” OR “ischaem*” OR “ischem*” OR “athero*” OR “angina” OR “steno*”
3	“telomere*” OR “*telomere length” OR “telomere shortening”
4	“leucocyte* OR “peripheral white blood cell” OR “white blood cell*” OR “TL” OR “LTL”
5	1 AND 2 AND 3 AND 4

The search was limited to studies conducted on humans and those that were available in English and was last run on 16.01.2014. Only studies that reported the association between telomere length in peripheral blood leucocytes and CAD were included. Exclusion criteria included: reviews, editorials, studies which included CVD other than CAD or only CVD risk factors. The reference lists of relevant articles (including reviews and editorials) were reviewed to identify additional articles that were potentially relevant. In cases where studies had duplicate publications, the most recent publication was used.

The data extracted from eligible articles included the publication date, study setting, study design, study population size and characteristics, LTL measurement method, main outcome of interest, and main findings (see Table 2)



**Figure 12. PRISMA Flow diagram of studies**

Table 2: Summary of studies: study design, sample, instruments, and main findings: leucocyte telomere length and coronary artery disease.

Lead author/ Country (publication year)	Study Design	Number of participants	Age of participants years (age range or SD)	Telomere Measurement method	Main Outcome indicator	Main Findings
Carquist (2013) USA Conference abstract	Cohort	3,569 patients undergoing angiography. 63.2% found to have CAD	62.9	Multiplex qPCR	Evidence of CAD and survival (follow up 9.2 years)	Univariate-longer LTL associated with decreased risk CAD and death. No association with MI or stroke. Multivariate – only association with death remained.
Ye (2013) Canada	Cohort	1,917 Population based	Shortest tertile – $41.7 \pm 18.0$ Middle tertile – $46.1 \pm 17.8$ Longest – $51.8 \pm 18.1$	PCR CoV 5% to 8%	Fatal/Non fatal CAD events (based on hospital discharges)	No association found. Non fatal CAD associated with the middle tertile (OR 1.63, 95%CI:1.07- 2.51, p=0.02) Small number of CAD events.
Perez-Rivera (2012) Spain	Cohort	203 males with ACS	50-74 (mean $62 \pm 7$ ) n=150 >75 (mean $82 \pm 5$ ) n=53	qPCR	Death Revascularisation Recurrent angina Heart failure Composite end point	Short telomere length is associated with a worse prognosis in men aged 50-(OR 2.56, 95% CI: 1.03-6.39, p=0.04) – note inflame markers
Ruff (2012) USA Conference abstract	Cohort	5,057 ACS	Unknown	qPCR	MACE	Strong independent association between LTL and MACE
Russo (2012) Italy	C-C	199 cases 190 controls	18-48 (period of most flattened LTL loss)	qPCR CoV 1.46%	AMI	No significant difference on univariate or multivariate analysis
Weischer (2012) Denmark	Cohort	19,284 Population based	36-74	RT-PCR CoV 2%	MI IHD Death	Short LTL is associated with only modest increases in risk of MI, IHD, and Death. HR MI: 1.10(1.01-1.19) IHD: 1.06 (1.00-1.11)
Fyhrquist (2011) USA	Cohort With HBP & LVH	1271		Southern Blot	CAD Framingham score MACE	Short LTL associated with CAD in males: OR 0.61, 95% CI 0.39–0.95), and transient ischemic attack in females (OR 0.62 95% CI 0.39–0.99). Proportion of short telomeres was associated with combined cardiovascular mortality, stroke or angina pectoris (HR 1.04, 95% CI 1.01–1.07)

<b>Lead author/ Country (publication year)</b>	<b>Study Design</b>	<b>Number of participants</b>	<b>Age of participants years (age range or SD)</b>	<b>Telomere Measurement method</b>	<b>Main Outcome indicator</b>	<b>Main Findings</b>
Wang (2011) China	C-C	CAD 163 Controls 112	CAD – 57.7±11.6 Controls 56.3±9.2	RT-PCR CoV 1.5%±1.2%	CAD Carotid femoral pulse wave velocity (PWV)	LTL shortening associated with PWV Correlation between LTL and PWV with CAD was stronger than in controls
Fitzpatrick (2011) USA Cardiovascular Health Study	Cohort	1,136 Population based 19.0% had CHD	73.9(4.7)	Southern Blot CoV 1.7%	Cause specific mortality	“Borderline” association for cardiac deaths 1.82 (95%CI:0.95-3.49, p=0.07)
Calvert (2011) UK	C-S PCI patients	170 stable angina or ACS	Upper tertile 59.9(11.0) Lower tertile 63.1(10.2)	qPCR	Plaque severity on virtual histology ultrasound	Shorter LTL is associated with high-risk plaque morphology but not total 3-vessel plaque burden.
Willeit (2010) Italy	Cohort	800 Population based	45-84 in 1995	qPCR intra-assay CoV 1.2%; interassay CoV 2.4%	Composite CVD endpoint	Baseline LTL is a significant and independent risk factor for composite CVD endpoint but not IC or stable CAD
Maubaret (2010) Europe	C-C	598 white patients who survived AMI 653 age- matched controls	<60	RT-PCR	CAD	Those with CAD had shorter LTL than controls
Spyridopoulos (2009) Germany	C-C	25 CAD 27 Controls	65	Flow-FISH Interindividual variability 5.5% and intraindividual variability 1.0%	CAD	LTL negatively associated with CAD
Njajou (2009) USA Health ABC study	Cohort	2,721 Population based Multi site	70-79	qPCR CofV 5.8%	Survival CAD mortality	LTL not associated with overall survival or CAD mortality

Lead author/ Country (publication year)	Study Design	Number of participants	Age of participants years (age range or SD)	Telomere Measurement method	Main Outcome indicator	Main Findings
Yang (2009) China	Cohort	388 patients with hypertension 379 healthy controls	30-80	qPCR Average SD for the triplicate measurement was 6.8%	CAD	Shorter LTL and hypertension are independent risk factors for developing CAD within 5 years
Mukherjee (2009) India	C-C	238 CAD (attending for CAD investigation or management) 238 Controls	29-82	RT-PCR CoV 1.5%	Confirmed CAD CAD severity	After adjustment T/S ratio was significantly lower in patients with a history of CAD. No association was found between CAD severity and LTL
Epel USA (2009)	Cohort McArthur Healthy ageing study	236 Population based	70-79	qPCR CoV 5%	CVD mortality over 3.5 years	Baseline LTL “weakly” predicted cardiovascular mortality, $p < 0.10$ In men, LTL change (shortening) associated with greater mortality from CVD OR 3.0, 95%CI:1.1- 8.2, $p < 0.04$ In women, baseline LTL was associated with greater mortality from CVD OR 2.3, 95%CI: 1.0- 5.3, $p < 0.05$
Zee (2009) USA	Nested C-C	337 white males AMI 337 Controls (USA physicians)	Cases $60.1 \pm 8.7$ Controls $60.2 \pm 8.7$	Modified qPCR CoV <5%	AMI	After adjustment (no lipids in model) LTL was significantly associated with the risk of MI (OR 1.6, 95%CI:1.14-2.30, $p = 0.007$ )
Farzaneh-Far (2008) USA	Cohort	780 with Stable angina	Quartile 1 – $70 \pm 10$ 2 – $68 \pm 11$ 3 – $67 \pm 11$ 4 – $65 \pm 11$	qPCR (single measurement) CoV 9.5%	Mortality MACE Heart failure Inflammatory markers	After adjustment lowest LTL quartile associated with significant increase in death, heart failure admissions but not MACE.
Starr (2007) UK	Nested C-C	620 patients with ischaemic heart failure 183 Controls	40-80	qPCR	Ischaemic heart failure	Univariate analysis – shorter LTL in ischaemic heart failure Shorter according to the severity of atherosclerosis Shorter LTL predicts death and hospitalisation

Lead author/ Country (publication year)	Study Design	Number of participants	Age of participants years (age range or SD)	Telomere Measurement method	Main Outcome indicator	Main Findings
Brouillette (2007) UK	Nested C-C WOSCOPS study	484 Patients with CAD 1,058 controls	Cases 56.9(5.1) Controls 56.7(5.2)	qPCR	CAD	Individuals in lowest tertile of LTL more likely to have future CAD. Risk was attenuated by use of Pravastatin.
Kurz (2006) Switzerland	C-C	64 patients with calcific aortic stenosis	>=70	Southern Blot Interassay variability 7.8%±7%	Calcific aortic stenosis CAD	No association found between CAD and LTL in neither cases or controls
Brouillette (2003) UK	C-C	203 premature AMI 180 Controls	Cases 46.8(6.2) Controls 47.2(5.9)	Southern Blot “Interassay variance in estimate of mean TRF was 3.3+/-2.7%”	Premature AMI	After adjustment – shorter quintile associated with 2.8-3.2 fold increase in premature MI
Obana (2003) Japan	C-C	78 CAD with hypercholesterolemia 62 No CAD with hypercholesterolemia 30 Controls	CAD 66±7.8 No CAD 64±7.1 Controls 64±46.4	Southern Blot Interassay CoV 2/1%	CAD	CAD had significantly shorter LTL compared with controls
Cawthon (2003) USA	Cohort	143 Population based	60-97	qPCR 5.8%	Death Death from CVD	3.18 fold higher mortality from heart disease (95% CI:1.36-7.45, p=0.0079)
Samani (2001) UK	C-C	10 Severe CAD 20 Controls	39-72	Southern Blot Interassay variation was 0.8% (SD 1.5%)	CAD	Cases had mean terminal restriction fragments 303 (SD 90) base pairs shorter. Equivalent to those 8.6 years younger.

ACS acute coronary syndrome; AMI acute myocardial infarction; CAD coronary artery disease; C-C case control; CI confidence interval; CoV coefficient of variance; CVD cardiovascular disease; Flow-FISH fluorescence *in situ* hybridization; HBP hypertension/high blood pressure; HR hazard ratio; LTL leucocyte telomere length; LVH left ventricular hypertrophy; MACE major adverse cardiac events; OR odds ratio; qPCR quantitative polymerase chain reaction; SD standard deviation; T/S ratio relative ratio of repeat to single copy number; WOSCOPS West of Scotland Coronary Prevention Study

#### 2.4.2. Findings

One thousand and fifty-one articles were identified by the electronic search, of which 248 were discarded as duplicates (Figure 12). Title review resulted in the exclusion of a further 554 articles. The abstracts of the remaining 249 were screened and an additional 181 were excluded. The full manuscripts of the remaining 68 were reviewed and 26 were judged eligible for inclusion in the review. The eligible studies were published between 2001 and 2013 inclusive. They included a total of 23,452 participants within the 26 studies. The oldest patient was reported as 97 years of age and the youngest as 18. Nine studies were case-controls, 3 were described as nested case-controls, 13 were cohorts, and one was cross-sectional. Of the cohort studies: 7 included population based participants; 2 had only participants who had recently had an AMI or ACS; 1 included participants with and without hypertension; 1 included participants with hypertension and left ventricular hypertrophy; 1 included participants attending for investigations or management of stable angina; 1 had participants who were undergoing PCI, and only 1 had participants who were undergoing angiography where some 63.2% were found to have CAD and were followed up for 9.2 years. Six studies used Southern Blot to measure LTL; 19 studies used some form of PCR (RT-PCR, qPCR, or “modified” PCR); and 1 study used “multicolor flow cytometry-fluorescent in situ hybridization”.

Of the 10 case-control and 2 nested case-control studies, 10 found an association between LTL and CAD. The first of these studies was a very small pilot study undertaken in 2001 by Samani *et al.*<sup>341</sup> They measured LTL, using the Southern Blot method (with interassay variation of 0.8 SD 1.5%), in 10 cases with severe CAD and 20 controls. The cases were 9 men and 1 woman with angiographically detected severe (>75% stenosis) triple vessel CAD. The controls were 10 men and 10 women with normal arteries on angiography which was preformed to investigate valvular heart disease (n=9) or chest pain of uncertain origin (n=11). An age-related decrease in mean telomere restriction fragment in blood leucocytes of 30-40 bb/year was found in individuals with CAD. On average, mean telomere restriction fraction was similar to controls who were 8.6 years older. The small numbers and sex difference between the groups was a major limiting factor in this study;

although the difference remained significant ( $p < 0.001$ ) when the analysis was restricted to males. The inter-assay variation of the LTL measurement was 0.8% (SD 1.5%).

The unselected consecutive cases in Mukherjee *et al.*'s<sup>342</sup> study also attended for coronary investigations or treatments of CAD (74% for diagnostic angiography, 20% for PCI, and 6% for CABG) to a single centre in India. They comprised of 195 males and 57 females aged 29-82 years. Controls were recruited from the general population of people living and working in the neighbourhood next to the hospital and were free of CAD (based on self-reported history, Rose's angina questionnaire and medications list). Real-time PCR was used to determine the T/S and the CoV was reported as being 1.5%. Correlations from a previous study were used to relate differences in T/S ratio to differences in mean telomere length. They found that the mean LTL in cases was significantly shorter after adjustment for clinical characteristics 1.34bp (95%CI:1.27bp – 1.42bp,  $p = 0.002$ ) with no relationship between T/S ratio and severity of disease. However, they did not find an association between LTL and age in either the cases nor the controls. This may be due to the wide age range or the Indian ethnicity of participants.

In 4 of the case control studies, all of the included cases had had an AMI. (Maubaret *et al.*,<sup>336</sup> Russo *et al.*,<sup>343</sup> Zee *et al.*,<sup>339</sup> and Brouillette *et al.* (in their 2003 study)<sup>344</sup> Russo *et al.*,<sup>343</sup> Maubaret *et al.*,<sup>336</sup> and Brouillette *et al.*<sup>344</sup> used cases that had premature AMIs. However, it is possible that LTL may adversely affect immediate prognosis following AMI meaning that survivors of AMI may have differences in LTL compared with those who died.

Maubaret *et al.*<sup>336</sup> in the HIFMECH (hypercoagulability and impaired fibrinolytic function mechanisms predisposing to myocardial infarction) study showed a significant relationship between LTL and AMI in patients <60 years of age. The study compared male survivors of AMI (598 white males <60 years of age) with population based controls of the same age and region of North or South Europe (598 white male patients (<60 years) who survived a first MI and 653 age-matched control subjects from across Europe). In addition, 413 coronary artery bypass graft patients and 2 groups of 461 patients with familial hypercholesterolemia (FH), of whom 162 had premature CHD, were recruited. LTL was measured using a real-time q-PCR. It was reported that LTL was significantly shorter in patients (7.85 kb) compared with control subjects (8.04 kb,  $P = 0.04$ ). In the coronary

artery bypass graft sub study, LTL was also significantly shorter compared with control subjects. In the FH patients, LTL was shorter in those with CHD (overall, 8.68 kb) compared with the non-CHD subjects (9.23 kb,  $P = 0.012$ ). On the other hand, LTL was not associated with any measured CHD risk factors except for age. These data confirm that subjects with CHD have shorter telomeres than control subjects, and extends this to those with monogenic and polygenic forms of CHD, such as FH.

Brouillette *et al.*<sup>344</sup> in 2003 also found a statistically significant association between mean terminal restriction fraction in 203 cases of AMI <50 years of age and 180 controls. Controls were recruited from 3 primary care practices in the same geographical area. Cases were 3/12 post AMI matched for age, sex and current smoking status. All participants were white and of Northern European origin. Southern Blot interassay variance in estimate of mean TRF was 3.3+/-2.7%. They found that having shorter than average mean leucocyte TRF length increased the risk of MI approximately 3-fold (2.79, 95%CI:1.53-5.11,  $p=0.001$ ). No correlation was found between age and LTL; however, the participants in the study were aged 18-48 years old. As discussed, this is thought to be the age range with the cardiovascular event. During multivariate regression analysis, the LTL determined at enrolment did not predict major cardiovascular events. The authors concluded that LTL did not represent a marker of acute MI in young patients and was not predictive during follow-up.<sup>36</sup> This could, however, have been influenced by the narrow age range (18-48 years is consistent with the most flattened area of the LTL age curve) and low number least telomere length attrition which may explain this finding.

In contrast, Russo *et al.*<sup>343</sup> compared LTL in 199 consecutive patients admitted to an Italian coronary care unit with a first diagnosis of AMI at  $\leq 48$  years of age to 190 age and sex matched controls. They describe their study as a prospective case-control, with cases being followed up for a median of 9 $\pm$ 5 years. No significant differences were observed in LTL between cases and controls (0.81 $\pm$ 0.14 vs 0.77 $\pm$ 0.2,  $p=0.46$ ). Real time quantitative PCR with a CoV of 1.46 5 was used to determine LTL. Little information is provided on controls and in all, 171 patients completed the average follow-up of 9 years, during which time 92 of them presented with a major of study participants as well as other

methodological shortcomings. For example, no correlation with chronological age and LTL was shown, as would have been expected.

Zee *et al.*<sup>339</sup> employed a nested-case-control design within a randomised, double blind, placebo-controlled trial of Aspirin and beta-carotene (a precursor of vitamin A). The study was initiated in 1982 among predominantly white male US physicians who were free of CVD at the time of enrolment. Unlike the other studies, incidence of AMI was assessed in 337 case-control pairs. The prospective nature of the original study reduces recall and confounding bias which is a common issue in case-control studies. A modified qPCR method was used to determine the LTL with a CoV of <5%. After adjustment, an association was found between LTL shortening and risk of incident AMI (OR MI 1.621 95%CI 1.14-2.304, p=0.007). As the participants were US physicians, there are issues with generalisability. In addition, potential confounders such as Triglycerides, LDL cholesterol, HDL cholesterol, glucose, and homocystine were not examined.

Similar to Zee *et al.*<sup>339</sup> Brouillette *et al.*(2007)<sup>11</sup> used a nested case-control design within the randomised controlled placebo controlled trial (WOSCOPS). All participants were from the West of Scotland, aged 45-64 years of age, had raised cholesterol at recruitment, and were statin-naïve. They were randomised to receive either a statin (Pravastatin 40mg daily) or placebo. The 484 cases had either a primary endpoint (non-fatal AMI or death from CHD) or underwent coronary revascularisation. They were matched on the basis of age, duration of follow-up and smoking status with two controls that remained event free for the duration of the study (mean follow-up period of 4.9 years). LTL was measured at recruitment using qPCR based technique which was shown to be correlated (r=0.65) with Southern Blot in a random sample of 18 participants. They found that individuals in the middle and lowest tertiles of LTL were more at risk of developing a CHD event than were individuals in the highest tertile (OR 1.51, 95%CI:1.15-1.98, p=0.0029 for the middle tertile and 1.44, 95%CI: 1.10-1.90, p=0.009 for the lowest). In addition, they reported that this risk was found not to be significant in those participants who had been randomised to receive the statin, suggesting an attenuation of risk. However, the end points used in this study meant that not all CAD could have been captured e.g. those being medically managed.

Case-control studies which included cases known to have CAD were also conducted by Wang *et al.*,<sup>345</sup> Obana *et al.*,<sup>346</sup> Syridopoulos *et al.*,<sup>323</sup> and Mukherjee *et al.*<sup>318</sup> Wang *et al.*<sup>321</sup> included 163 male Chinese Han patients with CAD attending a single centre hospital in Qingdao, China. They measured LTL and pulse wave velocity of large arteries (as a biomarker of ageing of the artery system which measures artery stiffness via the carotid and femoral arteries). The age range of participants was 40-73 years of age (less restrictive than in the WOSCOPS study) and quantitative PCR was used to measure LTL (CoV  $1.5\% \pm 1.2\%$ ). All cases had CAD confirmed on coronary angiography: this was defined as having  $\geq 50\%$  diameter narrowing 1 coronary artery or its major branches. Age and sex-matched controls were recruited from the same hospital (n=112). They attended for routine health examinations and were judged free of hypertension and cardiovascular disease according to history, clinical examination, ECG, and treadmill test. Therefore they may not be representative of the general population of Chinese Han. The T/S ratio as found to be significantly shorter ( $p < 0.001$ ) in patients with CAD ( $0.79 \pm 0.26$ ) than in control subjects, after adjustment for baseline characteristics including lipids and CRP.

Obana *et al.*<sup>346</sup> in Japan compared TL in patients with hypercholesterolaemia (n=91) and/or diabetes (n=84) (35 had both), according to the presence or absence of CAD. Controls were 30 healthy volunteers who were older than 50 years to adjust the mean age to be that of the patient's group. Cases were those with hypercholesterolaemia and/or diabetes found to have CAD by ECG or coronary angiography (n=62). LTL was measured using Southern Blot with an interassay coefficient of variance of 2.1%. LTL was significantly shorter in those with CAD than controls using ANOVA ( $6.1 \pm 0.9$  vs  $6.9 \pm 1.5$ ,  $p = 0.0014$ ). However, little information is given about the recruitment of controls or why only 30 were used. Of the baseline characteristics (age, gender, smoking status, BMI, systolic and diastolic blood pressure, total cholesterol) only serum glucose was found to be significantly different on using unpaired ttests (usually used to compare the means of a continuous variable between 2 unrelated groups – not the 3 (CAD+ with hypercholesterolaemia and/or diabetes, CAD – with hypercholesterolaemia and/or diabetes, and controls) used here. In addition, 64% of cases with CAD were male compared with 73% of controls, suggesting the small numbers may be an issue with statistical significance and that larger numbers with statistical adjustment may have been necessary.

Spyridopoulos *et al*<sup>347</sup>. cases included 25 men who had: coronary angiographic confirmed CAD; healed AMI (at least 3 months after infarction) and impaired left ventricular systolic function. Controls were 14 young (mean age  $26.7 \pm 1.8$  years) and 13 older ( $65.1 \pm 2.1$  years) healthy male volunteers. Medical history and blood tests were used to assess the health status of controls; with additional echocardiography and stress ECG testing in elderly controls. This was the only study that used Flow-FISH to determine LTL which had an inter-individual variability of 5.5% and an intra-individual variability of 1.0%. LTL was determined separately for all relevant leucocyte subtypes (n=12). A power calculation (to detect an 80% probability at the 5% significance level) was undertaken to determine sample size and cases were matched for age. However, no reference is provided for the expected difference between the groups. They found that LTL in cases was 500bp shorter than in age-matched healthy controls.

Mukherjee *et al*.<sup>342</sup> examined the association of LTL and CAD in Indian subjects (known to have a higher prevalence of CAD). Cases were 238 consecutive patients (aged 29-82 years) admitted to a single centre hospital for coronary investigation or treatment of CAD and controls were drawn from the general population (residing in the hospital catchment area) without a history of AMI, stroke or CAD. Presence of  $\geq 50\%$  stenosis on coronary angiography was considered diagnostic of CAD with severity classified according to the American Heart Association guidelines (33 cases did not fulfil this criteria). Cases were matched for: age, sex, BMI, and smoking history, with no statistically significant differences found between cases and controls in fasting lipids. LTL was measured using Real-time PCR with differences in T/S ratio matched to bp using their previous correlation of LTL measured by PCR and by Southern Blot.<sup>344</sup> Mean LTL was significantly shorter in cases compared with controls (1.21, 95% CI:1.16-1.42 vs 1.33, 95%CI:1.28-1.38,  $p=0.0003$ ) even after adjustment for baseline characteristics and excluding cases without significant CAD on coronary angiography. There was no relationship between LTL and the number of stenosis vessels ( $p=0.327$ ). However, no association was found between LT and age (despite the substantial age range in the study) in cases or controls. The authors suggest this may be related to the sample size or the ethnicity of participants (which the study was not designed to assess) rather than imprecision of the assay (CoV 1.5%).

In the nested case control study by Starr *et al.*,<sup>348</sup> participants were recruited from the Scottish Mental Survey and were all born in 1921. LTL measurement was undertaken in 190 participants at age 79 years using qPCR - mean LTL were determined following the Cawthon *et al.* (2003) method. Selection bias towards inclusion of participants from professional and semi-professional social classes and survivor bias were major limitations of this study. Heart disease was classified as definite, possible (e.g. possible angina), or absent (based on history and normal ECG). Definite or possible heart disease was reported in 33% of participants. Associations were examined by type of abnormality: conduction defect, ischaemia, and left ventricular hypertrophy. No association ( $F=0.021$ ,  $p=0.88$ ) was found between LTL shortening and with evidence of ischaemia on ECG.

Elderly participants (193 aged  $\geq 70$  years) were also used in Kurtz *et al.*'s<sup>349</sup> study. All participants were recruited prospectively from patients undergoing elective coronary angiography. Cases ( $n=64$ , mean age  $77.2 \pm 5.2$  years) were those undergoing investigation of CAD prior to valve replacement surgery for critical calcific aortic valve stenosis (CAC). Controls ( $n=129$ ) were those without CAC on direct measurement of the aortic valve pressures during coronary angiography (mean age  $75.9 \pm 4.3$  years). LTL was measured using Southern Blot with an interassay variability of  $7.8\% \pm 7\%$ . While there was an association between LTL and CAC, no significant association was found between CAD and LTL, in cases or controls, even after “investigating for possible confounding” (results not presented in the paper).

Of the 7 cohort studies which used population based participants, 4 restricted the age range to only elderly participants.<sup>352,353,354,355,356,357,358</sup> Epel *et al.*<sup>350</sup> measured telomeres in 236 elderly subjects at baseline and 2.5 years later (available in 134 participants). They measured both LTL and telomere attrition and studied their association with cardiovascular mortality (which included CAD) over 12 years. They state that baseline LTL “weakly” predicted cardiovascular mortality; however, this was not found to be statistically significant,  $p < 0.10$ . In men, LTL change (shortening) was associated with greater cardiovascular mortality OR 3.0, 95%CI: 1.1-8.2,  $p < 0.04$ . In women, baseline LTL was associated with greater cardiovascular mortality OR 2.3, 95%CI: 1.0-5.3,  $p < 0.05$ . The participants were a small atypical sample of white elderly men and women (restricted to age 70-79 years) from Boston, USA (all had good cognitive and physical functioning).

Analysis was performed using quantitative PCR and following Cawthon's process with reliability and validity being described as "high" – Cawthon suggests a CoV of 5%.<sup>121</sup> This study used CVD deaths as an outcome which did include CAD deaths; however, no details are provided on the breakdown of CVD deaths or how they were defined (e.g. inclusion of PAD or heart failure not stated). It is however, one of the few studies which assess telomere attrition over time.

Njajou *et al.*<sup>352</sup> also had an equally restrictive age group (70-79 years of age). This large (n=2,721) multi-site population based study assessed the association between LTL and cause-specific mortality as part of the Health ABC Study. Participants were all from the USA and eligible for Medicare (a national social insurance program, administered by the U.S. federal government). Similar to Epel *et al.*, exclusion criteria included deficits in physical and cognitive functioning. Baseline LTL was undertaken using qPCR with a high CoV of 5.8%. Multivariate linear regression was used to assess the mean terminal restriction fraction length based on the reference DNA sample. The mean follow up time was 8.2±2.3 years. Average LTL was marginally shorter in the 47% who were African Americans compared with white (4.77±1.3kbp vs 4.87±1.3kbp).

In contrast, Fitzpatrick *et al.*'s<sup>351</sup> USA study (Cardiovascular Health Study cohort (CHS)) had only 15% African American participants. However, they were less homogeneous in nature, had less physical and cognitive exclusions, and a less restrictive age group (65 years and over) than Njajou *et al.*<sup>352</sup> In addition, LTL measurement in the CHS, was undertaken by Southern Blot with a CoV of 1.7%. Cause of death was classified independently by physicians using medical records and death certification data, thus cause of death was more specific than in Njajou *et al.*'s study.<sup>352</sup> A "borderline" association between risk of death due to cardiac causes and shortest LTL quartile was found (HR 1.82, 95%CI:0.95-3.4). However, the p value was reported as p=0.07 and there was limited adjustment for traditional cardiovascular risk factors. Cardiac causes of death included: congestive heart failure, arrhythmia, cardiovascular procedures, or multiple mechanisms. No statistically significant associations were found for deaths from congestive heart failure or arrhythmias.

The first population based cohort to be undertaken was published in 2003 by Cawthon *et al.*<sup>121</sup> and was also conducted in USA citizens. Shorter leucocyte telomere length was associated with an increased age-adjusted risk for cardiovascular mortality in a convenience sample of 143 initially healthy subjects over the age of 60 years (3.18-fold higher mortality from heart disease (95CI:1.36-7.45,  $p=0.0079$ )). This was based on Cox's Proportional Hazard regression models for shortest half of telomere distribution after adjustment for age. No other adjustments were made for traditional cardiovascular risk factors. This was also the first to use qPCR to measure LTL (CoV 5.8%).<sup>353</sup> The vast majority of PCR techniques employed since then were based on (with subsequent modification) this initial approach. LTL was found to be associated with age with every 1 year of age being associated with a 0.0048 decrease in the relative T/S ratio.

Population based cohorts have also been undertaken with less restrictive age ranges. One of the most important studies in this area was undertaken by Ye *et al.*<sup>354</sup> with 1,917 healthy subjects aged >18 years. In univariate analysis participants with shorter telomeres appeared to have greater risk of incident CAD than those with longer LTL. Nevertheless, after adjustment for traditional cardiovascular risk factors and inflammatory markers, the risk of new ischaemic events was not linearly associated with LTL (HR 1.25 95%CI:0.82-1.90,  $p=0.30$  - shortest tertile of LTL compared with the longest). Subjects were relatively young (mean age 41.7( $\pm$ 18.0)-51.8( $\pm$ 18.1) depending on tertile of LTL) and the incidence of CAD in this group is low, with only 8.56% of participants presenting with events (fatal and non-fatal CHD events) which were assessed using robust hospitalisation data.

One of the largest population based cohorts was conducted by Weischer *et al.*<sup>337</sup> in Copenhagen, Denmark. Randomly invited participants numbered 19,383 with a follow up of median 17 years assessing incident AMI ( $n=929$ ) and CAD ( $n=2038$ ). LTL decreased linearly with age; although, age explained only 6% to 11% of the variation. Per 1000 bp decrease in LTL with the adjusted HR for AMI and CAD was: 1.10 95%CI:1.01-1.19 and 1.1.06 (1.00-1.11) respectively, suggesting only modest increases. Follow up was complete and the 100% interassay coefficient was 2%. All participants were white and of Danish descent and participants diagnosed with an end point were excluded from that particular analysis.

Willeit *et al.*<sup>355</sup> included 800 men and women aged 45-84 years (in 1995) in the Bruneck Study (South Tyrol, Italy) of prevention of cardiovascular and cerebrovascular disease. Participants were also randomly chosen from the general population and were exclusively white but of heterogeneous origin – Italian, German and Austrian background. During the 10 years follow up, 43 participants experienced an AMI. Hospitalisation data was used to assess endpoints. Each 1-SD decrease in  $\log_e$  –transformed relative to T/S ratio was associated with 41% increase risk of AMI (95%CI:1.02-1.96). Intra-assay CoV of 1.2% and interassay of 2.4% was reported.

The association between LTL and adverse outcomes in populations known to have CAD were explored in Farzaneh-Far *et al.*,<sup>356</sup> Perez-Rivera *et al.*,<sup>357</sup> and Ruff *et al.*<sup>355</sup> Both Ruff *et al.* and Perez-Rivera *et al.* used populations who presented with ACS and used qPCR. While Farzaneh-Far included outpatients with stable CAD. The Spanish cohort<sup>357</sup> consisted of 150 males aged 50-75 years and 53 aged >75 years of age and used the same primers for the qPCR telomere length analysis as Cawthon *et al.*<sup>121</sup> Cardiovascular prognosis was self-reported at interviews. In men aged 50-75 years there was a statistically significant worse prognosis in patients with shorter LTL (log-rank: 5.22,  $p < 0.05$ ). Cox's analysis was used to confirm the independence of this relationship: HR 2.56 95%CI:1.03-6.39,  $p = 0.04$ . Ruff *et al.*<sup>358</sup> followed up 5,057 participants who were enrolled in the MERLIN-TIMI 36 and PROVE-IT TIMI 22 ACS trials, for 1.2 years. After adjustment for baseline characteristics, short LTL was strongly associated with a subsequent AMI (HR 1.81 95%CI:1.36-2.42,  $p < 0.001$ ). While Farzaneh-Far *et al.*<sup>356</sup> in the San Francisco, USA based Heart and Soul Study also used self-reported outcomes; This study also reviewed medical records, death certification and coroners' reports. Seven hundred and eighty participants were followed up for a mean time of 4.4 years. Exclusions included previous history of AMI in the last 6 months, those who deemed themselves unable to walk 1 block, or those who were planning to move out of the area. After adjustment for clinical, inflammatory, and echocardiographic risk factors, participants in the lowest quartile of LTL remained at significantly increased risk of death and heart failure admission, but not a subsequent cardiovascular event (which included MI, stroke or CVD death) Adj HR 1.5 95%CI:0.9-2.6,  $p = 0.11$ ). The CoV of the qPCR was reported as 9.5%.

The only cross-sectional study was undertaken by Calvert *et al.*<sup>359</sup> and included 170 patients with stable and unstable angina who were referred for PCI and underwent 3-vessel virtual histology intravascular ultrasound. Shorter LTL was found to be associated with high-risk plaque morphology but not total 3-vessel burden, adding to the evidence that leucocyte ageing is involved in vulnerable plaque formation.

To examine how shorter LTL might be associated with unstable plaques, age- and sex-matched healthy controls were also used as an extension of the cross-sectional study. However, no further information is provided on the controls. LTL was significantly shorter in CAD patients vs controls  $\sim 0.45\text{kb}$ ,  $p < 0.01$ . qPCR was used to measure LTL and compared to a reference DNA of known telomere length (by Southern Blot). This is a highly selective patient group and exclusion criteria included: previous PCI, unsuitability for virtual histology intravascular ultrasound, active inflammation, and any form of surgery 3 months before the procedure.

Two of the cohort studies were focused on patients with hypertension. Fyhrquist *et al.*<sup>360</sup> measured LTL in 1271 subjects (aged 55-80 years) with hypertension and left ventricular hypertrophy participating in the Lifestyle Interventions and Independence For Elders (LIFE) study. This was a post hoc analysis of participants who were in a randomised double blind controlled trial which was set up to compare different medical treatments. Southern Blot (CoV reported as 3.70%) was used to measure LTL at recruitment. At baseline, short mean LTL was associated with CAD in males (OR 0.61 95%CI:0.39-0.95) but not in females. During the 4 year follow up, development of angina, but not AMI, was associated with short mean telomere length. Antihypertensive medication given in the trial (adjustments were only made for age and sex although differences in LTL were noted between the treatment arms noted between LTL and treatment ). Lack of controls and the post hoc nature of the study may have influenced the baseline mean LTL.

Hypertensive patients in a study of 767 30-80 year olds,<sup>337</sup> were found to have 7 shorter LTL than healthy controls (0.57 vs 0.67,  $p < 0.001$ ). Exclusion criteria were history of alcohol abuse, diabetes, secondary hypertension, and renal disease. After 5 years follow up of 411 subjects, those with shorter LTL were at higher risk of developing CAD than individuals with longer LTL (OR 3.315 95%CI:1.662-6.609,  $p < 0.001$ ). Multivariate

analysis showed that LTL and hypertension were independent risk factors for developing CAD (adjusted for age and gender OR 6.413,  $p=0.008$ ). LTL measurement was undertaken using qPCR with a CoV of 6.8%. Follow up drop out participants were similar in terms of age, gender, and conventional risk factors.

In 2013, Carlquist *et al.*<sup>361</sup> presented a poster on the association between LTL and cardiovascular outcomes in 3,569 patients attending for coronary angiography at a single centre in Utah, USA. LTL was measured using multiplex qPCR with patient information being extracted from electronic records and survival status being verified by the National Death Index. Some 63.2% of patients had CAD on coronary angiography and the mean age was 62.9 years; 91.3% were white and 63.8% were male. LTL was found to be correlated with age ( $r=-0.244$ ,  $p<0.001$ ). After adjustment for: severity of CAD; traditional cardiovascular risk factors; and heart medication use, LTL was associated with risk of death but not AMI during follow up (median 9.2 years). Longer LTL was associated with a decreased risk of CAD (OR=0.54,  $p<0.0001$ ) but not after adjustment.

### 2.4.3. Conclusion

In 2003, Cawthon *et al.*<sup>121</sup> first reported that shorter LTL was associated with an increase in age-adjusted risk for cardiovascular mortality in a population based sample. Since then there have been a number of studies which have reported discordant findings, with some studies reporting a weak or non-existent association between LTL and CAD in population based cohorts. Others have reported a modest association. Many of the studies have notable limitations such as restricting enrolment to the very elderly, leading to possible survival bias; the inclusion of participants with previous CVD; and limited adjustment for traditional cardiovascular risk factors. Further studies are needed to explore the link between LTL, and CAD and its subsequent adverse outcomes. These should include longitudinal studies which can provide evidence for a causal association and whether or not LTL could be used to predict adverse outcomes in patients with CAD. Comparison between the different methods of measuring LTL is difficult; therefore, consensus on the most appropriate way of analysing LTL would be useful. Alternatively, the development of a statistical method which produces a common scale could be explored. As there is still

debate as to whether LTL reflects telomere length in arterial endothelial cells, additional studies in this area are warranted.

This literature review could be described as “near” systematic review<sup>362</sup> because it was undertaken following the PRISMA guidelines for searching and synthesising the literature on LTL and CAD. In addition, it was undertaken to identify, critically appraise, and synthesise the existing literature relating to LTL and CAD. This is to provide background and a basis for comparison of results for the biological ageing in CAD study in Chapter 4.4. In addition, a near systematic review does not include 2 independent reviewers assessing the studies against the inclusion/exclusion criteria or independently reviewing the quality of the studies using a formal checklist. Therefore, a meta-analysis has not been undertaken at this stage. However, this is a potential area for future research.

## 3. METHODS

### 3.1. *Secondary data analysis*

CAD remains the most commonly reported limiting longstanding illness and accounts for around a quarter of deaths in men and women. Morbidity and mortality from CAD are strongly associated with chronological age. A secondary data analysis was undertaken to examine whether elderly patients undergo coronary angiography at a more advanced stage of disease, whether they are less likely to proceed to revascularisation if CAD is confirmed and whether any inequalities in management appear justified by a high risk of peri-procedural complications in elderly patients.

#### 3.1.1. Data sources and study population

##### 3.1.1.1 Scottish Coronary Revascularisation Register (SCRR)

Since 1997, the Scottish Coronary Revascularisation Register (<http://www.scs-online.org.uk/cardreg.php>) has routinely collected detailed information prospectively on all coronary angiographies, PCIs, and CABGs performed in Scottish hospitals. Data are entered prospectively by a combination of clinical and administrative staff according to a predefined set of standardised data definitions, and centrally collated to form the Scottish Coronary Revascularisation Register. The database is annually validated for completeness and consistency. Information collected includes: demography (including age, gender, and deprivation); co-morbidity (e.g. previous history of MI, stroke, chronic lung disease, diabetes, and hypertension); clinical presentation (e.g. indication for PCI, procedural priority); CAD severity (using CCS); procedural details (type of lesion, length of lesion, diameter of treated vessel, type of stent) and in-hospital/in-catheter lab complications. In Scotland, relatively few angiographies or revascularisation procedures are performed in the

private sector. Therefore, the analysis was restricted to the publicly funded NHS hospitals that performed angiographies or coronary revascularisation procedures during the period of study.

Consecutive patients who underwent diagnostic coronary angiography in Scotland between April 2001 and March 2010, and PCI between 1998 and 2008 were included in the secondary data analysis. These were the most recent database downloads available from the SCRR system, prior to it being decommissioned. Decommissioning of the SCRR system was undertaken in stages (related to funding) and the coronary angiography database was the last to return from ISD with linked follow up. This resulted in more recent data being available for the coronary angiography analyses, compared with PCI. Informed consent to collate and use data for audit and research purposes is routinely obtained from patients prior to angiography or coronary revascularisation. Both studies were approved by the Scottish Coronary Revascularisation Registry Steering Committee and the NHS Privacy Advisory Committee. All patient and operator data were stripped of unique identifiers prior to analysis.

#### 3.1.1.2 Scottish Morbidity Record (SMR01)

The Scottish Morbidity Record (SMR01) collects information on all discharges (and therefore admissions) to acute hospitals in Scotland, including disease and procedure codes (<http://www.datadictionaryadmin.scot.nhs.uk/isddd/9065.html>). SMR01 has episode-based patient records that relate to all acute inpatient and day cases. Every record in SMR01 data reflects one episode of care. An SMR01 record is generated every time a patient completes an episode of inpatient or day case care. Completion of an episode includes: discharge home; transfer to another consultant in either; the same or a different hospital; a change of specialty under either the same or a different consultant; or death. The data that are collected to describe each episode include clinical (diagnoses and procedures) and non-clinical (demographic information, episode management details) information. Details of diagnoses on discharge are recorded, by a clinician, in the patient's medical notes. These are then translated into codes using the ICD-10, Office of Population

Censuses and Surveys' Classification of Surgical Operations version-4 (OPCS-4) codes, and National Interim Clinical Imaging Procedure Codes.

### 3.1.1.3 General Register Office for Scotland (GROS)

The GROS collates death certificate data across Scotland, including cause of death. Diseases are recorded using the International Classification of Diseases (ICD) and procedures using the Operating Procedure Codes (OPCS). It has since merged with the National Archives of Scotland to become the National Records of Scotland (NRS). Any death which occurs in Scotland must be registered within eight days of the date of death by the Registrar of Births, Deaths and Marriages.

The SCRR is linked annually to the SMR01 and death certificate data at an individual level, providing information on fatal and non-fatal events that occur following discharge. The last linkage, available via the SCRR system prior to it being decommissioned, provided follow-up events up to 31 March 2007 for the PCI data, and 31st March 2011 for the diagnostic angiography data, inclusive.

### 3.1.2. Definitions

Elderly was defined as  $\geq 75$  years of age, and the younger group as  $< 75$  years of age. Angina severity was graded using the CCS classification: (from 0 (no symptoms) to 4 (most severe). CAD was defined in the register as angiographic evidence of significant stenosis ( $\geq 70\%$  diameter stenosis of at least one major epicardial artery segment or  $\geq 50\%$  diameter stenosis in the left main coronary artery). MVD was defined as significant stenoses in two or more coronary arteries. MVD with left main stem (LMS) involvement was allocated to a separate category. Current smoking was defined in the registry as regular smoking of one or more cigarettes per day in the month preceding coronary angiography. Obesity was defined as a body mass index (BMI)  $> 30$ . The Scottish Index of Multiple Deprivation

(SIMD; [www.scotland.gov.uk/Topics/Statistics/SIMD](http://www.scotland.gov.uk/Topics/Statistics/SIMD)) was used to measure socioeconomic status. This ranks data zones of residence (mean population of 750) to produce deprivation quintiles for the general population. These were applied to the study cohort using their postcode of residence. Diabetes included both type 1 and type 2. Hypertension was defined as a systolic blood pressure  $\geq 140$ mmHg, diastolic blood pressure  $\geq 90$ mmHg or use of anti-hypertensive therapy. Extra-cardiac arteriopathy included peripheral artery disease, previous stroke, recurrent transient ischaemic attacks and carotid stenosis  $\geq 70\%$ . Impaired renal function was defined as serum creatinine  $> 200$ mmol/l or use of renal replacement therapy. Impaired left ventricular function was defined as an ejection fraction of  $< 50\%$ . Multiple co-morbidity was defined as the presence of two or more of these conditions.

Follow on PCI were procedures which were undertaken immediately (with the patient still on the catheterization table) following diagnostic coronary angiography, rather than a staged procedure performed during a different session - these are also known as *ad hoc* PCIs. Any revascularization within 30 days included follow on PCI. Isolated coronary angiography was defined as an angiogram that was not associated with follow-on PCI. Data for outcomes are presented by diagnostic coronary angiography, follow-on PCI, and

any revascularisation within 30 days, given the increased risks associated with revascularisations.<sup>3</sup> In addition, data are broken down by presences and severity of CAD (SVD, MVD, LMS) because this influences how patients are subsequently managed.<sup>3</sup>

Peri-procedural complications were defined as adverse events (including: death; abrupt closure of a coronary artery; anaphylaxis; coronary artery perforation or dissection; cardiac arrhythmias; cardiopulmonary arrest; bleeding; and peripheral vessel haematoma) that occurred during or following the procedure, but prior to leaving the catheter laboratory. Thirty-day outcomes were defined as events that occurred during the procedure or up to 30 days follow-up, and included events that occurred both in hospital and post discharge. The binary outcomes studied were: PCI; CABG; coronary revascularisation (PCI or CABG); all cause death; fatal/non-fatal AMI, fatal/non-fatal cerebrovascular event (stroke or transient ischaemic attack) and a composite outcomes of major adverse cardiovascular event (MACE) comprising death, AMI or cerebrovascular event.

The coverage of troponin measurement varied by hospital and over time; therefore peri-procedural AMI was defined as electrocardiographic evidence of a Q wave AMI rather than including those with the more recent definition of ACS: STEMI, NSTEMI or unstable angina.

### 3.1.3. Statistical analyses

All statistical analyses were undertaken using STATA 11. Baseline characteristics were compared between elderly and younger patients using: Pearson's Chi-Squared tests  $\chi^2$  tests for binary variables; independent t tests for continuous variables; and Cuzick's non-parametric test for trend for ordinal data.<sup>363</sup>

Thirty-day outcomes were compared using univariable and multivariable binary logistic regression models, and longer-term outcomes using Cox's proportion

regression hazard model.<sup>364</sup> Logistic regression was used as the outcomes of interest were binary in nature e.g. death/no death. Logistic regression models the relationship between a dependent and one or more independent variables, and allows us to look at the fit of the model as well as at the significance of the relationships (between dependent and independent variables). The goodness of fit of the models was assessed using Hosmer and Lemeshow's test<sup>365</sup> applied to 8, 10 and 12 groups of observed and predicted binary outcomes. Logistic regression, being based on the probability of an event occurring, allows us to calculate odds ratios (OR). OR is defined as the odds that an outcome will occur in one group, compared to the odds of the outcome occurring in another. Where odds is the ratio of the probability of an event occurring to the probability that it does not.

Cox proportional regression hazard model was used to determine the unadjusted and adjusted hazard ratio (HR) for elderly versus younger patients, with the younger patient group forming the referent population. HR is a measure of how often a particular event happens in one group compared to how often it happens in another group, over time. It assumes that hazard ratio is constant over time. The proportional hazard assumption was tested using a residual based inference method.<sup>366</sup> The assumptions of the test were met for all models presented in the results section (Chapter 4.1).

The multivariable models provided a method for adjusting for the potential confounding effects of sex, smoking status, diabetes, hypertension, extra-cardiac vascular disease, renal impairment, impaired left ventricular function, disease severity, SIMD deprivation quintile, urgency of the procedure, access site, year of procedure and hospital.

An interaction term between year of procedure and age-group was introduced, in order to determine whether the association between age-group and outcome changed over time after adjusting for the potential confounding factors listed above. This was thought to be a possible likely interaction given the advances in coronary angiography and PCI over the time period.

In addition, the MACCE and revascularisation outcomes following coronary angiography analyses were re-run using imputed variables with missing data and using multiple imputation by chained equations. The chained equations used the same variables as the full models and included outcome variables. Five imputed datasets were created using the ICE package in STATA.<sup>367</sup> Multiple imputation is a statistical technique which has been used for missing data in observational studies using secondary data.<sup>368</sup> However, as it assumes that the data is missing randomly and normally distributed, this technique is less useful with missing binary variables such as diabetes status. In addition, it been considered less useful with large datasets where power is often not an issue.<sup>369</sup>

Multiple imputation was used to explore the effects of missing data in the coronary angiography outcomes study, as missing values were higher in this dataset. Most common variables with missing data were BMI (44.6%), diabetes status (23.6%) and left ventricular dysfunction (25.4%). Goodness of fit was assessed using a plot of observed versus expected values as well as Hosmer-Lemeshow tests. Where the tests showed that the models were an adequate fit for the data, the multiple logistic regressions were re-run. However, using the imputed dataset resulted in little change in the association between age group and MACE or revascularisation outcomes. Therefore, multiple regression models are presented with complete cases.

General population age-group specific rates were calculated using the 2006 mid-census population estimates from General Registrar for Scotland. This was used because the study used PCI data from 2000 – 2010 inclusive. This estimate can be applied to all the years and was more likely to best reflect the breakdown of the population than the 2001 census.

## **3.2. *Methods for Revascularisation and Quality of Life (ReQoL)***

### **3.2.1. Aims and objectives**

As discussed, PCI is generally undertaken in elderly patients to alleviate the signs and symptoms of CAD. The systematic review of the literature, in Chapter 2.3, suggested that QoL in older patients improves at least as much as younger patients and they may gain more in the areas of physical functioning and improved angina status. The benefits appear to be greatest in the first 6 months and may continue until at least 3 years. However, only a small number of studies were included in the systematic review (11 studies with a total of 700 octogenarians). Therefore, assessing the benefits of PCI on QoL for elderly patients is an important area for further study.

In addition to undertaking secondary data analysis to investigate the risk of peri-procedural complications and MACCE outcomes in elderly patients, QoL improvements following PCI were also assessed as part of this thesis as the ReQoL study.

The main objectives were:

- To assess the baseline QoL in patients undertaking PCI using generic and disease specific tools;
- To assess QoL at 3 months post PCI using generic and disease specific tools;
- To compare differences between baseline and 3 months QoL
- To compare differences and changes in QoL between elderly and younger patients
- To assess the impact of frailty on outcomes.
- To use routine hospital data to assess baseline characteristics (such as demographics, comorbidity etc.) and severity of CAD

### 3.2.2. Participants

Patients undergoing non-emergency PCI for CAD were recruited from the West of Scotland Regional Heart and Lung Centre at the Golden Jubilee National Hospital (GJNH). The GJNH is one of the largest regional centres undertaking PCIs in the West of Scotland; with around 5,000 diagnostic angiograms and 2,000 PCIs undertaken per annum. The majority of patients attend as day cases (68%) rather than inpatients, with some (96%) being undertaken using the radial approach rather than via the femoral or brachial artery. This allows many patients to be discharged as “rapid discharge” (~ 4 hours) following the modified Amsterdam criteria.<sup>154</sup>

The study group comprised consecutive patients attending for non-emergency PCI with CAD who did not have the following exclusion criteria:

- Emergency admission
- Attending for investigation/management following ACS
- Unable to read/write in English (as QoL questionnaires were validated in English)
- Unwilling or unable to provide informed consent

Potential participants were identified through the GJNH procedure list. The Cardiac Research Nurses approached potential participants after routine admission to the unit to discuss potential involvement in the study. They were provided with participant information leaflets and given sufficient time to consider their involvement. Following written, informed consent participants undertook a baseline questionnaire in the unit with face-to-face support by the cardiac research nurses. The 3-month questionnaires were sent to the participant’s contact address using a pre-paid reply envelope. Participants were sent reminders 1 month following a non-returned questionnaire.

### 3.2.3. Ethical considerations

Ethical approval for this study was granted from the West of Scotland Ethics Committee (REC 2) and was supported by the GJNH Research and Development department (see Appendix 7.1).

Ethical considerations for patients were carefully considered and these included:

- Taking part in this study had no impact on clinical care as it involved self-reported QoL questionnaires;
- The research involved NHS patients and included administration of questionnaires;
- Participation in this research did include an additional burden for patients as completion of the questionnaires took between 20 and 30 minutes;
- Sensitive questions – no sensitive questions were included in the QoL questionnaire;
- Confidentiality – The top sheet of the questionnaire, which included the participant contact details, was detached and stored separately from the questionnaires. Questionnaires contained only the study number (and no other identifiable data) which was not available to the researcher. Contact details were exclusively used for follow-up and only an experienced and professionally qualified administrator had access to them. All data was kept separately and password protected on University of Glasgow computers in the Department of Public Health.
- Informed consent – Information about studies being undertaken at the GJNH are provided in the pre-admission information provided by the GJNH. It routinely states that: “As a regional centre for the West of Scotland in the treatment of heart disease, the Golden Jubilee Hospital participates in a number of research studies’ and “During your stay, you may be approached by a member of the Cardiology research team and invited to take part

in such a study. Participation in research is entirely voluntary and will not affect the standard of care you receive". The cardiology research nurses provided potential participants with a participant information leaflet (see Appendix 7.2) and sufficient time to decide whether or not to take part in the study. Informed consent was gained using a consent form (see Appendix 7.3).

#### 3.2.4. Power calculation

Seto *et al.*<sup>281</sup> assessed QoL in 1445 patients following PCI using SF-36 and SAQ. They compared QoL at baseline, 6 months and at 1 year between elderly ( $\geq 70$  years of age) and non-elderly ( $< 70$  years of age). They demonstrated that elderly patients had an increase of 8.6 points in the median SF-36 physical functioning score compared with a 10.5 increase in non-elderly. If we wish to demonstrate a statistically significant difference at the 5% level and achieve a power of 95%, with a conservative difference of only median of 1.9 points, we need at least 360 in each group.

Initially, age groups were defined as elderly ( $\geq 75$  years of age) and non-elderly ( $< 75$  years of age) to be consistent with the secondary data analysis in Chapter 4.2. However, following slow recruitment, a revised sample size calculation was undertaken based on the analysis of the first 100 participants, revised age groups (elderly as  $\geq 70$  years of age and non-elderly as  $< 70$  years of age), and a power of 90%. This suggested 252 in each group should be sufficient to demonstrate statistical significance.

#### 3.2.5. Questionnaire

Following informed written consent, all eligible patients attending for non-emergency revascularisation procedures are asked to complete a questionnaire on admission to the GJNH (see Appendix 7.4). The questionnaire included:

Two generic QoL instruments:

**EQ-5D:** This is a widely used simple and validated general instrument to measure QoL in a standard way. This instrument is used in a wide range of conditions, treatments and

interventions. It is also used extensively in health economics to derive a quality adjusted life year (QALY). The EQ-5D descriptive system consists of five dimensions (mobility, self-care, usual activities, pain/discomfort, anxiety/depression) with three levels each (no problem, some problems, extreme problems), thus defining 243 distinct health states. Low scores on the 5 items of EQ-5D reflect a favourable assessment of each component. Responses were used to derive the single index value based on the algorithm provided by EuroQoL. This single index represents the overall health status by applying preference weights to participant responses. The index-based score is typically interpreted along a scale where 1 represents best possible health and 0 represents dead, with some health states being valued as worse than dead ( $<0$ ). In addition, EQ-5D contains a visual analogue scale (VAS). The endpoints of the VAS are labelled 'best imaginable health state' and 'worst imaginable health state', at 100 and 0, respectively. Respondents were asked to indicate how they rated their health by drawing a line from an anchor box to the point on the VAS which best represented their own health that day. Permission to use the EQ-5D was obtained from EuroQol([www.euroqol.org](http://www.euroqol.org)). This widely used QoL tool was chosen because it is simple for researchers to administer, score and interpret. It also imposes minimal burden on participants, as it is a brief, simple measure to understand and to complete (with resultant high completion rates. In addition, the generation of the utility index based health status are useful for both clinical outcomes and economic evaluation.

**SF-12:** This is a commonly used health status questionnaire and is a shortened version of the SF-36. It appears to be more sensitive to differences in health status for people with less morbidity. Like the SF-36, summary scores for physical and mental health status can be derived from the SF-12, referred to as the Physical Component Summary (PCS-12) and the Mental Component Summary (MCS-12), respectively. The summary scores of the SF-12 have also been shown to closely represent the summary scores of the SF-36.<sup>343</sup> The PCS-12 and MCS-12 scores have also been found to be virtually identical to the equivalent SF-36 summary scores in indicating the level of health and are equally sensitive to changes in health status among patients with various conditions. Permission to use the SF-12<sub>v2</sub> was obtained from QualityMetric Incorporated ([www.qualitymetric.com](http://www.qualitymetric.com)). This is the most commonly used generic QoL tool for

assessing QoL following PCI in both young and elderly patients (see Chapter 2.3). This was chosen, rather than other instruments that are thought to be more appropriate to use in older people, because the questionnaire will be used for both elderly and younger patients. In addition, it allows multiple comparisons to be made with existing literature on QoL outcomes following PCI.

**MacNew:** Disease specific QoL was assessed using the MacNew health-related QoL questionnaire. It was designed to assess how heart disease affects daily physical activities and emotional and social functioning. It has been widely used and validated in cardiology patients with AMI, angina and heart failure at the time of designing the ReQoL study. It was chosen primarily because it had not been utilised for assessing QoL following PCI in the UK. However, it has been used to assess QoL following PCI outwith the UK e.g. Sipötz *et al.*<sup>372</sup> It contains 27 items (questions), the responses to which, map to 3 domains: physical limitations, emotional function, and social functioning. Global QoL score and individual domain subscale scores can be calculated. Scores are from 1 (low QoL) to 7 (high QoL). In addition, MacNew has an established the minimal important difference (MID) of  $\geq 0.5$ . Permission to use the MacNew questionnaire was obtained from MacNew ([www.macnew.org](http://www.macnew.org)).

Frailty questions were devised based on Rockwood's 4 levels of progressive impairment in the Rockwood frailty index.<sup>187</sup> This is a brief clinical instrument that was originally developed to target patients in hospital, who may be eligible for specialised geriatric intervention. However, it has subsequently been used in community dwelling residents who self-report deficits. The 4 Rockwood levels are:

- (0) Those who walk without help, perform basic activities of daily living (eating, dressing, bathing, bed transfers), are continent of bowel and bladder, and are not cognitively impaired

- (1) bladder incontinence only
- (2) one (two if incontinent) or more of needing assistance with mobility or activities of daily living, has cognitive impairment with no dementia, or has bowel or bladder incontinence
- (3) two (three if incontinent) or more of totally dependent for transfers or one or more activities of daily life, incontinent of bowel and bladder, and diagnosis of dementia

This was used rather than the Fried Frailty Index because it does not include variables which require an independent physical assessment e.g. grip strength and walk test. There were a number of reasons for excluding physical assessments in this study, including: these assessments were not part of routine admission to day care (would require additional assessment); difficulties for patients who may wish to complete the questionnaire following their PCI (dominant hand may well be used to gain radial artery access) and concerns raised by clinicians that the walk test may not be appropriate to assess a patient attending for PCI (potential to produce angina symptoms).

Therefore, the self-reported questions included in the study questionnaire were

1. During the past 2 weeks, how much of the time did you:

Have difficulty reasoning and solving problems, for example: making plans, making decisions, learning new things?

*All of the time/ A good bit of the time/ Some of the time/ A little of the time/ None of the time*

Forget, for example things that happened recently, where you put things, your appointments?

*All of the time/ A good bit of the time/ Some of the time/ A little of the time/ None of the time*

Have difficulty doing activities involving concentration and thinking?

*All of the time/ A good bit of the time/ Some of the time/ A little of the time/ None of the time*

2. Have you ever been diagnosed with dementia? *Yes/No/Don't Know*

3. How would you describe your current living arrangements?

*Living alone, living with partner or spouse, living with family, living in a nursing home, living in supported accommodation?*

4. When you need help with tasks, can you count on someone who is willing and able to meet your demands? *Yes/No/Don't Know*

5. Have you fallen in the last 3 months? *Yes/No/Don't Know*

6. Do you have a problem with losing control of urine or your bowel when you don't want to? *Yes/No/Don't Know*

7. Have you recently lost weight such that your clothing has become looser? *Yes/No/Don't Know*

8. At times do you forget to take your prescriptions? *Yes/No/Don't Know*

Questionnaire administration was facilitated by 2 cardiac research nurses in the Golden Jubilee National Hospital. Facilitation included identifying eligible participants, explaining the study, gaining informed consent, giving out questionnaires, and assisting in completing the questionnaires (from answering questions about the completing the questionnaire to completing the questionnaire for participants unable to complete it themselves due to issues like iv access or radial catheter on dominant hand). Further questionnaires were posted to patients at 3 months with a pre-paid addressed return envelope for self-completion.

### 3.2.6. Statistical analysis

Descriptive analysis included frequencies, means, medians, Pearson's Chi-Squared tests  $\chi^2$ , t tests or Mann-Whitney for non-parametric values. Paired ttests or Willcoxon signed rank tests were used to compare before and after QoL scores in both groups. The Spearman rank correlation coefficients of the QoL score were calculated. The association between MacNew total scores and the EQ-5D index score, and the MacNew emotional and physical scores and the MCS and the PCS respectively, were assessed using Spearman's correlation by constructing a correlation matrix of the QoL questionnaire. Internal consistency reliability of the scales was assessed by calculating Cronbach's alpha coefficient using Nunnally's criterion of 0.7 or greater to indicate good internal consistency.<sup>369</sup> Test re-test reliability was not assessed as the questionnaire was completed before and after PCI; an intervention designed to have an effect on QoL. Multivariate linear regression was used to assess the differences in change of QoL following PCI between elderly and younger patients after adjusting for baseline characteristics. For this, the absolute change in QoL following PCI was used (QoL at 3 months – QoL at baseline). An alternative is to use the percentage change from baseline as a measure of relative change in QoL. While this method takes account of the baseline score and can increase comparability across subjects, it normalises the data, which may alter its distribution and may introduce additional complexity to the analysis.<sup>370</sup>

Multivariate models were developed with and without the inclusion of baseline QoL measurements as debate exists in the literature as to which is the most appropriate approach.<sup>371</sup> Models, which include baseline scores, take account of the variation in 3 months scores that arise from the variation in baseline scores. In addition, baseline QoL score is a potential confounder as it is associated with both age and changes in QoL. However there are concerns that this method may introduce regression-to-the mean bias if baseline function is measured with error.<sup>371</sup> Adjusting for baseline QoL score is the most common method used in the literature.<sup>31,291,292,</sup> Interactions were explored and goodness of fit of models were assessed using the likelihood ratio test. A p-value of <0.05 was considered statistically significant.

### **3.3. *Methods for Biological Ageing***

CAD is associated with both chronological and biological ageing processes. However, conflicting evidence exists as to whether LTL is an appropriate marker of biological ageing in CAD.

#### **3.3.1. Participants**

Participants were recruited from consecutive patients attending the West of Scotland Regional Heart and Lung Centre at the GJNH for non-emergency coronary angiography between October 2011 and August 2012.

Inclusion criteria were: patients attending electively (as in-patients or day cases) for coronary angiography.

As ACS is associated with an acute inflammatory response, which could have an effect on measurement of biomarkers and inflammatory markers, patients were excluded if they were <48 hours following an acute ACS event. Additional exclusion criteria included: patients who were unwilling or unable to provide informed consent and those with insufficient English comprehension to complete the consent form.

Potential participants were approached by the interventional cardiologist to answer any questions regarding the study and to complete the consent form; this was undertaken at the same time as routinely consenting the patient for the coronary angiogram. Participant information was collated and transferred to the Western Infirmary and via the hospital postal system. Manual collection from the Western Infirmary to the University of Glasgow Public Health Department was undertaken on a weekly basis. Participant details were imputed into an access database held on the University of Glasgow secured server by an experienced professional administrator.

Baseline participant characteristics were retrieved by data linkage with the Cardiac, cardiology and Thoracic Health Information system (CaTHI). Data linkage was deterministic in nature. A deterministic data linkage requires a high quality formal identifier common to both datasets to

exactly match between the datasets. In this study CHI was used as the unique identifier. In addition, date of procedure allowed collection of information for the correct coronary angiography event (given that participants may have multiple entries). Where deterministic linkage was unable to match for CHI; manual case record search was undertaken by the cardiac research nurses (n=3). CaTHI provides clinical information for all cardiac surgery, cardiology, and thoracic specialties. It captures data throughout the full patient journey from assessments, surgical procedures to discharges, and follow-ups. It is used for administrative processes and assists with the monitoring of clinical performance, waiting times, and facilitates research. CaTHI collects data similar to the SCRR.

Data are entered prospectively by a combination of clinical and administrative staff according to a predefined set of standardised data definitions. Information collected includes: demography (including age, gender, and deprivation); comorbidity (e.g. previous history of MI, stroke, chronic lung disease, diabetes, and hypertension); clinical presentation (e.g. indication for PCI, procedural priority); CAD severity (using CCS).

### 3.3.2. Definitions

Baseline definitions were the same as those within SCRR (see Chapter 3.1.2).

Severity of CAD was assessed in 2 different ways. The first was similar to SCRR where CAD was defined as angiographic evidence of significant stenosis ( $\geq 70\%$  diameter stenosis of at least one major epicardial artery segment or  $\geq 50\%$  diameter stenosis in the left main coronary artery). MVD was defined as significant stenoses in two or more coronary arteries. MVD with left main stem (LMS) involvement was allocated to a separate category.

The second used the SYNTAX score.<sup>149</sup> Two independent interventional cardiologists reviewed and coded the angiographies using the SYNTAX scoring system. They were both blinded to the study outcomes.

In addition, if a follow on PCI was undertaken then procedural details (type of lesion, length of lesion, diameter of treated vessel, type of stent) are also recorded in the CaTHI system.

### 3.3.3. Power calculation

Aviv *et al.*<sup>92</sup> used data from the UK Twins Study to study sample size and power issues with regard to telomere attrition rates. Their study provides sample sizes for research into ageing involving telomere attrition. Their results suggest that for patients in the age-group of those undergoing coronary angiography, if we wish to demonstrate as statistically significant at the 5% level and achieve 95% power, with a conservative difference of only 10 base pairs per annum we require at least 525 patients in each group. Applying a 25% margin of error produces a figure of 656 in each group. From the Scottish Coronary Revascularisation Register we know that ~40% of patients undergoing elective diagnostic coronary angiography will be found to have normal coronary arteries. Therefore, we planned to recruit 1802 patients prior to catheterisation with a view to analysing the blood samples of 656 with normal coronary arteries and the first 656 of the 1,531 with CAD. From the register, we knew that there are 5392 coronary angiograms performed per annum at the Golden Jubilee National Hospital. Some 2476 are recorded as being “elective” and therefore would be eligible for recruitment (given that the inclusion criteria for this study includes >48hrs post ACS - this is a conservative estimate as these aren’t usually defined or coded as “elective”). Therefore, assuming a recruitment rate of 88%, we estimated that it would take around one year to recruit the required number of participants.

However, recruitment reached the appropriate predetermined sample size by 11 months.

### 3.3.4. Telomere length determination

Participant blood was drawn by the interventional cardiologist, after artery sheath insertion and before administration of any drugs e.g. heparin. A 20 ml syringe was used, which was handed to the assisting catheterisation laboratory nurse. From this syringe, 4 vacutainer tubes were filled (to allow for analysis of DNA (EDTA), plasma, RNA, and serum) and mixed well by inverting the samples 10 times. Samples were labeled using patients CHI sticky labels and transferred to a dedicated research fridge. On a twice-daily basis, the

cardiology research nurses checked the participant blood samples for labeling accuracy and centrifuged the serum and plasma samples prior to transferring them to a dedicated research -20° freezer. All samples were transferred from the GJNH to the Shiels lab on a weekly basis in specialized containers to maintain sample temperatures. The EDTA tube containing 5mls of blood was used for the LTL analysis.

Laboratory analysis of telomere length was undertaken by Shiels lab based in the University of Glasgow department at the NHS Western Infirmary. The lab was blinded to the outcomes of participants for analysis and only age and sex was provided post analysis for validation purposes.

DNA was extracted from peripheral blood leucocytes using the Maxwell automated purified system (Promega, WI, USA). Telomere length determination was performed blindly using a Roche Light Cycler LC480 (Roche Diagnostics, Indianapolis, Indiana, USA) by qPCR. Analyses were undertaken in triplicate for each sample, using a single-copy gene amplicon primer set (acidic ribosomal phosphoprotein, 36B4) and a telomere-specific amplicon primer set. A standard deviation (SD) of 0.15 of the threshold cycle (Ct) for sample replicates was used as a cut-off for quality control parameters for the amplifications. The Ct is the intersection between an amplification curve and an amplification threshold line. Its value indicates the fractional PCR cycle number for which the fluorescence is greater than the threshold. Samples were reanalysed at a SD above 0.15. The average SD across plates was 0.05. When telomere and control gene assays resulted in similar amplification efficiencies, relative telomere length was estimated from Ct scores using the comparative Ct method. This method determined the ratio of telomere repeat copy number to single copy gene number (T/S) ratio in experimental samples relative to a control sample DNA. The normalized T/S ratio was used as the estimate of relative telomere length (Relative T/S). The interassay variation was tested by comparing relative T/S ratios across assays for the positive controls on every assay plate. The average inter-assay CoV was 0.51% for telomere length and 0.18% for 36B4. The average of triplicates was used for further analysis. This has shown to be an effective measure of the average telomere length. For a copy of the full laboratory protocol see Appendix 7.5.

### 3.3.5. Statistical analysis

Using the Categorical baseline data displayed in Table 16 were analysed using the Pearson's  $\chi^2$  test or Fisher's exact test where any cell had an expected count of <1 or >20% of cells <5. Those with and without CAD were analysed using t-tests for independent samples assuming unequal variance. Comparisons between patients with one-, two- and three-vessel disease were made using one-way analysis of variance (ANOVA). Adjusted mean differences in T/S ratio were calculated using multiple linear regression models. Models were constructed and interactions explored using dependent variables e.g. age, sex, current smoking status, deprivation, comorbidities, angina severity, obesity, and family history. The data were inspected visually; with the assumption of parallel lines for the relationship between the dependent variables and covariates. Thus the values quoted are the coefficients for the difference between those with and without CAD after adjustment for age, sex, smoking status, and level of deprivation. All continuous results are expressed as mean (standard deviation) unless otherwise stated. Statistical significance was accepted when  $P < 0.05$ .

### 3.3.6. Ethical considerations

Ethical approval for this study was granted from the West of Scotland Ethics Committee (REC 5) and was supported by the GJNH Research and Development department (see Appendix 7.6).

Ethical considerations for patients were carefully considered and these included:

- Taking part in this study had no impact on clinical care as it involved taking 20mls of blood through the artery access sheath which is used routinely for coronary angiography.
- The research involved NHS patients and included taking a blood sample

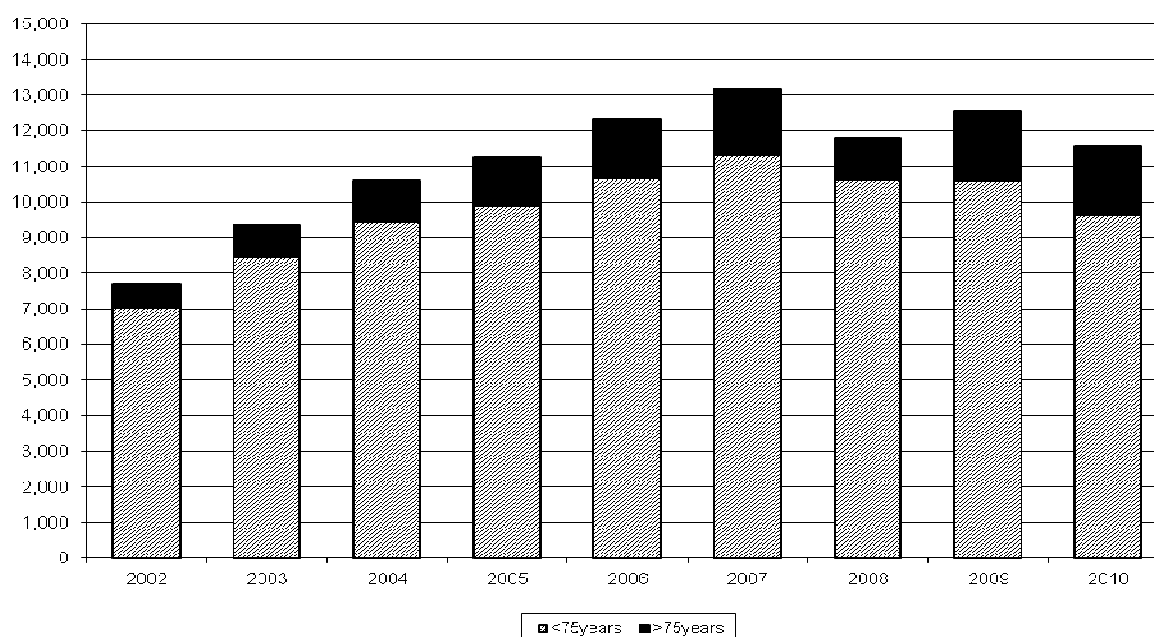
- Participation in this research did not include an additional burden for patients as blood was drawn at the same time as artery access; therefore, no additional needles were required.
- Confidentiality – A top sheet containing participant details (name, date of birth, date of procedure, and CHI number) was sent directly to the University of Glasgow Public Health Department via the NHS internal mail system and collected in person on a weekly basis. This information was entered into a Microsoft Access database by a qualified professional administrator. This allowed continuous monitoring of recruitment levels. It also provided the information required for data linkage to both the Shields lab data and the CaTHI system. The Access database was password protected on University of Glasgow computers and kept secure in the department of Public Health. Following linkage, CHI and participant names were removed prior to statistical analysis using STATA.
- Informed consent – Information studies being undertaken at the GJNH are provided in the pre-admission information provided by the GJNH. It routinely states that: “As a regional centre for the West of Scotland in the treatment of heart disease, the Golden Jubilee Hospital participates in a number of research studies”” The interventional cardiologist who was undertaking the coronary angiography explained the study and gained written consent (Appendix 7.7). All participants were provided with a participant information sheet (see Appendix 7.8).

## 4. RESULTS

### 4.1. *Diagnostic coronary angiography among elderly versus younger patients.*

#### 4.1.1. Number of coronary angiograms

Over the nine-year period (2001-2010), 126,726 non-emergency coronary angiograms were performed on 101,057 patients in Scotland (total population around 5.2 million). Of the 101,057 first procedures 13,344 (13.2%) were performed on elderly patients. The overall number of angiograms performed each year increased by 60% from 7,714 in 2001 to 13,175 in 2007 (Figure 13).



**Figure 13:** Number of angiography procedures per annum by age group

Thereafter, the number fell slightly to 11,567 in 2010 mainly due to a reduction in angiograms among younger patients (a similar trend was found when patients with multiple coronary angiograms were included). The absolute number of coronary angiograms performed on elderly patients increased from 669 in 2001 to 1,945 in 2010. The elderly accounted for an increasing proportion of coronary angiograms: from 8.7% in 2001 to 16.8% to 2010 ( $\chi^2$  test for trend,  $p<0.0001$ ). The incidence of coronary angiography among the elderly general population increased three-fold from 198/100,000 per annum in 2001 to 629/100,000 per annum in 2008. In comparison, among the general population aged 35 to 74 years, the incidence of coronary angiography increased by only 56% from 284/100,000 per annum to 444/100,000 per annum.

#### 4.1.2. Case-mix

Compared with younger patients, the elderly were more likely to be female, have severe (CCS grade IV) angina, have multiple co-morbidities and have a past medical history of AMI, PCI or CABG (Table 3).

Table3. Characteristics of patients undergoing diagnostic coronary angiography by age-group

	≥75 years	<75 years	
	n (%)	n (%)	
	<b>n=13,344</b>	<b>n=87,713</b>	<b>P value*</b>
Male	7,507 (56.3)	55,758 (63.6)	<0.001
Current smoker	3,820 (37.8)	33,823 (52.6)	<0.001
CCS angina score			
0 (none)	1,730 (18.8)	10,205 (17.1)	<0.001
I	1,632 (17.7)	16,480 (27.5)	
II	2,033 (22.1)	14,064 (23.5)	
III	1,264 (13.7)	6,571 (11.0)	
IV (severe)	2,542 (27.6)	12,514 (20.9)	<0.001
Procedural urgency			
Urgent	5,240 (38.5)	25,928 (29.1)	
Routine	8,103 (60.7)	61,774 (70.4)	<0.001
Obese	1,359 (18.1)	15,853 (33.1)	<0.001
Diabetes mellitus	1,510 (14.7)	9,553 (14.6)	0.911
Hypertension	5,753 (55.7)	30,019 (46.1)	<0.001
Extra-cardiac arteriopathy	1,551 (15.4)	5,979 (9.4)	<0.00
Renal impairment	294 (2.9)	949 (1.5)	<0.001
Left ventricular dysfunction	2,246 (50.4)	12,386 (40.3)	<0.001
Multiple comorbidity	2,714 (20.3)	12,981 (14.8)	<0.001
Previous AMI	3,053 (29.6)	15,784 (24.1)	<0.001

	<b>≥75 years</b>	<b>&lt;75 years</b>	<b>P value*</b>
	<b>n (%)</b>	<b>n (%)</b>	
	<b>n=13,344</b>	<b>n=87,713</b>	
Previous PCI	814 (8.0)	3,036 (4.7)	<0.001
Previous CABG	1,121 (10.7)	4,633 (7.0)	<0.001
Family history CAD	1,945 (14.6)	23,493 (26.8)	<0.001
Deprivation (SIMD)			
Most deprived quintile	2,333 (17.9)	22,001 (25.6)	<0.001
Least deprived quintile	2,800 (21.4)	13,496 (15.7)	<0.001

\*Pearson's  $\chi^2$  test

CCS Canadian Cardiovascular Society scoring; AMI: acute myocardial infarction; PCI percutaneous coronary intervention; CAD coronary artery disease; SIMD: Scottish Index of Multiple Deprivation. CABG: coronary artery bypass grafting

In addition, elderly patients were less likely to live in the most deprived SIMD quintile, be a current smoker, be obese; or report a family history of CAD. At coronary angiography, elderly patients were more likely to have significant coronary stenoses detected (76.1% vs 63.51%,  $p<0.001$ ) and more likely to have MVD with or without LMS (39.3% vs 27.1%,  $p<0.001$  and 13.0% vs 5.9%,  $p<0.001$ , respectively) (Table 4).

#### 4.1.3. Adverse outcomes

Overall (diagnostic only plus follow-on), angiography was associated with a low crude rate of peri-procedural complications which occurred in 1.7% of patients. By 30 days follow-up, 1.1% of patients had suffered a MACE; 0.5% had suffered an AMI, 0.1% a cerebrovascular event, and 1.0% had died. The elderly were more likely, than their younger counterparts, to have a peri-procedural complication (2.0% vs 1.6%,  $p<0.001$ ) and a MACE (2.9% vs 0.8%,  $p<0.001$ ) within 30 days of their angiogram.

Among all patients who underwent isolated coronary angiography, the elderly group experienced similar rates of peri-procedural complications (1.4% vs 1.2%,  $p=0.168$ ) but higher rates of AMI (1.1% vs 0.2%,  $p<0.001$ ), death (2.3% vs 0.6%,  $p<0.001$ ) and MACE (2.6% vs 0.7%,  $p<0.001$ ) within 30 days. In those without significant CAD at angiography, peri-procedural complications and adverse 30-day outcomes were rare but still more common in the elderly (peri-procedural complication: 1.9% elderly vs 1.1% young,  $p<0.001$ ; and MACE: 1.2% vs 0.4%,  $p<0.001$ ). In those with significant CAD, peri-procedural complications following isolated angiography were generally no more common in elderly patients regardless of severity of disease. However, adverse 30-day outcomes were more common (Table 4).

**Table 4.** Comparison of crude thirty day outcomes by type of procedure, age-group and coronary angiographic findings.

	Diagnostic coronary angiography only,								
	no PCI /CABG within 30 days.			Follow on PCI			Any revascularization within 30 days		
	n=36,115			n=19,857			n=26,230		
	n (%)			n (%)			n (%)		
	≥75	<75	P value	≥75	<75	P value*	≥75	<75	P value*
<b>NO CAD</b>									
Peri-procedural complications	59 (1.9)	363 (1.1)	<0.001						
All-cause death	34 (1.1)	107 (0.3)	<0.001						
AMI	11 (0.4)	15 (0.1)	<0.001						
CVA/TIA	4 (0.1)	28 (0.1)	0.355						
Death, AMI or CVA/TIA	38 (1.2)	129 (0.4)	<0.001						
<b>SVD</b>									
Peri-procedural complications	17 (1.0)	363 (1.1)	0.258	45 (4.2)	262 (2.4)	<0.001	51 (3.9)	297 (2.4)	0.001
All-cause death	29 (1.7)	107 (0.3)	<0.001	13 (1.2)	41 (0.4)	<0.001	24 (1.9)	64 (0.5)	<0.001
AMI	14 (0.8)	15 (0.1)	<0.001	8 (0.8)	31 (0.3)	0.011	12(0.9)	42 (0.3)	0.001
CVA/TIA	10 (0.6)	28 (0.1)	<0.001	1 (0.1)	12 (0.1)	0.674	1 (0.1)	15 (0.1)	0.554
Death, AMI or CVA/TIA	38 (2.2)	129 (0.4)	<0.001	136 (1.2)	51 (0.5)	0.001	24 (1.9)	77 (0.6)	<0.001

	Diagnostic coronary angiography only, no PCI /CABG within 30 days.			Follow on PCI			Any revascularization within 30 days		
	n=36,115			n=19,857			n=26,230		
	n (%)			n (%)			n (%)		
MVD without LMS									
Peri-procedural complications	44 (1.3)		0.343	60 (4.7)	212 (3.5)	0.033	65 (3.6)	252 (3.0)	0.199
All-cause death	90 (2.7)	119 (0.8)	<0.001	49 (3.8)	79 (1.3)	<0.001	66 (3.7)	114 (1.4)	<0.001
AMI	46 (1.4)	55 (0.4)	<0.001	25 (2.0)	56 (0.9)	0.001	35 (2.0)	75 (0.9)	<0.001
CVA/TIA	6 (0.2)	21 (0.1)	0.629	8 (0.6)	7 (0.1)	<0.001	10 (0.6)	7 (0.1)	<0.001
Death, AMI or CVA/TIA	95 (2.9)	139 (1.0)	<0.001	57 (1.9)	85 (1.4)	<0.001	76 (4.3)	120 (1.5)	<0.001
LMS	1,145(25.6)	3,334(74.4)		157(31.3)	344(68.7)		548(24.7)	1,670(75.3)	
Peri-procedural complications									
All-cause death	13 (1.1)	45 (1.3)	0.579	10 (6.4)	22 (6.4)	0.991	17 (3.1)	50 (3.0)	0.898
AMI	63 (5.5)	82 (2.5)	<0.001	13 (8.3)	16 (4.7)	0.107	38 (6.9)	46 (2.8)	<0.001
CVA/TIA	31 (2.7)	49 (1.5)	0.006	11 (7.1)	14 (4.1)	0.161	21 (3.9)	31 (1.9)	0.008
Death, AMI or CVA/TIA	1 (0.1)	8 (0.2)	0.463	1 (0.2)	0 (0.0)	0.313	1 (0.2)	1 (0.1)	0.433
	64 (5.6)	89 (2.7)	<0.001	14 (9.0)	16 (4.7)	0.062	39 (7.2)	47 (2.9)	<0.001

	Diagnostic coronary angiography only, no PCI /CABG within 30 days.			Follow on PCI			Any revascularization within 30 days		
	n=36,115			n=19,857			n=26,230		
	n (%)			n (%)			n (%)		
Overall									
Peri-procedural complications	133 (1.4)	728 (1.2)	0.049	115 (4.6)	496 (2.9)	<0.001	133 (3.7)	599 (2.7)	<0.001
All-cause death	216 (2.3)	363 (0.6)	<0.001	75 (3.0)	136 (0.8)	<0.001	128 (3.5)	224 (1.0)	<0.001
AMI	102 (1.1)	144 (0.2)	<0.001	44 (1.8)	101 (0.6)	<0.001	68 (1.9)	148 (0.7)	<0.001
CVA/TIA	21 (0.2)	65 (0.1)	0.002	10 (0.4)	19 (0.1)	<0.001	12 (0.3)	23 (0.1)	<0.001
Death, AMI or CVA/TIA	235 (2.5)	422 (0.7)	<0.001	84 (3.4)	152 (0.9)	<0.001	139 (3.9)	244 (1.1)	<0.001
*Pearson's $\chi^2$ test . AMI: acute myocardial infarction; PCI: percutaneous coronary intervention; CVA/TIA: cerebrovascular accident/transient ischaemic attack; CABG: coronary artery bypass grafting; MVD: multi-vessel disease; SVD: single vessel disease; CAD: coronary artery disease									

Overall follow-on PCI was associated with a higher crude risk of adverse outcomes than isolated coronary angiography (peri-procedural complications 3.1% vs 1.4%,  $p<0.001$ ; 30 day MACE 1.5% vs 1.2%,  $p=0.012$ ). In those undergoing follow-on PCI for SVD and MVD without LMS, differences in adverse outcomes between elderly and younger patients were similar to that seen for isolated coronary angiography. Elderly patients experienced higher rates of AMI, MACE and all-cause death within 30 days of follow-on PCI. While crude 30-day MACE outcomes appeared more common among elderly, compared with younger, patients with LMS (9.0% vs 4.7%,  $p=0.062$ ), this was not found to be statistically significant.

In the univariable logistic regression analysis (Table 5), being elderly was associated with an increased risk of peri-procedural complications in those patients with no significant stenosis (OR 1.64, 95% CI: 1.24, 2.17,  $p<0.001$ ), but this did not persist after adjustment for potential confounding factors (Table 1) (adjusted OR 0.86, 95% CI: 0.34, 2.05,  $p=0.159$ ). Potential confounders included in the model were those, which were prospectively collected by the SCRR, and were associated with age and the outcome of interest: sex, smoking status, diabetes, hypertension, non-cardiac vascular disease, renal impairment, left ventricular dysfunction, cardiogenic shock, co-morbidity, disease severity, deprivation quintile, and year of procedure. The covariates which reduced the estimates of the risk of peri-procedural in patients with no significant stenosis, for elderly compared to younger persons when included in the model as potential confounders, were left ventricular dysfunction, renal disease, and final access site. In those with SVD or LMS, univariable analysis suggested that being elderly was associated with an increased risk of all-cause death, AMI and having a MACE. However, after adjusting for potential confounders (see above), being elderly was not found to be associated with adverse outcomes in these patients. In those with MVD, being elderly was associated with an increased risk of all-cause death, AMI, CVA/TIA and composite outcomes. Risk of all-cause death, CVA/TIA and composite outcomes remained statistically significant following adjustment. The covariates which reduced the estimates of the risk of MACE outcomes in patients with SVD stenosis, for elderly compared to younger persons when included in the model as potential confounders, were left ventricular dysfunction, renal disease, and diabetes. In MVD and LMS left ventricular dysfunction, renal disease, diabetes, and smoking reduced the estimates most.

**Table 5.** Complication rates and any revascularisation rates for those with CAD: Overall (elderly referent to young):

	Univariable			Multivariable*		
	OR/HR	95% CI	P value	OR/HR	95% CI	P value
<b>No significant stenosis &lt;75%</b>						
Peri-procedural complications	1.64	1.24, 2.17	<0.001	0.86	0.34, 2.05	0.159
All-cause death within 30 days	3.21	2.18, 4.73	<0.001	1.49	0.56, 3.53	0.155
AMI within 30 days	2.01	1.24, 2.77	<0.001	1.82	0.85, 4.48	0.916
CVA/TIA within 30 days	1.67	0.58, 4.83	0.341	1.04	0.02, 47.51	0.984
Death, AMI or CVA/TIA within 30 days	2.98	2.07, 4.29	<0.001	2.20	0.32, 15.08	0.422
<b>SVD</b>						
Peri-procedural complications	1.22	0.94, 1.58	0.126	1.47	0.91, 2.38	0.114
All-cause death within 30 days	3.66	2.64, 5.08	<0.001	1.57	0.52, 4.76	0.420
AMI within 30 days	3.17	2.01, 5.00	<0.001	2.12	0.58, 7.77	0.256
CVA/TIA within 30 days	3.31	1.64, 6.69	0.001	0.30	0.02, 5.47	0.415
Death, AMI or CVA/TIA within 30 days	3.60	2.66, 4.86	<0.001	1.14	0.39, 3.36	0.811

	Univariable			Multivariable*		
	OR/HR	95% CI	P value	OR/HR	95% CI	P value
<b>SVD</b>						
PCI within 30 days	0.66	0.61, 0.71	<0.001	0.70	0.56, 0.86	0.001
CABG within 30 days	0.71	0.66, 0.76	<0.001	0.55	0.13, 2.34	0.424
Any revascularisation within 30 days	1.93	1.55, 2.40	<0.001	0.69	0.56, 0.86	0.001
PCI within 1 year	0.71	0.66, 0.76	<0.001	0.62	0.50, 0.78	<0.001
CABG within 1 year	1.93	1.55, 2.40	<0.001	0.90	0.59, 1.39	0.643
Any revascularisation within 1 year	0.69	0.64, 0.75	<0.001	0.55	0.43, 0.71	<0.001
<b>MVD without LMS</b>						
Peri-procedural complications	1.17	0.95, 1.45	0.144	1.00	0.62, 1.63	0.978
All-cause death within 30 days	3.05	2.48, 3.74	<0.001	1.82	1.03, 3.20	0.038
AMI within 30 days	2.82	2.13, 3.73	<0.001	1.68	0.81, 3.47	0.163
CVA/TIA within 30 days	2.55	1.38, 4.72	0.003	5.01	1.09, 22.9	0.038
Death, AMI or CVA/TIA within 30 days	3.03	2.49, 3.68	<0.001	2.05	1.21, 3.46	0.007
PCI within 30 days	0.92	0.86, 0.99	0.020	0.91	0.78, 1.06	0.242
CABG within 30 days	1.07	0.94, 1.21	0.317	0.97	0.64, 1.45	0.868
Any revascularisation within 30 days	0.95	0.89, 1.01	0.088	0.90	0.77, 1.06	0.208
PCI within 1 year	0.82	0.77, 0.87	<0.001	0.92	0.79, 1.08	0.331
CABG within 1 year	0.72	0.67, 0.77	<0.001	0.73	0.60, 0.89	0.002

	Univariable			Multivariable*		
	OR/HR	95% CI	P value	OR/HR	95% CI	P value
<b>MVD without LMS</b>						
Any revascularisation within 1 year	0.62	0.58, 0.66	<0.001	0.70	0.58, 0.85	<0.001
<b>LMS</b>						
Peri-procedural complications						
All-cause death within 30 days	2.41	1.85, 3.16	<0.001	1.80	0.61, 5.28	0.288
AMI within 30 days	1.95	1.37, 2.78	<0.001	1.06	0.27, 4.17	0.935
CVA/TIA within 30 days	0.66	0.14, 3.04	0.592	0.73	0.03, 15.17	0.837
Death, AMI or CVA/TIA within 30 days	2.32	1.79, 3.01	<0.001	1.46	0.50, 4.25	0.488
PCI within 30 days	1.40	1.18, 1.67	<0.001	1.59	1.09, 2.34	0.018
CABG within 30 days	0.79	0.69, 0.90	<0.001	0.97	0.60, 1.59	0.912
Any revascularisation within 30 days	0.96	0.85, 1.07	0.448	1.48	1.05, 2.10	0.027
PCI within 1 year	1.07	0.92, 1.26	0.367	1.30	0.90, 1.89	0.163
CABG within 1 year	0.51	0.45, 0.57	<0.001	0.49	0.34, 0.70	<0.001
Any revascularisation within 1 year	0.50	0.44, 0.56	<0.001	0.58	0.38, 0.89	0.012

OR: odds ratio, used for 30 day outcomes; HR: hazards ratio, used for 1 year outcomes; CI: confidence interval; AMI: acute myocardial infarction; PCI: percutaneous coronary intervention; CVA/TIA: cerebrovascular accident/transient ischaemic attack.

\*adjusted for sex, smoking status, diabetes, hypertension, non-cardiac vascular disease, renal impairment, left ventricular dysfunction, cardiogenic shock, co-morbidity, disease severity, deprivation quintile, year of procedure, and final access site.

#### 4.1.4. Management of patients with confirmed coronary artery disease

In the univariable analyses, among those patients in whom angiography demonstrated evidence of CAD, the elderly were less likely to proceed to follow-on PCI than younger patients (24.9% vs 31.4%,  $p<0.001$ ) and less likely to undergo revascularisation within one year, irrespective of the severity of CAD found at diagnostic angiography (60.1% vs 68.8%,  $p<0.001$ ) (presented by SVD, MVD, and LMS in Table 6). As shown in Table 6, those with SVD, elderly patients were more likely to undergo CABG by 1 year than younger patients but this was offset by a lower number undergoing PCI; resulting in a lower overall use of revascularisation (61.9% vs 70.1%,  $p<0.001$  at 1 year). Among those with LMS, elderly patients were more likely to undergo early but not later PCI than younger patients (11.1% vs 8.0%,  $p<0.001$  at 30 days and 13.8% vs 13.0%,  $p=0.429$  at 1 year). This was offset by a lower percentage undergoing CABG, resulting in a lower overall use of revascularisation at 1 year (61.2% vs 76.0%,  $p<0.001$ ) (presented by SVD, MVD, and LMS in Table 6). Among those with MVD excluding LMS, elderly patients were less likely to undergo both PCI and CABG.

In the multivariable analyses, elderly patients with evidence of CAD were less likely than younger patients to have had a revascularisation procedure by one year (OR 0.68, 95% CI 0.65–0.71,  $p<0.001$ ) even after adjusting for baseline characteristics (adjusted OR 0.60, 95% CI 0.52–0.69,  $p<0.001$ ) (data not shown in Table 6). This was found to be the case regardless of severity of disease (Table 6).

**Table 6.** Comparison of revascularization rates following diagnostic coronary angiography by age-group and angiographic findings

	≥75 years n=9,853	<75 years n=52,492	Total 62,345	P value
<b>SVD</b>	<b>3,035 (11.0)</b>	<b>24,625 (89.0)</b>	<b>27,660</b>	
Follow on PCI	1,067 (35.7)	10,881 (44.2)	11,948	<0.001
PCI within 30 days (including FO)	1,076 (35.5)	11,267 (45.8)	12,343	<0.001
Any revascularization within 30 days	1,296 (42.7)	12,635 (51.3)	13,931	<0.001
PCI within 1 year (including FO)	1,379 (45.4)	14,568 (59.2)	15,947	<0.001
CABG within 1 year	414 (13.6)	2,060 (8.4)	2,474	<0.001
Any revascularization within 1 year	1,880 (61.9)	17,263 (70.1)	19,143	<0.001
<b>MVD without LMS</b>	<b>5,125 (18.3)</b>	<b>22,863 (81.7)</b>	<b>27,988</b>	
Follow on PCI	1,279 (25.0)	6,129 (26.8)	7,408	0.007
PCI within 30 days (including FO)	1,327 (25.9)	6,370 (27.9)	7,697	0.004
Any revascularization within 30 days	1,793 (35.0)	8,288 (36.3)	10,081	0.088

	≥75 years	<75 years	Total	P value
	n=9,853	n=52,492	62,345	
<b>MVD without LMS</b>				
PCI within 1 year (including FO)	1,736 (33.9)	8,949 (39.1)	10,685	<0.001
CABG within 1 year	1,257 (24.5)	7,102 (31.1)	8,359	<0.001
Any revascularization within 1 year	3,077 (60.0)	16,177 (70.8)	19,254	<0.001
<b>LMS</b>	<b>1,693 (25.3)</b>	<b>5,004 (74.7)</b>	<b>6,697</b>	
Follow on PCI	157 (9.3)	344 (6.9)	501	0.001
PCI within 30 days (including FO)	188 (11.1)	402 (8.0)	590	<0.001
Any revascularization within 30 days	548 (32.4)	1,670 (33.4)	2,218	0.448
PCI within 1 year (including FO)	233 (13.8)	651 (13.0)	884	0.429
CABG within 1 year	803 (47.4)	3,201 (64.0)	4,004	<0.001
Any revascularization within 1 year	1,036 (61.2)	3,801 (76.0)	4,837	<0.001

\*Pearson's  $\chi^2$  test . AMI: acute myocardial infarction; PCI: percutaneous coronary intervention; CVA/TIA

## Strengths and Limitations

Data for this study was based on a national register and, therefore, included all patients who underwent coronary angiography over the study period. The registry data are detailed and comprehensive,<sup>373</sup> and are collected prospectively by staff at the patient's hospital. In Scotland, the data on outcomes are obtained via linkage to routine hospital and death records which have been shown to be as complete and accurate as those obtained using conventional follow-up methods. The vast majority of patients were successfully linked to the follow-up databases (99%), which is in keeping with similar studies using similar registry data.<sup>256</sup> Previous studies and quality assurance exercises, suggest that systematic bias is unlikely. Only 1% could not be linked and there was no reason to suspect a systematic bias in successful linkage. In addition, post hoc analysis confirmed no significant statistical differences according to whether or not linkage was achieved.

This section of the thesis utilised statistical methods to adjust for known differences in baseline characteristics between elderly and younger patients. However, residual confounding due to unknown or unrecorded differences cannot be excluded. This is a recognised limitation of all observational studies. In any cohort study, there is always the potential for loss to follow-up due to, for example, emigration from Scotland. Linkage with SMR01 and GROS would not provide admission data for events and deaths occurring outside Scotland. However, this is unlikely to impact significantly on the early (30 day) complications reported here.

Age-stratified general population incidence rates were based on a single year being used as the denominator – 2006. This could have resulted in a modest underestimate of the incidence rate as the elderly probably increased in the overall population during the study period.

#### 4.1.5. Discussion of the main findings

The total numbers and rates of coronary angiography undertaken in Scotland have increased until the mid 2000s. This may be explained by increasing prevalence of CAD and changes in the threshold for investigation by coronary angiography. Elderly patients accounted for an increasing number and proportion of first diagnostic coronary angiograms, over this time period. Age-stratified general population incidence rates of coronary angiography increased more in the elderly group, suggesting the threshold for investigation by coronary angiography may have decreased over time. Compared with younger patients, the elderly were more likely to have severe angina, angiographic evidence of any CAD, or MVD with or without LMS, suggesting that the threshold for investigation is probably higher.

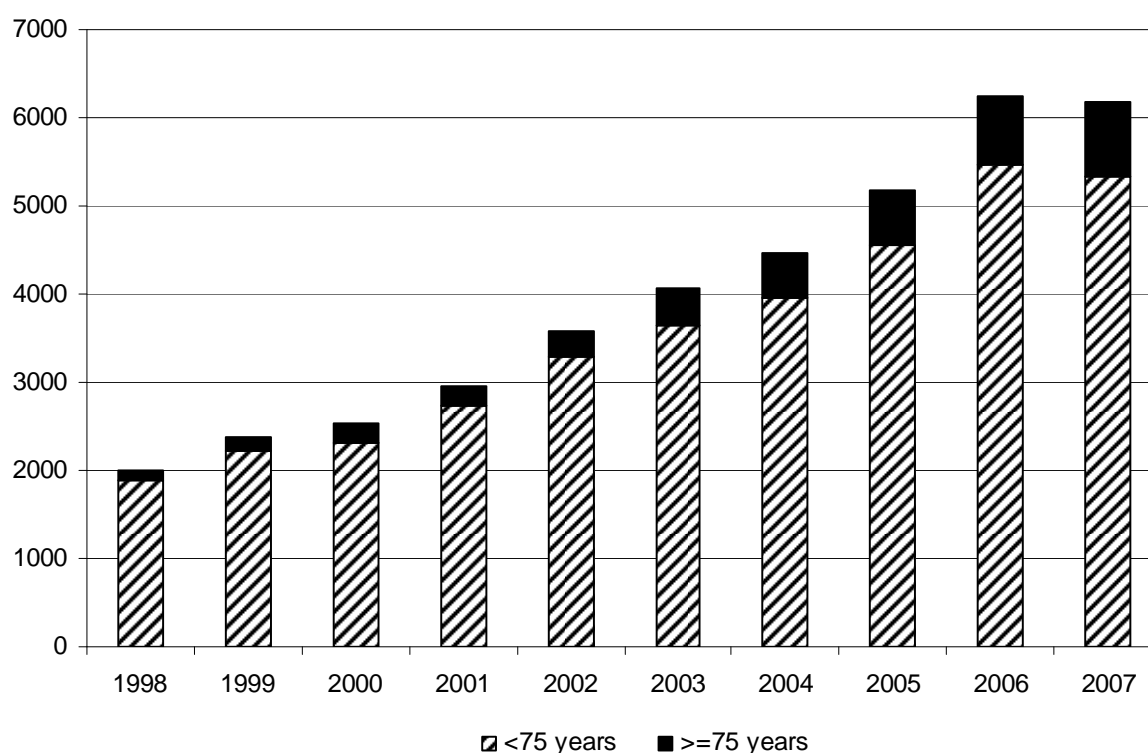
Elderly patients had a higher risk of early complications but their crude risk was nonetheless low (2.0% of elderly patients suffered complications, compared with 1.6% of young patients ( $p<0.001$ ) suggesting that coronary angiography is a safe procedure to perform in the elderly. This suggests that concerns about investigating elderly patients with suspected CAD using coronary angiography may not be justified.

Elderly patients were less likely to proceed to revascularisation, either as a follow-on procedure, or over the subsequent year, suggesting a higher threshold for intervention even among those investigated. Further investigation of this apparent higher threshold for revascularisation in elderly patients has been undertaken in this thesis by examining PCI data from the SCRR in more depth (the findings of which are presented below in Chapter 4.2). In addition, trends have been examined to assess changes over time in case-mix in patients undergoing PCI.

## 4.2. PCI in the elderly: changes in case-mix and periprocedural outcomes

### Overall comparison

Of the 46,774 patients who underwent PCI in Scotland between 1998 and 2008, 40,933 (87.6%) were classed as non-emergencies. Of these, 4,544 (11.1%) were performed in elderly patients and 36,387 (88.9%) in younger patients. Compared with younger patients, the elderly were more likely to be female, have MVD disease, have multiple comorbidity, and have a past medical history of AMI or CABG (Table 7).



**Figure 14.** Numbers of non-emergency percutaneous coronary interventions per annum by age-group.

Of the 40,933 patients, 5,912 (14.4%) were excluded from the outcome analysis because of missing or incomplete follow-up data. There were no significant differences between those included and excluded in terms of age, sex, SIMD quintile, disease severity or presence of comorbidities. In the remaining cohort of 35,021 (85.6%) patients, the overall crude risk of MACE within 30 days of PCI was 4.5% in the elderly

compared with 2.8% in younger patients ( $\chi^2$  test,  $p<0.001$ ). In the logistic regression model, the increased risk among the elderly (unadjusted OR 1.61, 95% CI 1.30–1.91,  $p<0.001$ ) was attenuated after adjustment for differences in case-mix, but remained statistically significant (adjusted OR 1.36, 95% CI 1.07–1.75,  $p<0.001$ ).

### Time-trends

The overall number of non-emergency PCI procedures performed each year tripled, from 2,009 in 1998 to 6,244 in 2006 (Figure 14). Thereafter, the number fell slightly to 6,180 in 2008, mainly due to a reduction in procedures among younger patients. The absolute number of PCIs performed on the elderly increased from 101 in 1998 to 792 in 2008. The incidence of PCI among the elderly general population increased from 29/100,000 per annum in 1998 to 219/100,000 per annum in 2008. Among the general population aged 35-74 years, the incidence of PCI increased from 71/100,000 per annum to 173/100,000 per annum.

The elderly accounted for an increasing proportion of non-emergency PCIs, from 2.5% in 1998 to 11.1% in 2008 ( $\chi^2$  test for trend,  $p<0.0001$ ). Among elderly patients, there was a significant increase in the prevalence of all comorbid conditions, other than obesity (Table 8).

**Table 7.** Comparison of characteristics of patients undergoing non-emergency percutaneous coronary intervention by age-group.

	≥75 years	<75 years	P value*
	n (%)	n (%)	
Male	2,702 (59.6)	25,900 (71.2)	<0.001
Current smoker	1,415 (34.8)	10,873 (32.8)	0.013
MVD	2,480 (56.3)	14,628 (41.5)	<0.001
Obese	569 (18.9)	7,595 (30.6)	<0.001
Diabetes mellitus	817 (18.8)	6,342 (18.0)	0.202
Hypertension	2,303 (55.8)	15,150 (45.4)	<0.001
Extra-cardiac arteriopathy	602 (13.3)	2,757 (7.6)	<0.001
Renal impairment	120 (3.0)	409 (1.25)	<0.001
Left ventricular dysfunction	2,063 (51.8)	13,651 (42.1)	<0.001
Multiple comorbidity	821 (18.1)	4,841 (13.3)	<0.001
Previous PCI	603 (14.5)	5,294 (15.7)	0.060
Previous CABG	681 (16.1)	3,644 (10.7)	<0.001
Previous AMI	1,679 (39.8)	12,233 (35.4)	<0.001
Family history	1,022 (25.4)	14,034 (42.6)	<0.001

\* $\chi^2$  test

n number, PCI percutaneous coronary intervention, CABG coronary artery bypass grafting, AMI acute myocardial infarction, MVD multivessel disease

**Table 8.** Time trends in case-mix of patients undergoing non-emergency percutaneous coronary intervention by age-group.

	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	P value*
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	
<b>≥75 years</b>												
male	64 (63.5)	92 (71.3)	124 (63.3)	130 (59.6)	141 (52.2)	212 (54.5)	257 (60.2)	340 (61.1)	418 (61.1)	440 (58.5)	484 (61.1)	0.790
current smoker	34 (37.4)	52 (48.6)	65 (44.5)	84 (42.9)	85 (35.6)	127 (38.8)	130 (31.9)	208 (38.0)	310 (33.9)	213 (32.0)	207 (28.5)	<0.001
MVD	48 (51.1)	56 (54.4)	85 (59.0)	115 (55.6)	140 (52.4)	212 (55.5)	244 (55.6)	312 (56.0)	384 (56.6)	407 (54.5)	477 (60.5)	0.090
obesity	13 (16.0)	18 (18.0)	39 (30.2)	30 (18.1)	31 (16.2)	53 (21.5)	51 (19.0)	68 (17.3)	87 (17.7)	98 (19.5)	81 (18.1)	0.193
diabetes mellitus	10 (9.9)	35 (27.1)	61 (31.4)	31 (14.8)	44 (16.5)	64 (17.3)	86 (20.0)	77 (13.8)	121 (18.4)	147 (21.0)	141 (19.0)	<0.001
hypertension	27 (28.4)	35 (32.4)	46 (31.3)	78 (39.4)	103 (43.6)	177 (51.5)	212 (51.5)	327 (59.6)	403 (64.2)	434 (63.9)	461 (62.8)	<0.001
extra-cardiac arteriopathy	-	-	11 (5.6)	33 (15.1)	33 (12.2)	60 (15.7)	48 (10.8)	66 (11.7)	103 (15.1)	124 (16.5)	115 (14.5)	<0.001
renal impairment	0 (0.0)	0 (0.0)	1 (5.3)	1 (4.3)	0 (0.0)	1 (2.8)	3 (6.1)	5 (7.8)	10 (11.4)	5 (4.7)	3 (2.2)	0.020
left ventricular dysfunction	32 (35.2)	38 (38.8)	55 (37.7)	106 (51.2)	124 (46.8)	203 (53.4)	240 (55.3)	279 (54.1)	291 (52.9)	346 (55.4)	349 (51.8)	<0.001
multiple comorbidity	-	-	12 (6.1)	29 (13.3)	35 (13.0)	61 (16.0)	70 (15.7)	107 (19.0)	150 (21.9)	165 (21.9)	178 (22.5)	<0.001

	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	P value*
previous PCI	16 (16.7)	14 (12.7)	24 (15.8)	34 (16.4)	47 (17.9)	57 (16.6)	53 (12.8)	82 (14.9)	91 (14.6)	93 (14.0)	92 (12.7)	0.730
previous CABG	15 (16.0)	12 (11.2)	27 (19.6)	29 (14.0)	46 (17.4)	72 (19.5)	67 (15.5)	93 (16.6)	90 (14.0)	123 (18.2)	107 (14.7)	0.710
previous AMI	48 (47.5)	49 (38.0)	58 (29.6)	101 (46.3)	132 (48.9)	159 (41.5)	166 (37.3)	221 (39.1)	256 (37.4)	260 (34.6)	229 (28.9)	<0.001
family history	43 (46.7)	50 (47.6)	73 (50.7)	60 (30.8)	54 (23.6)	81 (25.2)	88 (22.3)	141 (26.1)	151 (24.5)	159 (23.9)	122 (16.9)	<0.001

### <75 years

male	1,167 (62.5)	1,167 (67.7)	1,290 (67.2)	1,854 (68.3)	2,132 (70.7)	2,385 (72.4)	2,609 (72.3)	2,958 (71.5)	3,431 (70.9)	3,375 (72.2)	3,292 (73.0)	<0.001
current smoker	664 (43.6)	810 (48.0)	705 (45.2)	829 (37.0)	835 (31.2)	923 (30.5)	985 (28.9)	1,305 (32.4)	1,435 (31.4)	1,267 (29.8)	1,115 (26.4)	<0.001
MVD	859 (55.2)	995 (58.9)	847 (53.2)	1,026 (40.5)	1,061 (35.7)	1,223 (37.5)	1,473 (41.5)	1,656 (40.4)	1,920 (39.8)	1,835 (39.4)	1,733 (38.7)	<0.001
obesity	294 (21.3)	380 (24.4)	362 (25.5)	567 (30.1)	637 (30.8)	647 (30.1)	576 (28.9)	946 (32.9)	1,185 (33.7)	1,085 (33.7)	916 (33.6)	<0.001
diabetes mellitus	326 (18.9)	399 (20.8)	612 (30.2)	429 (16.9)	472 (16.9)	519 (16.3)	569 (16.0)	626 (15.4)	879 (18.6)	773 (17.6)	738 (17.3)	<0.001
hypertension	490 (31.5)	606 (35.5)	595 (37.7)	916 (40.4)	1,015 (37.8)	1,300 (42.4)	1,450 (42.3)	2,005 (49.7)	2,403 (52.5)	2,193 (51.6)	2,177 (51.7)	<0.001
extra-cardiac arteriopathy	-	-	97 (4.7)	222 (8.5)	246 (8.2)	276 (8.4)	250 (6.9)	336 (8.1)	434 (9.0)	402 (8.6)	364 (8.1)	<0.001

	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	P value*
renal impairment	0 (0.0)	2 (0.7)	3 (1.2)	6 (2.4)	2 (0.7)	3 (0.9)	12 (3.3)	6 (1.4)	22 (3.7)	13 (1.9)	18 (2.0)	<0.001
left ventricular dysfunction	545 (39.9)	501 (33.2)	559 (35.1)	1,029 (41.1)	1,180 (40.1)	1,412 (43.5)	1,412 (40.2)	1,603 (41.8)	1,691 (42.1)	1,959 (48.9)	1,760 (45.1)	<0.001
multiple comorbidity	-	-	162 (7.9)	313 (12.0)	360 (11.9)	415 (12.6)	453 (12.6)	616 (15.3)	788 (16.3)	730 (15.6)	724 (16.1)	<0.001
previous PCI	246 (15.7)	290 (16.8)	331 (20.2)	439 (18.1)	569 (19.3)	440 (14.4)	453 (13.1)	541 (13.4)	696 (15.2)	661 (15.7)	628 (15.0)	<0.001
previous CABG	192 (12.4)	228 (13.8)	211 (13.3)	334 (13.7)	339 (11.4)	371 (11.7)	358 (10.1)	425 (10.4)	427 (9.2)	396 (9.4)	363 (8.7)	<0.001
previous AMI	673 (39.0)	718 (37.4)	597 (29.0)	992 (37.9)	1,148 (38.1)	1,251 (38.0)	1,214 (33.6)	1,390 (33.6)	1,635 (33.8)	1,396 (29.5)	1,221 (27.1)	<0.001
family history	804 (52.9)	769 (46.2)	769 (49.4)	1,076 (48.3)	1,122 (42.2)	1,229 (41.1)	1,319 (39.0)	1,801 (44.8)	1,987 (43.6)	1,671 (39.6)	1,447 (34.7)	<0.001

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\* $\chi^2$  test for trend

n number, PCI percutaneous coronary intervention, CABG coronary artery bypass grafting, AMI acute myocardial infarction, MVD multi-vessel disease

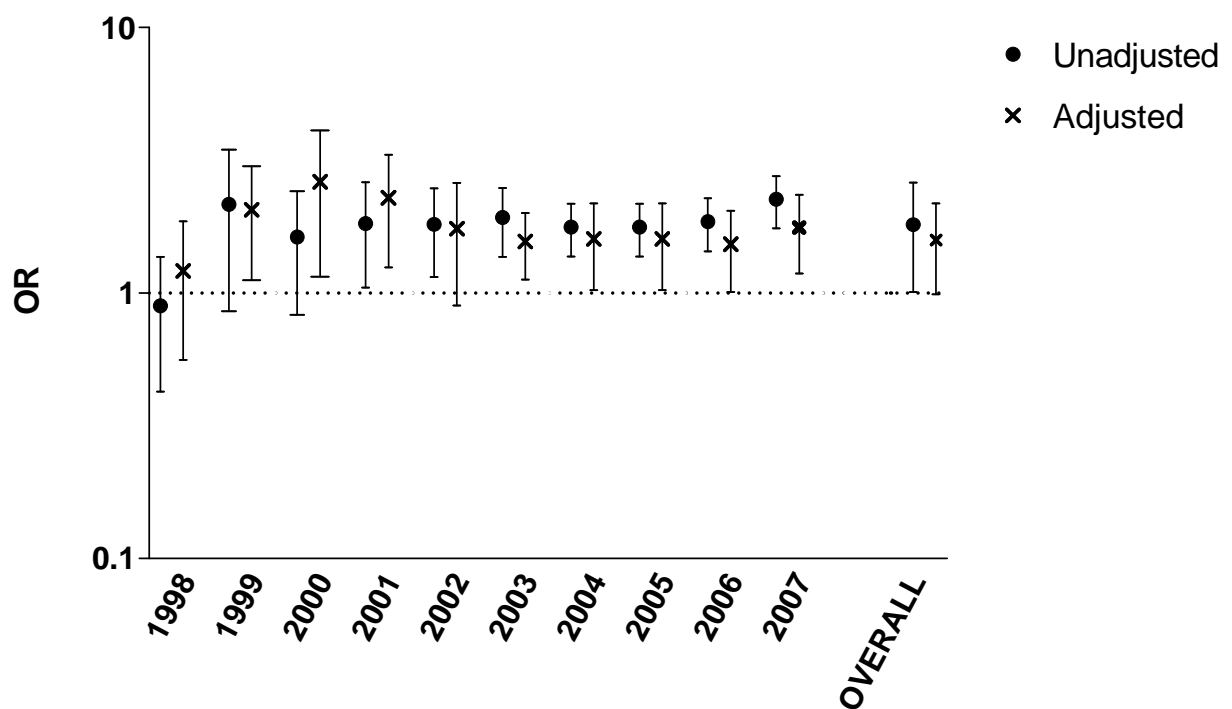
There was a four-fold increase in multiple comorbidity in elderly patients, compared with only a 35% increase in younger patients. The elderly were also characterised by increasing severity of cardiac disease with the prevalence of left ventricular dysfunction increasing from 35.2% to 51.8% ( $p<0.001$ ). The prevalence of MVD increased by 9.4% in the elderly, compared with a 16.5% fall in younger patients.

The adjusted risk of MACE among the elderly referent to the young did not change significantly over time (Figure 15). Among younger patients, the crude risk of MACE within 30 days of PCI fell significantly over the ten-year period from 6.3% to 2.4% (Table 9). By contrast, there was no significant change among elderly patients. Overall, the crude risk of MACE fell from 5.8% to 2.7% ( $\chi^2$  test for trend,  $p=0.019$ ).

**Table 9.** Time trends in crude 30 day outcomes following non-emergency percutaneous coronary intervention by age-group.

	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	P value*
<b>≥75 years</b>											
All-cause death	1 (1.0)	2 (1.6)	2 (1.0)	2 (1.0)	4 (1.5)	6 (1.6)	10 (2.3)	7 (1.3)	10 (1.5)	16 (2.1)	0.260
AMI	2 (2.0)	4 (3.2)	1 (0.5)	7 (3.5)	6 (2.3)	15 (4.0)	13 (2.9)	20 (3.7)	25 (3.7)	27 (3.7)	0.07
Cardiac surgery	2 (2.0)	1 (0.8)	2 (1.0)	0 (0.0)	2 (0.8)	1 (0.3)	2 (0.9)	4 (0.7)	3 (0.4)	3 (0.4)	0.213
MACE	4 (4.0)	6 (4.7)	5 (2.6)	7 (3.5)	10 (3.7)	18 (4.8)	20 (4.5)	29 (5.2)	31 (4.6)	36 (4.9)	0.216
<b>&lt;75 years</b>											
All-cause death	21 (1.2)	10 (0.5)	10 (0.5)	10 (0.4)	12 (0.4)	18 (0.6)	26 (0.7)	27 (0.7)	26 (0.5)	25 (0.6)	0.357
AMI	53 (3.1)	39 (2.1)	25 (1.7)	34 (1.4)	54 (1.8)	65 (2.0)	83 (2.3)	98 (2.3)	104 (2.2)	83 (1.8)	0.909
Cardiac surgery	45 (2.6)	18 (0.9)	11 (1.1)	21 (0.8)	16 (0.5)	22 (0.6)	23 (0.6)	25 (0.6)	15 (0.3)	22 (0.5)	<0.001
MACE	101 (6.3)	56 (3.1)	42 (2.2)	58 (2.4)	72 (2.5)	90 (2.8)	111 (3.2)	127 (3.2)	127 (2.7)	107 (2.4)	<0.001

\* $\chi^2$  test for trend, n number, AMI acute myocardial infarction, MACE major adverse cardiovascular event



Adjusted for: sex, smoking status, diabetes, hypertension, extra- cardiac vascular disease, renal impairment, impaired left ventricular function, disease severity, SIMD deprivation quintile, urgency of the procedure, access site, year of procedure, and hospital.

**Figure 15.** Unadjusted and adjusted risk of major adverse cardiovascular events within 30 days of non-emergency percutaneous coronary intervention in the elderly referent to younger patients by year of procedure.

#### 4.2.1. Strengths and Limitations

The cohort used in this analysis comprised all patients undergoing PCI (between 1998 and 2008) in Scottish NHS hospitals, this is not a selected sample and therefore avoids selection bias. The registry data are detailed and comprehensive, and are collected prospectively by clinical staff at the patient's hospital. As discussed above, the SCRR has a high proportion of successful linkage (98% for this analysis) to follow-up databases (99%) and robust quality assurance mechanisms that make systematic bias is unlikely. Statistical methods were utilised to adjust for known differences in baseline characteristics between elderly and younger patients. However, residual confounding due to unknown or unrecorded differences cannot be excluded. This is a recognised limitation of all observational studies. Age-stratified general population incidence rates were based on a single year being used as the denominator – 2006. This could have resulted in a modest underestimate of the incidence rate as the elderly probably increased in the overall population during the study period. In any cohort study, there is always the potential for loss to follow-up due to, for example, emigration from Scotland. Linkage with SMR01 and GROS would not provide admission data for events and deaths occurring outside Scotland. However, this is unlikely to impact significantly on the early (30 day) complications reported here. Unfortunately, it was not possible to assess long-term outcomes in this cohort.

The vast majority of patients were successfully linked to the follow-up databases. Fourteen percent could not be linked but, as mentioned above, there is no reason to suspect a systematic bias in the success of linkage, and analysis confirmed no significant differences in case-mix according to whether or not linkage was achieved. We had baseline data up to March 2008 but outcome until only March 2007 due to follow-up data only being updated on an annual basis. Data on non-cardiac arteriopathy were not collected in the first two years. Therefore, this could not be included as a covariate in the multivariate analysis and multiple comorbidity was only calculated for the subsequent years.

#### 4.2.2. Discussion of the main findings

Similar to the findings in the coronary angiography analysis, there was an increase in the number and percentage of PCIs undertaken in elderly patients - from 196 (8.7%) in 2000 to 752 (13.9%) in 2007. Compared with younger patients, the elderly were more likely to have multivessel disease, multiple comorbidity, and a history of myocardial infarction or coronary artery bypass grafting ( $\chi^2$  tests, all  $P < 0.001$ ).

Over the 7 years, there was a significant increase in the proportion of elderly patients who had multiple comorbidity ( $\chi^2$  test for trend,  $P < 0.001$ ). Despite this, the underlying risk of complications did not change significantly over time in the elderly ( $\chi^2$  test for trend,  $P = 0.142$ ), or overall ( $\chi^2$  test for trend,  $P = 0.083$ ). This suggests that even though the threshold for undertaking PCI appears to have changed over time (likely as a result of earlier revascularization for milder disease and use PCI in patients with more severe disease but deemed high risk for surgery), the risk of complications after PCI has not.

Compared with younger patients, elderly patients having PCI were more likely to have multivessel disease, multiple comorbidity, and a history of myocardial infarction or coronary artery bypass grafting ( $\chi^2$  tests, all  $p < 0.001$ ). The elderly had a higher risk of MACE within 30 days of PCI (4.5% versus 2.7%,  $\chi^2$  test  $p < 0.001$ ). However, similar to the findings in coronary angiography, crude risk was low.

As discussed, PCI is generally undertaken in elderly patients to alleviate the signs and symptoms of CAD. Therefore, assessing the benefits of PCI on QoL is as important as investigating the risk of peri-procedural complications or the longer term MACCE outcomes found for follow on PCI in the coronary angiography analysis. To do this, QoL improvements following PCI were assessed as part of this thesis in the ReQoL study (for results, see Chapter 4.3 below).

### **4.3. *ReQoL (Revascularisation and quality of life)***

Four hundred and fifty-one participants were recruited from consecutive patients attending the GJNH for elective PCI between September 2009 and August 2011. Of these, 437 fully completed the baseline questionnaire and had baseline characteristics and procedural information available. The Cardiac Research Nurses did not record actual response rate; however, based on data available from the National Golden Jubilee Hospital, this is likely to represent 18.2% of the eligible study population (based on ~1200 PCI procedures per year being undertaken as daycase procedures, with daycases being used as a proxy of the eligible population). The average age of participant was 61.1 (SD 10.3), with a range from 26 to 86 years of age. The most common presentation was with a CCS angina score of III (44.7%). The participant characteristics are shown in Table 10.

**Table 10. Baseline characteristics of participants enrolled in the ReQoL study (n= 437)**

Mean age (SD)	61.1 (10.3)
Median age (range)	62 (26-86)
	n(%)
Male	318 (72.8)
Smoker	117 (28.5)
CCS angina score	
0	8 (1.9)
I	12 (2.8)
II	92 (21.8)
III	187 (44.7)
IV	124 (29.3)
Obese	176 (43.7)
Diabetes mellitus	73 (16.9)
Hypertension	287 (68.5)
Extra cardiac arteriopathy	56 (13.4)
Renal impairment	4 (0.9)
Left ventricular dysfunction	217 (57.3)
Multiple co-morbidity	357 (81.7)
Previous AMI	233 (56.3)
Previous PCI	89 (22.6)
Previous CABG	32 (8.1)
Family History CAD	288 (69.1)
SIMD Deprivation	
Most deprived	122 (28.2)
Least deprived	72 (19.7)
Severity of disease	
SVD	254 (58.1)
MVD	183 (41.88)

PCI percutaneous coronary intervention; CABG coronary artery bypass grafting; AMI acute myocardial infarction; SVD single vessel disease; MVD multi-vessel disease; CAD coronary artery disease; SIMD Scottish Index of Multiple Deprivation; CCS Canadian Cardiovascular Society classification

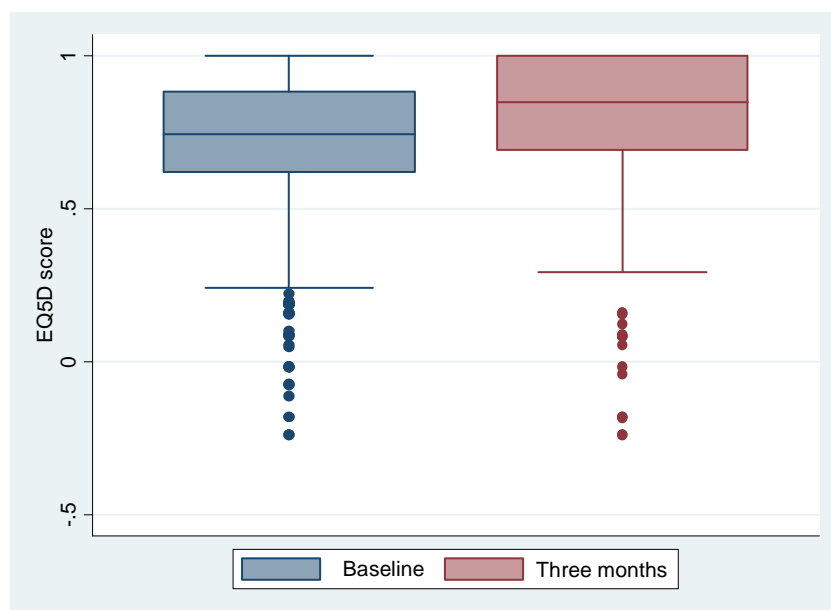
There were 103 (23.6%) participants aged  $\geq 70$  years and 334 (76.4%)  $< 70$  years of age. Elderly participants were more likely to smoke, have more severe disease, a family history of CAD, and have had a previous CABG. They were less likely to smoke or live in the most deprived SIMD quintile (see Table 11)

Table 11. ReQoL: Baseline characteristics of 437 ReQoL patients by age group

	$\geq 70$ years n (%) 103	$< 70$ years n (%) 334	P value*
Male	73 (70.9)	245 (73.3)	0.621
Current smoker	9 (10.0)		
CCS angina score			
0 (none)	0 (0.0)	8 (2.5)	
I	3 (3.0)	9 (2.8)	
II	14 (14.1)	78 (24.1)	
III	52 (52.5)	135 (41.7)	
IV (severe)	30 (30.3)	94 (29.0)	0.122
Obese	36 (37.5)	140 (45.6)	0.362
Diabetes mellitus	22 (22.0)	51 (15.4)	0.124
Hypertension	71 (73.0)	216 (67.1)	0.256
Extra-cardiac arteriopathy	17 (17.5)	39 (12.1)	0.170
Renal impairment	1 (0.0)	3 (0.9)	0.927
Left ventricular dysfunction**	52 (60.5)	165 (56.3)	0.494
Multiple comorbidity	85 (82.5)	272 (81.4)	0.183
Previous AMI	32 (34.2)	74 (22.6)	0.082
Previous PCI	27 (29.3)	62 (20.5)	0.077
Previous CABG	18 (19.3)	14 (4.6)	$< 0.001$
Family history of CAD	55 (57.3)	233 (72.6)	0.004
Deprivation (SIMD quintile)			
Most deprived	16 (15.7)	106 (32.0)	0.001
Least deprived	22 (26.5)	50 (17.7)	0.075
Severity of disease			
SVD	49 (47.6)	205 (61.4)	
MVD	54 (52.4)	129 (38.6)	0.013

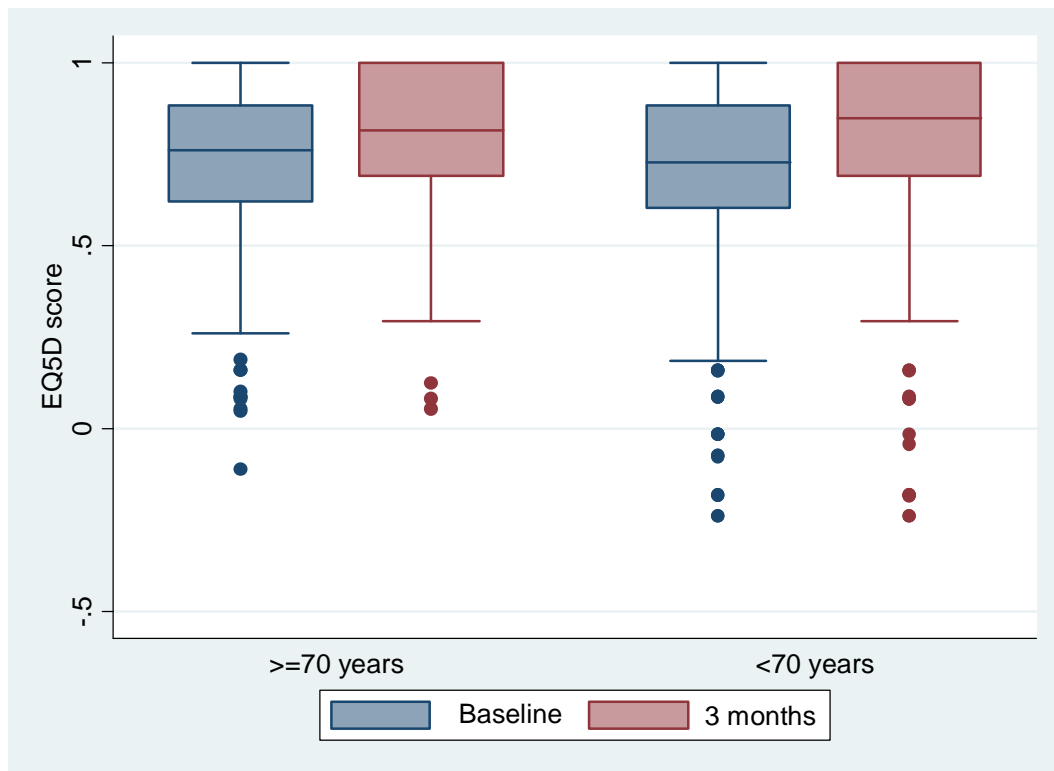
\* Person's  $\chi^2$  test/nptrend/ranksum for trend; \*\*75% missing; PCI percutaneous coronary intervention; CABG coronary artery bypass grafting; AMI acute myocardial infarction; SVD single vessel disease; MVD multi-vessel disease; CAD coronary artery disease; SIMD Scottish Index of Multiple Deprivation; CCS Canadian Cardiovascular Society classification

Three hundred and nine participants completed both the baseline and 3 months questionnaire. This equates to an attrition rate of 29.3%. There were 79 patients  $\geq 70$  years of age and 213 patients  $< 70$  years of age. Patients who did not complete the 3 months assessment were more likely to be younger (32.6% vs 18.5% dropped out from younger and older groups respectively,  $p=0.006$ ) and smoke (38.5% of the drop-outs vs 25.3,  $p=0.008$ ); however, they were no more likely to have severe disease ( $p=0.469$ ), or be from the most deprived SIMD quintile (0.063).



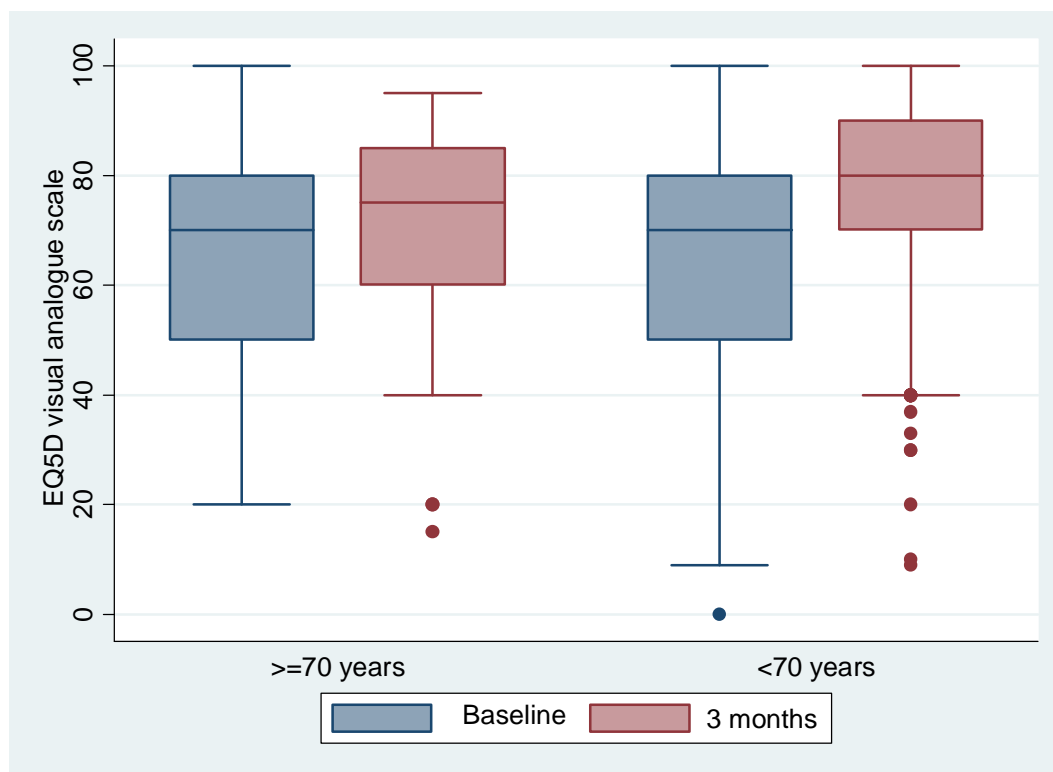
**Figure 16. Baseline and 3 months EQ-5D index utility score in all participants**

Baseline average EQ-5D index utility score was similar in elderly and younger patients (0.7,  $p=0.561$ ). Overall, the score increased following PCI (0.7 to 0.8,  $p < 0.001$ ). The greatest increase appeared to be in younger patients (0.075 vs 0.071); however, this was not found to be statistically significant  $p=0.911$ .



**Figure 17. Baseline and 3 months EQ-5D index utility score, by age group.**

The EQ-5D visual analogue scale showed that the mean score increased from 67.8 (SD 18.8) to 73.9 (SD17.8),  $p<0.001$ , following PCI. The mean difference for older patients in the EQ-5D visual analogue was 3.2 (SD 19.8) compared with 7.2 (SD19.0) in younger patients; again, this was not found to be statistically significant ( $p=0.141$ )



**Figure 18. Baseline and 3 months EQ-5D visual analogue scale, by age group.**

The breakdown of EQ-5D domains suggests that baseline anxiety and depression is much less in elderly patients ( $p=0.009$ ). However, none of the other variables at baseline showed statistical significance.

Overall mean SF.12<sub>v2</sub> PCS and MCS increased following PCI (39.0 vs 44.6 and 47.8 vs 50.9,  $p<0.001$ ). While baseline PCS appeared to be lower in elderly patients compared with younger (37.6 vs 39.7,  $p=0.106$ ) and MCS higher (50.0 vs 46.7,  $p=0.027$ ), only the MCS was statistically significant. Both elderly and younger patients showed statistically significant increases in both PCS and MCS following PCI (see table 12).

**Table 12. Baseline and 3 months quality of life score, by measurement tool and age group**

$\geq 70$ years of age			< 70 years of age			
	Before	After	P value	Before	After	P value*
EQ-5D Index**	0.7 (0.3)	0.8 (0.2)	0.009	0.7 (0.3)	0.8 (0.0)	<0.001
EQ-5D visual scale	67.7 (19.1)	70.9 (18.6)	0.187	68.0 (18.5)	75.3 (17.7)	<0.001
EQ-5D domains***						
Mobility	1.5 (0.5)	1.4 (0.5)	0.672	1.4 (0.5)	1.3 (0.5)	0.093
Self-care	1.1 (0.3)	1.1 (0.3)	0.132	1.1 (0.3)	1.1 (0.3)	0.1797
Usual activity	1.6 (0.6)	1.4 (0.5)	0.011	1.7 (0.7)	1.4 (0.6)	<0.001
Pain or discomfort	1.6 (0.7)	1.5 (0.6)	0.061	1.6 (0.6)	1.4 (0.6)	<0.001
Anxiety or depression	1.2 (0.5)	1.2 (0.4)	0.808	1.4 (0.6)	1.4 (0.6)	0.584
SF-12 v2						
PCS	37.6 (10.1)	41.9 (10.1)	<0.001	39.7 (10.0)	45.6 (10.8)	<0.001
MCS	50.0 (10.4)	53.0 (11.9)	0.035	46.7 (11.1)	49.7 (11.1)	<0.001
MacNew Global	4.7 (1.3)	5.6 (1.1)	<0.001	4.4 (1.3)	5.4 (1.3)	<0.001
MacNew						
Emotional	4.9 (1.3)	5.7 (1.2)	<0.001	4.5 (1.4)	5.3 (1.4)	<0.001
Physical	4.4 (1.4)	4.2 (1.4)	<0.001	4.2 (1.4)	5.5 (1.4)	<0.001
Social	4.7 (1.4)	5.7 (1.1)	<0.001	4.3 (1.4)	5.6 (1.4)	<0.001

\*paired ttest/Wilcoxon signrank test depending on skewness of data

\*\* As a utility index score

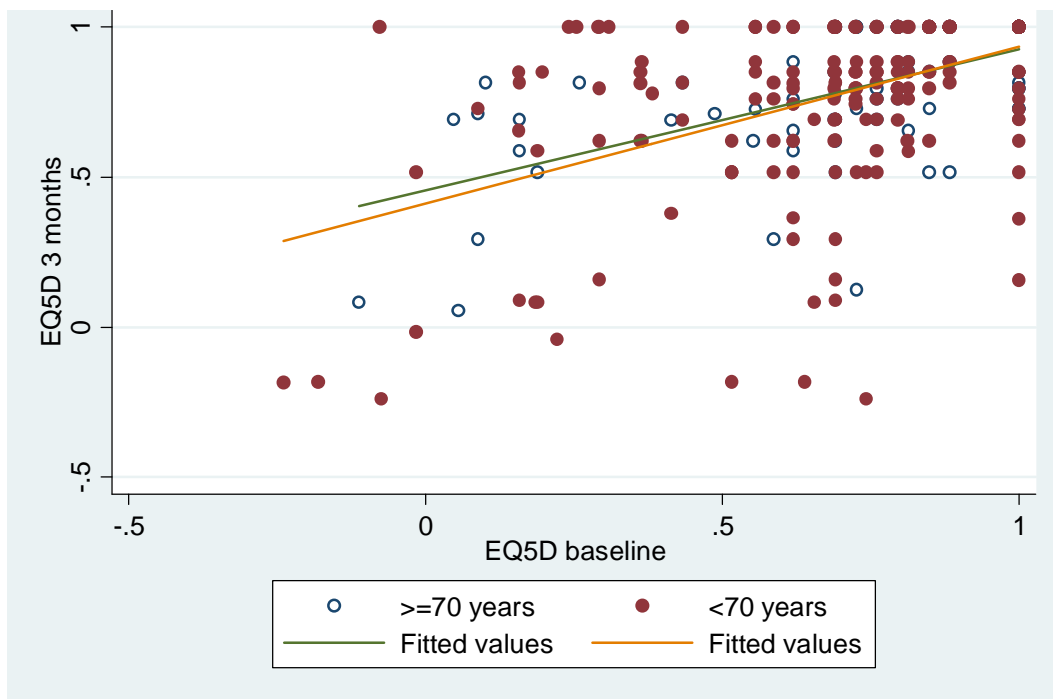
\*\*\*low scores on the 5 items of EQ-5D reflect a favourable assessment of each component

PCS: Physical component score; MCS: Mental component score; SF-12<sub>v2</sub>: Short Form Questionnaire

Similar to the EQ-5D VAS, the MacNew showed that global baseline QoL score was better in elderly patients compared with younger patients (4.7 vs 4.4,  $p=0.024$ ) and that all the MacNew domains (emotional, physical and social) showed statistically significant increases following PCI.

There was no statistically significant difference between elderly and younger patients in the proportion of patients who achieved the MID (73.8% vs 77.9%,  $p=0.393$ ) in MacNew score between baseline and 3 months.

In the MacNew questionnaire there is an optional question asking: “How often during the last 2 weeks have you felt your heart problem limited or interfered with sexual intercourse?” One hundred and twenty-five patients answered this question (15 aged  $\geq 70$  years) in the follow up questionnaire. Sixty-eight patients responded as “all the time” or “most of the time” at baseline compared with only 34 at 3 months ( $p < 0.001$ ) suggesting a significant improvement.



**Figure 19. Scatterplot of baseline and 3 months EQ-5D utility index score, by age group.**

Following adjustment for baseline characteristics (sex, disease severity, smoking status, obesity, comorbidity, and deprivation) using multivariate linear regression there was no statistically significant difference between elderly and younger patients in mean increase in EQ-5D score, PCS, MCS or any of the MacNew domains.

**Table 13. Multivariate linear regression (elderly referent to younger), by QoL measurement tool and with or without baseline score adjustment.**

QoL questionnaire	$\beta$ coefficient (95% CI), p value	
	Without baseline score	With baseline score
EQ-5D utility index	0.12 (-0.06, 0.09), 0.757	0.03 (-0.60, 0.66), 0.917
PCS	1.34 (-1.61, 4.30), 0.373	2.27 (-0.35, 4.89), 0.090
MCS	0.77 (-2.58, 4.12), 0.652	0.70 (-3.80, 42.01), 0.546
MacNew Global	0.20 (-0.28, 0.69), 0.406	0.01 (-0.43, 0.24), 0.992

Likelihood ratio testing suggested a good fit for the model and that fit was not improved following the introduction of interaction terms e.g. sex and deprivation, age and sex

Reliability testing suggested good internal consistency reliability for all the questionnaires:

**Table 14. Cronbach alpha, by QoL measurement tool**

Questionnaire	Cronbach's alpha
EQ-5D	0.70
PCS	0.86
MCS	0.84
MacNew Global	0.95

Spearman's correlation coefficient was assessed between EQ-5D utility index and MacNew global; PCS and MacNew Physical; and MCS and MacNew emotional.

**Table 15. Correlation matrix of QoL measurement tools**

Spearman's rho, p value			
	EQ-5D utility index	PCS	MCS
MacNew global	0.52, <0.001		
MacNew physical		0.65, <0.001	
MacNew Emotional			0.74, <0.001
		PCS	MCS

The coefficients were found to be larger between the related dimensions (e.g. PCS and MacNew Physical functioning) than the global scores (EQ-5D and MacNew global).

At baseline, a small number of patients were found to have conditions associated with significant frailty: 5 had dementia (1 in the  $\geq 70$  year old group); 2 were confined to bed (1 in the  $\geq 70$  year old group); and 39 were incontinent of bowel or bladder (11 in the  $\geq 70$  year old group). Using the “usual activities” domain of EQ-5D as a proxy for ADL; 10.4% participants reported that they were unable to perform their usual activities at baseline. Overall, there was a statically significant difference in usual activities score between baseline and 3 months (10.4% vs 3.2%,  $p < 0.001$ ). Comparisons between change in score in elderly and younger patients was not possible due to small numbers (e.g. the assumptions of the statistical tests were not met).

#### 4.3.1. Strengths and Limitations

Assessment of QoL was undertaken using both generic and disease specific QoL questionnaires. The aim was to recruit consecutive patients in real life practice. Cardiac Research Nurses at GJNH were responsible for recruitment and baseline data collection. Although, the nurses did not record actual response rate, routine PCI data from the GJNH suggest that an estimated 18% of the eligible study population was recruited. Contributing factors to the lower than anticipated recruitment rates include the length of the questionnaire and the rapid same day discharge process. This resulted in loss of potential participants due to time constraints, which could potentially result in issues of generalizability. In addition, selection bias may have occurred, especially if there were systematic differences between participants and non-participants. However, in keeping with data from the secondary data analysis of SCRR (see Chapters 4.1 and 4.2), elderly patients enrolled in ReQoL were more likely to have severe disease, a family history of CAD, and have had a revascularisation procedure in the past, suggesting the sample could be reasonably representative of the target population. As discussed, there could be issues of generalisability in the study, finding similar patterns in the baseline differences between elderly and younger patients, suggests that comparisons in outcomes between elderly and younger patients can still be undertaken. This would allow comparisons in outcomes between elderly and younger patients.

There were advantages associated with the administration of the baseline questionnaire by the Cardiac Research Nurses, for example improved completion rates. Participants were able to ask

for clarification and assistance with questionnaire, factors that may be particularly important in elderly patients who may have problems with vision or who may have found the length of the questionnaire challenging. While participants completed the 3 months questionnaire without such support, the initial facilitation may also have increased confidence in participants' perceived ability to self-complete the same questionnaire three months later. However, potential limitations include information bias; the Cardiac Research Nurses were not blinded to the research question and their support may have influenced responses. In addition, nurse facilitation of the questionnaire at baseline could have impacted on responses to potentially sensitive items, for example, the question about sexual activity in the MacNew questionnaire; or the question on continence.

This was also a single-centre study, and it is possible that unique characteristics of the patients, the physicians, or the institution may limit the generalizability of these results. However, it does improve consistency of management and recording of baseline characteristics.

An additional concern is that only 79.7% of the patients involved in baseline analyses completed 3-months follow-up. However, loss to follow-up is not uncommon in studies of QoL following PCI. Sperus *et al*<sup>201</sup> had an attrition rate of 31% and may be offset by the benefit of a cohort that is more representative of the general patient population undergoing PCI than a secondary analysis of patients enrolled in a clinical trial. Although conducting these analyses in the context of clinical trials may allow higher rates of follow-up, the selection bias of who gets enrolled into the clinical trials may significantly offset this benefit.

Data on clinical outcomes, such as MACCE were not available for this cohort. these may wellhowever, these may well be influenced by adverse clinical outcomes such as MACCE.

#### 4.3.2. Discussion of the main findings

Following PCI, mean QoL improved in both elderly and younger patients. Using the SF-12<sub>v2</sub>, elderly participants had higher baseline MCS but lower PCS. After adjusting for baseline differences, QoL (both physical and mental component) in elderly patients improved as much as younger patients, following PCI: MCS 50.0(SD 10.4) to 53.0(SD 11.9) vs 46.7(SD 11.1) to 49.7(SD 11.1),  $p=0.652$ ; and PCS 37.6(SD 10.1) to 41.9(SD 10.1) vs 39.7(SD 10.0) to 45.6(SD 10.8),  $p=0.373$ ). Similar findings were seen when the EQ-5D or MacNew tools were used to assess QoL. These findings suggest that elderly patients benefit just as much from PCI than younger patients, irrespective of the measurement tool.

A Cronbach's alpha of 0.95 was found for MacNew which suggests that the questionnaire had good internal consistency and the Spearman's correlation coefficient suggests that the physical and emotional domains correlate well with the mental and physical domains of the SF-12<sub>v2</sub>.

The questions designed to assess frailty were found to be of limited use in this population; given the small numbers with significant frailty. The likely explanations for this are (i) this is a group already selected to have an invasive procedure by their clinician; (ii) the question on incontinence failed to map to the Rockford levels as it did not distinguish between bowel and bladder incontinence, and (iii) no physical assessment of frailty, such as, grip strength or walk test was used. The usual activities question from the EQ-5D were used as a proxy of ADL and did show a statistically significant improvement after PCI; however, numbers were too small to allow comparisons between elderly and younger patients.

The findings from the analysis of coronary angiography and PCI secondary data analysis suggest that there is a higher threshold for the investigation and management of CAD in elderly patients and that elderly patients have a higher risk of adverse outcomes. In keeping with the systematic review of the literature in Chapter 2.3, ReQoL confirms the QoL benefit of PCI for elderly patients. However, deciding who will most benefit vs risk is challenging. Currently many tools are used to assist with clinical decision making include, chronological age e.g. PTP. However, there is growing interest in the use of biological markers of ageing (such as LTL) to predict incidence, prognosis, and who would most benefit from invasive investigations and/or management of CAD.

The first stage in assessing whether LTL could be used for this purpose is to investigate the association between LTL and the presence and severity of CAD. This association was assessed in this thesis in the Biomarkers of Ageing in CAD study (for results of this study, see Chapter 4.4 below).

## 4.1. Biological ageing in CAD

One thousand eight hundred and fifty-two participants were recruited at the GJNH from

**Table 16. Baseline characteristics of study participants by presence or absence of**

	CAD 1,350 (73.1)	No-CAD n=496 (26.9)	P value*
Age (mean, SD)	63.0 (10.7)	62.6 (11.4)	0.487
Male	972 (72.0)	378 (59.2)	<0.001
Current smoker	374 (30.9)	94 (22.1)	0.001
CCS angina score			
0 (none)	128 (9.9)	151 (32.0)	
I	124 (9.6)	54 (11.4)	
II	486 (37.5)	169 (35.8)	
III	424 (32.7)	66 (14.0)	
IV (severe)	134 (10.3)	32 (6.8)	<0.001
Obese	505 (40.1)	193 (42.1)	0.541
Diabetes mellitus	268 (20.3)	77 (16.0)	0.041
Hypertension	899 (71.2)	297 (64.9)	0.011
Extra-cardiac arteriopathy	236 (18.7)	82 (17.9)	0.707
Renal impairment	23 (1.8)	7 (1.5)	0.671
Left ventricular dysfunction **	200 (43.1)	12 (15.6)	<0.001
Multiple comorbidity	1031 (76.4)	332 (67.0)	<0.001
Previous AMI	749 (63.5)	-	-
Previous PCI	323 (29.4)	-	-
Previous CABG	123 (11.4)	-	-
Family history CAD	865 (70.1)	292 (65.2)	0.054
Deprivation (SIMD <sup>2</sup> )			
Most deprived quintile	467 (34.8)	162 (32.7)	0.406
Least deprived quintile	201 (17.5)	79 (18.4)	0.670

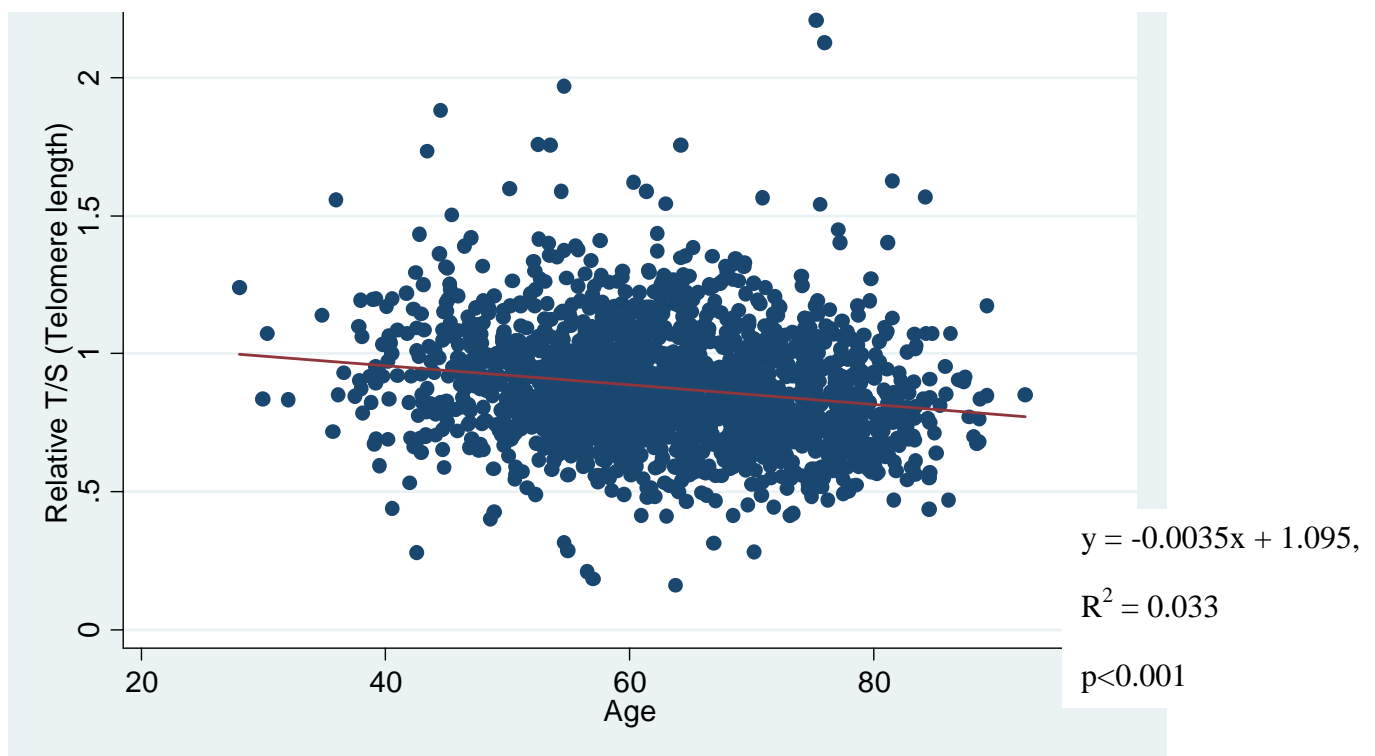
\*Pearson's  $\chi^2$  test \*\*70% values missing

CCS Canadian Cardiovascular Society; AMI: acute myocardial infarction; PCI: percutaneous coronary intervention; CAD: coronary artery disease; CABG: Coronary artery bypass grafting. SIMD: Scottish Index of Multiple Deprivation

consecutive patients attending for coronary angiography. Of these, LTL was successfully measured in 1846 participants. Of these 1846, 73.1% (n=1350) had evidence of CAD on angiography or were known to have had a history of AMI, PCI or CABG. The baseline characteristics for these participants is shown in Table 16.

Those with CAD were more likely to be male, currently smoke, have a higher CCS angina score, and have multiple comorbidity. They were no more likely to be older, have a family history of CAD or be from the most deprived SIMD quintile.

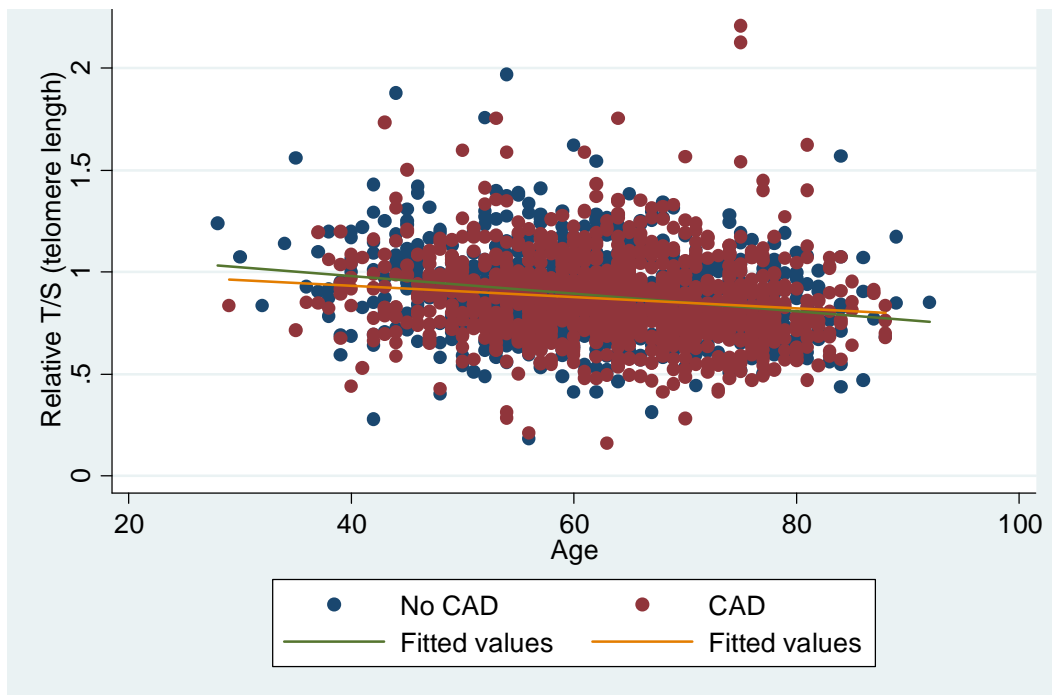
Mean relative T/S for the whole cohort was 0.875 (SD 0.210) with a range of 0.160 to 2.208. Decreased relative T/S was associated with increasing age (see Figure 20) however only 3.3% of the variance in relative T/S can be attributed to age.



**Figure 20. Scatterplot of age and leukocyte telomere length (determined by by relative T/S)**

On univariate analysis, mean relative T/S appeared to be higher in patients with CAD compared to those without CAD (0.890 (SD 0.207) vs 0.869 (0.214). However, this was not found to be statistically significant,  $p=0.064$ .

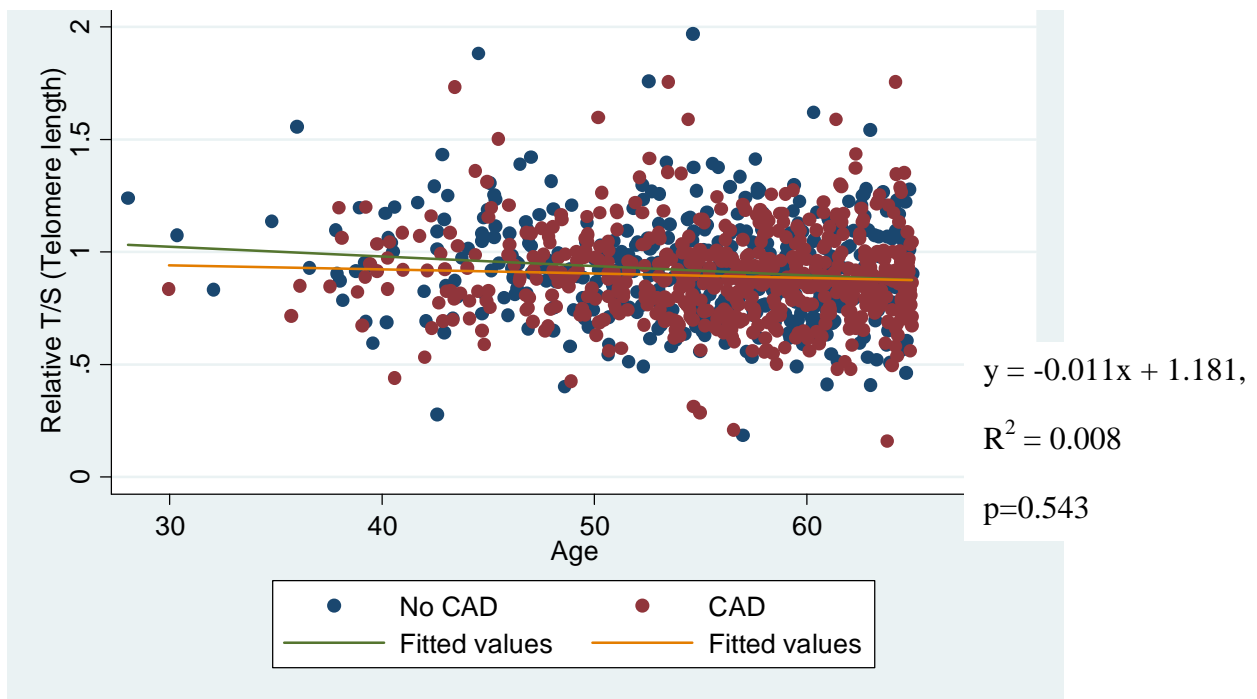
No statistically significant association was found between LTL and sex ( $p=0.068$ ), smoking ( $p=0.239$ ), deprivation ( $p=0.382$ ), or comorbidity ( $p=0.268$ ).



**Figure 21. Scatterplot of age and leukocyte telomere length, by presence or absence of coronary artery disease.**

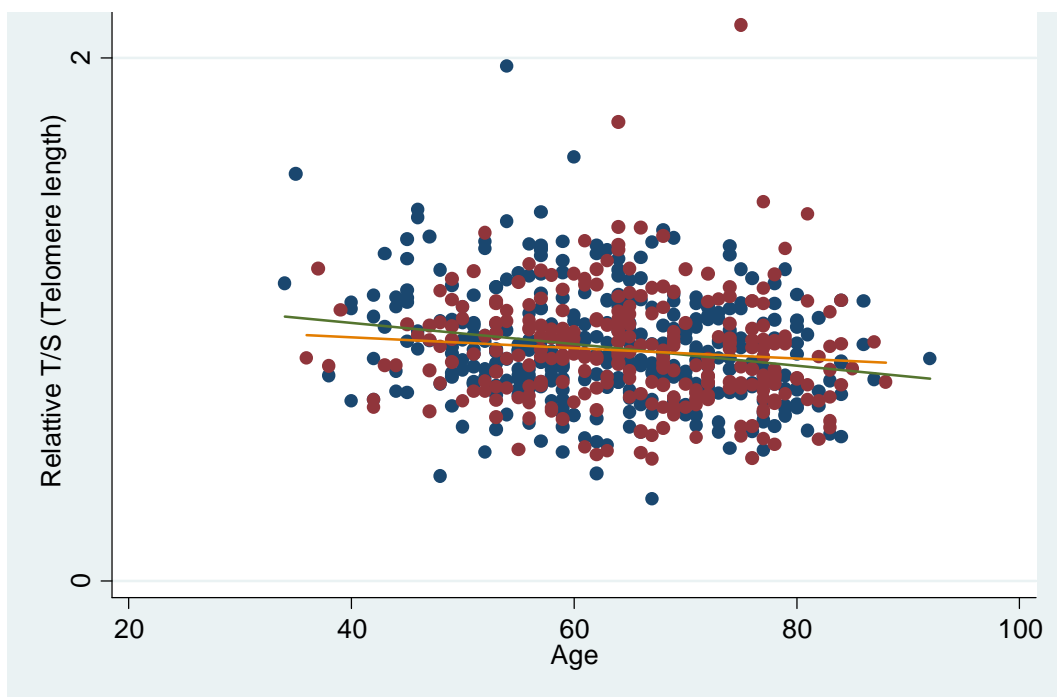
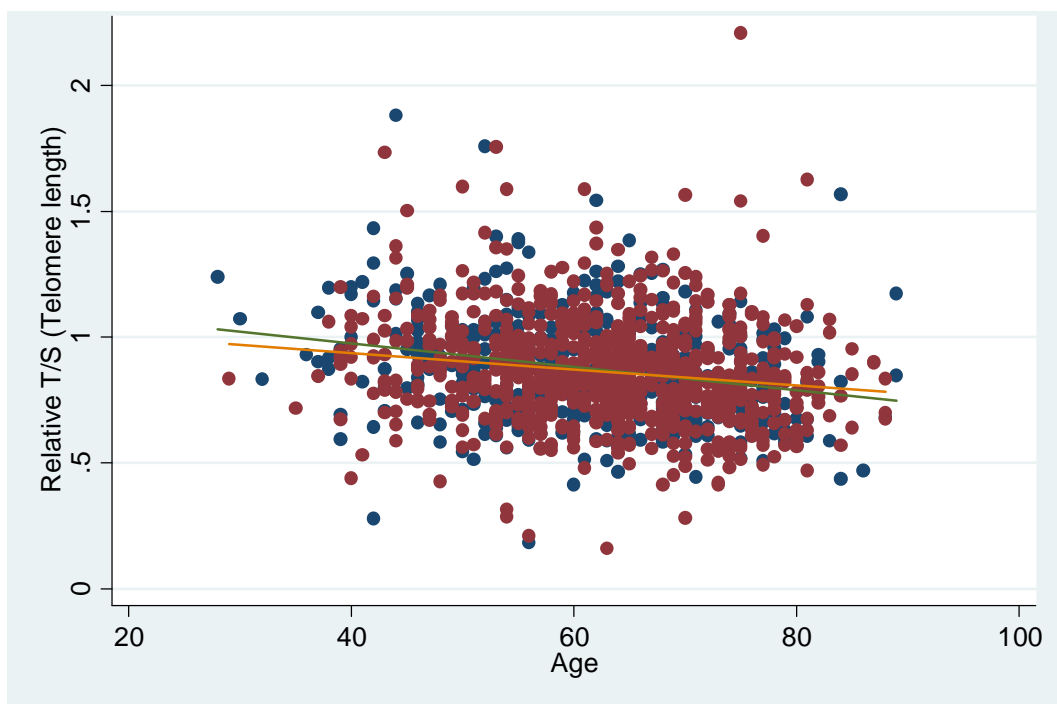
After adjusting for age, sex, current smoking status, obesity, comorbidity, deprivation, and family history, no association was found between CAD and relative T/S  $y=-0.009x + 1.137$ , adjusted  $R^2=0.033$ ,  $p=0.365$ .

No statistically significant difference was found after truncation at  $\leq 65$  years of age or by stratifying by gender, either on univariate or multivariate analysis.



**Figure 22. Scatterplot of age and leukocyte telomere length, by presence or absence of coronary artery disease (age truncated at 65 years).**

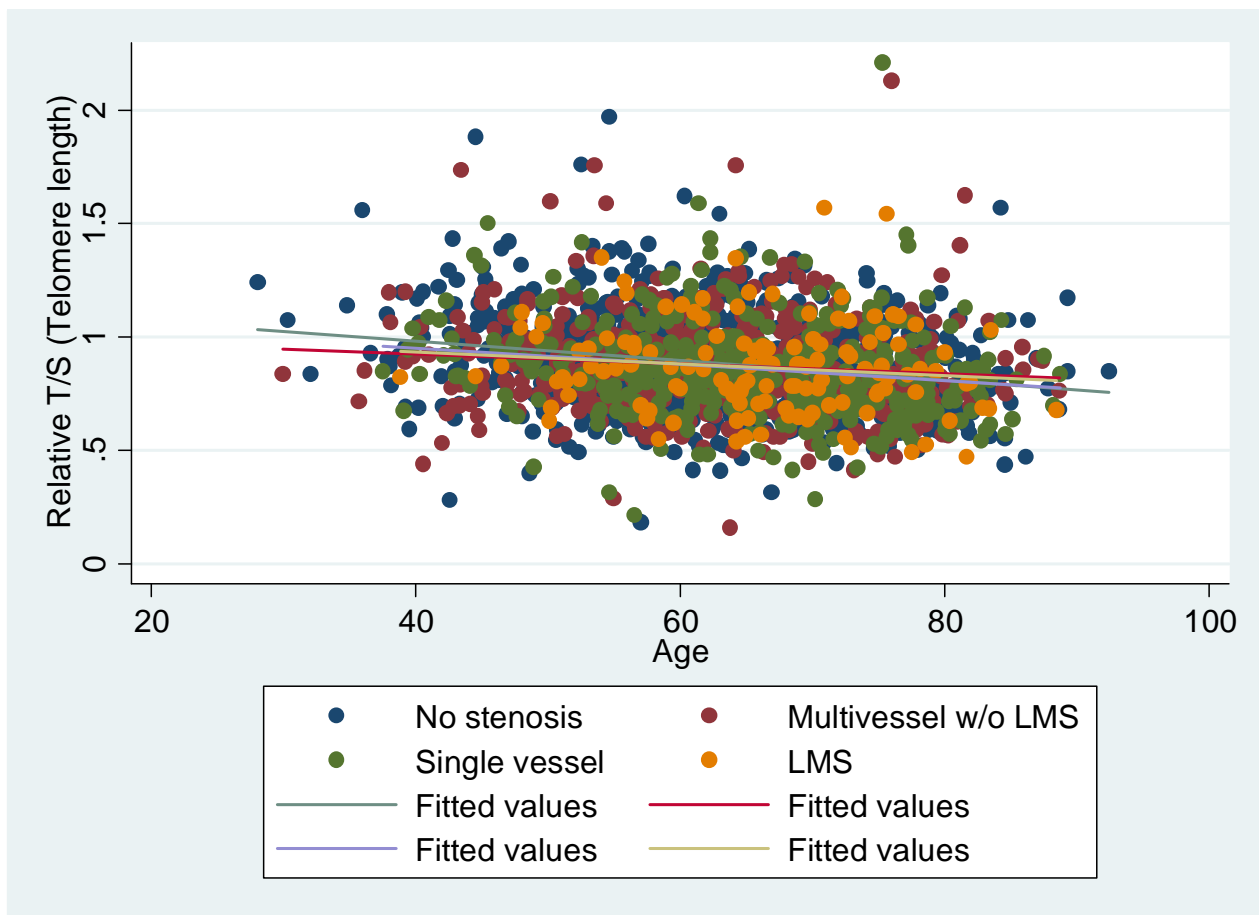
On univariate analysis, there was a statistically significant difference in the mean age of those with and without CAD: 55.9 (SD 6.74) years vs 54.8 (SD 7.10) years of age,  $p=0.007$ . However, no such difference was found in mean T/S ratio: 0.893 (SD 0.202) vs 0.918 (SD 0.226),  $p=0.063$ .



**Figure 23. Scatterplot of age and leukocyte telomere length, by presence or absence of coronary artery disease: (a) females (b) males**

Of the 1,350 patients known to have CAD (by angiography or by clinical history of AMI, PCI, or CABG), 262 (19.5%) had no evidence of significant stenosis on angiography. Of the others, 508 (37.7%) had single vessel disease; 445 (33.0%) had multivessel disease; and 132 (9.8%) had LMS involvement. There was no statistically significant difference in relative T/S length by severity of disease: 0.875 (SD 0.211) vs 0.875 (SD 0.212) vs 0.860 (SD 0.203) vs 0.867 (SD 0.200),  $p=0.670$

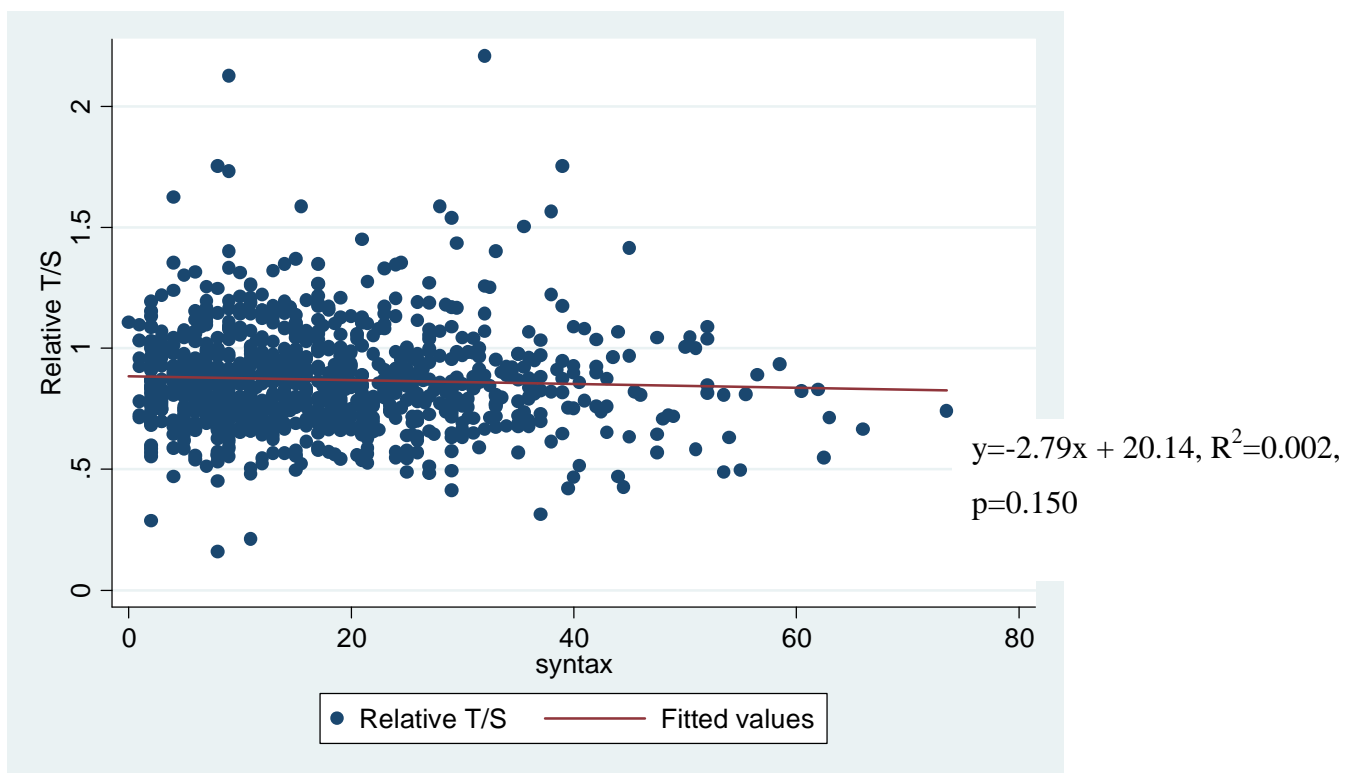
After adjusting for age, sex, current smoking status, obesity, comorbidity, deprivation, and family history, no association was found between CAD and relative T/S  $y=-0.002x + 1.086$ , adjusted  $R^2=0.0217$ ,  $p=0.891$ .



**Figure 24. Scatterplot of age and leukocyte telomere length, by severity of coronary artery disease (by number of stenosed vessels).**

Overall there were 16 (0.88%) inlab complications. No deaths occurred in the cohort. There was one infarction leading to a VF/VT arrest which was successfully managed within the catheter lab. The most common complication was occlusion or dissection of the coronary artery (n=9). No association was found between the occurrence of inlab complication and telomere length (0.874 (SD 0.005) vs 0.915 (SD 0.542),  $p=0.4395$ ).

SYNTAX score was available for 922 of the participants who had evidence of stenosis on angiography. Syntax score ranged from 0 – 73.5, with a mean score of 17.7 (median 15). On univariate and multivariate analysis, SYNTAX score was not found to be associated with relative T/S.



**Figure 25. Scatterplot of age and leukocyte telomere length, by severity of coronary artery disease (by SYNTAX score).**

#### 4.1.1. Strengths and Limitations

This is a large cross-sectional study of unselected consecutive cases attending a single large regional institution. While this may raise issues around generalisability, it has the advantage that there is consistency of approach to managing patients (following the most up-to-date European guidelines from the ESC). In addition, consistency in approach to recruitment and data collection, reduces selection and information bias. Recruitment at the GJNH finished well within the timeframe expected, suggesting a high recruitment rate which will reduce selection bias.

The analysis adjusted for common confounders (as highlighted in the introduction to this thesis, as being associated with both CAD and LTL) such as, age, sex, current smoking status, obesity, comorbidity, deprivation, and family history. However, it was not possible to adjust for those variables not routinely collected by the database. For example, individual socioeconomic status, rather than the area-based measures in the database have been associated with LTL.<sup>33</sup> In addition, inflammatory markers (IL-6 and CRP) and lipid levels were not available at the time of analysis. It was also not possible to assess the influence of cardiovascular drugs on the association between LTL and CAD. Therefore, residual confounding in the study cannot be excluded.

A limitation of the study is that the comparison group comprised of those undergoing angiography for symptoms suggestive of CAD, rather than using a sample from the general population. However, this study design has made it possible to assess the association between LTL and CAD in a real life population referred for, and attending for, an invasive investigation for CAD. In addition, coronary angiography is considered as the “gold standard” investigation for the diagnosis of CAD, therefore, reducing misclassification bias.

Generally data completeness was >90% for most covariates, such as smoking status and quality assurance of the data in the clinical system is undertaken on a regular basis for audit purposes. However, not all patients had undergone echocardiography prior to coronary angiography, resulting in a low proportion of patients with a measurement of their left ventricular ejection fraction. This is likely to be less of an issue for future studies using this database because the ESC guidelines now recommend echocardiography prior to coronary angiography. The case definition of CAD took past medical history of AMI, CABG, and PCI into consideration as well as the

angiographic appearance. Severity of CAD was assessed using 2 methods: number of stenosed vessels and SYNTAX score.

LTL was measured using Real-Time qPCR by Shiels lab. The CoV was found to be 0.51%, which shows a high degree of precision for the technique. The LTL was based on all populations of leucocytes rather than a specific sub-population; however, it is not clear if this is strength or a possible limitation of the study.

#### 4.1.2. Discussion of the main findings

This cross sectional study design was used to investigate the association between LTL and the presence, and severity, of CAD. Those with CAD were more likely to be male, currently smoke, have a higher CCS angina score, and have multiple comorbidity. This suggests that males are more likely to be diagnosed with CAD in this population. Incidence and prevalence of CAD is known to increase with age; although, age was not associated with an increased likelihood of having a CAD in this study. However, this is a population who have presented with symptoms suggestive of CAD, been referred for coronary angiography, and agreed to be in the study; making comparisons with general population difficult. In addition, these findings suggest that the character and severity of pain (based on the CCS) is an important indicator of the presence of CAD on coronary angiography.

No statistically significant difference was found in mean LTL T/S ratio between those with and without CAD (0.87 (SD 0.21) vs 0.89 (SD 0.21),  $p=0.91$ ), even after adjusting for baseline characteristics. Therefore there is no evidence of an association between LTL and CAD in this population. LTL was no better than chronological age at predicting the presence of CAD; suggesting it would be of little use as a biomarker of ageing in this population. No significant difference was found between LTL T/S ratio and severity of disease irrespective of which method was used (number of stenosed vessels or SYNTAX score).

Similar to the findings in Chapter 4.1 of this thesis, there was a low complication rate (0.88%) associated with coronary angiography, suggesting that coronary angiography is a safe investigation.

## **5. OVERALL DISCUSSION.**

### ***5.1. Discussion of thesis findings***

Chapter 4.1 and 4.2, of this thesis, demonstrated that elderly people account for an increasing number and proportion of patients presenting for the investigation and management of CAD, however, Chapter 4.1 and 4.2 also demonstrated that the threshold for investigation, via coronary angiography, and subsequent intervention, using PCI, were higher among this increasingly large group of patients, even after taking account of co-morbidities. This suggests a chronological age-based inequality in access to investigation and treatment. Inequality might, nonetheless, be justified if the risks were unacceptably high or benefits less among elderly patients. However, this is not the case. Section 4.2 demonstrated that elderly patients do have a higher risk of early complications than younger patients, but their absolute risk was, nonetheless, low suggesting that coronary angiography and PCI are safe procedures to perform in the elderly.

PCI is performed to improve symptoms and thereby, QoL, rather than survival. The systematic review undertaken in section 2.3 demonstrated that QoL following PCI in octogenarians improves at least as much as younger patients. In addition, section 4.3 the primary research project, ReQoL, showed that following PCI, the QoL of elderly patients improved at least as much as in younger patients. Therefore, PCI is both a safe and effective procedure for elderly patients.

There is currently much interest in whether biomarkers of ageing may provide a better measurement of “miles on the clock.”<sup>33</sup> If so, biomarkers of ageing may be a better basis for clinical decision-making (than chronological age) and provide a better means of judging equality of access based on equivalent need or the potential for equivalent benefit. A number of biomarkers of ageing have been explored, including telomere length. However, Section 4.4 demonstrated that

telomere length is no better than age at predicting the presence of CAD and therefore is unlikely to be a useful biomarker of ageing in CAD.

## **5.2. Comparison of thesis findings with the literature**

Chapter 4.1 demonstrated that the total numbers and rates of coronary angiography undertaken in Scotland had been increasing until the mid 2000s. This may be explained by increasing prevalence of CAD and changes in the threshold for investigation by coronary angiography. Other countries, such as the USA,<sup>374</sup> Israel<sup>375</sup> and Pakistan, have also seen similar patterns of increases in coronary angiography.<sup>376</sup>

Also in keeping with other studies, Chapters 4.1 and 4.2 demonstrated that elderly patients (aged  $\geq 75$  years of age) represent an increasing number and proportion of those who undergo coronary angiography and PCI in Scotland. Groarke *et al.*<sup>377</sup> used comprehensive routine national Irish hospital inpatient data and found that the proportion of those having coronary angiography who were aged  $>70$  years increased from 19% in 1997 to 31% in 2007. Rajani *et al.*<sup>378</sup> also found that the number and proportion of elderly patients (defined as  $\geq 80$  years of age) attending the Royal Sussex County Hospital between 2000 and 2008 for PCI, increased from 5.8% to 12.2%.

In common with the findings of this thesis, previous studies have shown that older age was associated with an increased likelihood of finding evidence of both, any CAD, and more advanced CAD.<sup>379,380,381,382</sup> Kotecha *et al.*<sup>379</sup> found that those with angiographically-confirmed disease were older than those with no or non-obstructive CAD (mean age  $61.1 \pm 11.3$  years vs  $67.0 \pm 9.9$  years,  $p < 0.001$ ) and the odds of having more severe disease increased with every 10 years of age (OR 1.57, 95% CI: 1.57 – 1.95,  $p < 0.001$ ). Their study used a 10% random sample ( $n=539$ ) of coronary angiograms, from three interventional centres in Australia, which were reviewed by two experienced, blinded operators and defined stenosis as  $\geq 50\%$  in a native major coronary artery or a main tributary. while in

Chapters 4.1 and 4.3 stenosis was defined as  $\geq 70\%$  diameter stenosis of at least one major epicardial artery segment or  $\geq 50\%$  diameter stenosis in the left main coronary artery.

In a study by Veeranna *et al.*<sup>381</sup> based on 631 consecutive patients aged 65 years or over who underwent coronary angiography, 21.7% were found to have a Dukes Myocardial Jeopardy (DMJ)<sup>353</sup> score of  $< 2$ . In our study, 24.2% of elderly patients were found to have no evidence of stenosis on coronary angiography; using a comparable definition of angiographic disease (significant stenosis:  $\geq 70\%$  diameter stenosis of at least one major epicardial artery segment; or  $\geq 50\%$  diameter stenosis in the left main coronary artery). DMJ score was not available in the data from the SCRR.

The high rates of CAD and more severe disease in the elderly likely reflect a combination of delayed presentation, delayed investigation and more stringent referral or investigation criteria. Sekhri *et al.*<sup>383</sup> found that, even when deemed appropriate, coronary angiography was less likely to be performed in patients over 64 years of age compared with those aged under 50 years (HR 0.6, 95% CI: 0.4 – 1.0,  $p=0.031$ ).

As presented in Chapter 4.2, elderly patients were also more likely to have more severe disease when undergoing revascularisation. The time trends presented suggest that the prevalence of multivessel disease decreased only slightly in the elderly ( $p=0.098$ ), compared with a 16.5% fall in younger patients ( $p<0.001$ ). This suggests that the increasing numbers in younger patients may reflect use of PCI in less severe cases that may previously have been treated by medical therapy alone. However, no significant change in the threshold for management appears to exist for elderly patients undergoing PCI. In contrast, the Royal Sussex County Hospital cohort<sup>378</sup> found an increase in the proportion of calcified, tortuous and LMS disease treated in the octogenarians over time. Detailed data on lesion characteristics were not available at the time of the analysis presented in Chapter 4.2 (therefore MVD or SVD was used) while the Royal Sussex County Hospital cohort<sup>380</sup> used a different cut-off for elderly ( $\geq 80$  years old) and included primary and rescue PCI. These may be responsible for the differences in findings.

Elderly patients have been shown to have an increased risk of early and late complications following coronary angiography and PCI. Studies have reported a 4-5 fold increased risk of in-hospital death,<sup>32,201</sup> and a 4-6 fold increased risk of in-hospital MACE,<sup>5,32,201</sup> among elderly patients undergoing PCI. This thesis adds to this literature by comparing their risk of early complications following coronary angiography. The evidence presented in Chapter 4.1 and 4.2 confirms that elderly patients do have an increased risk of early complications following diagnostic angiography alone; follow-on PCI; and PCI. However, the crude risk is nonetheless low suggesting that coronary angiography and subsequent PCI are safe procedures to perform in the elderly.

Among elderly patients the baseline risk profile increased over time. For example, the presence of multiple comorbidity in elderly patients undergoing PCI increased more than 4-fold from 2000-2007 (6.1% to 21.9%,  $p<0.001$ ) and the increase in left ventricular dysfunction 37.7% to 55.4% ( $<0.001$ ). In spite of these trends over time, the risk of adverse events has not increased in the elderly, and was shown to fall overall. This suggests increasing willingness to use PCI in patients who might previously have been managed with medical therapy alone. In contrast, Rajani *et al*<sup>378</sup>. found that rates of MACCE for elderly patients declined between 2000 and 2008; however, no information on trends in comorbidity is presented, only morphology of lesions.

Over the period studied in both Chapter 4.1 and 4.2, many technical developments, new devices and adjuvant therapies, have been shown to be effective at improving coronary angiography and PCI outcomes,<sup>6,32,260,271,272,273</sup> and have been adopted into routine clinical practice in Scotland, as elsewhere. For example, over the period studied, deployment of coronary stents increased from 60% to 90% among elderly patients (47% to 93% among younger patients) and the use of ticlopidine or clopidogrel increased from 33% to 67% among elderly patients (19% to 68% among younger patients). In 1998, radial access was used in only one patient. In 2008, it was used in 37% of younger patients and 41% of elderly patients. These findings suggest that these advances have been sufficient to offset the effect of worsening risk profiles among patients.

In contrast to the USA based study by Good *et al.*<sup>384</sup> in which 96% of patients had “*ad hoc*” (follow- on PCI), only 24.1-34.5% of Scottish patients with CAD had a follow-on PCI. European based clinical guidelines recommend that follow-on PCI is “reasonable for many patients but not desirable for all” following potential indications e.g. lesions with high risk morphology.<sup>3</sup> Elderly patients were less likely to proceed to revascularisation, either as a follow-on procedure or over the subsequent year, suggesting a higher threshold for intervention even among those with angiography confirmed disease. This finding is also in keeping with the literature on referral and management patterns for CAD in elderly patients.<sup>300,386</sup>

Most studies have only been able to report in-hospital complications.<sup>5,32,201,256</sup> This has the potential to introduce bias, since length of stay in hospital is longer in elderly patients<sup>32</sup> and has fallen over time due to an increase in day-case procedures.<sup>294</sup> Through linkage to routine data we were able to obtain outcomes up to 30-days of follow-up for patients undergoing PCI and 1 year for those having coronary angiography, in both the elderly and younger patients. In Scotland, the follow-up information derived from SMR01 has been shown to be as complete and accurate as that obtained using conventional follow-up methods.<sup>294</sup> Unlike previous studies,<sup>5,32,201,351</sup> we excluded patients presenting with AMI since the use of primary and rescue PCI has significantly increased over time and varies by age.<sup>375</sup>

Chapter 4.1 and 4.2 examine increased risks associated with coronary angiography and PCI. These are undertaken to either investigate CAD with a view to revascularisation or carry out the revascularisation itself. PCI is generally undertaken to relieve the signs and symptoms of myocardial ischemia. Therefore, given these risks, it is important to assess whether or not PCI improves symptoms and QoL in elderly patients. These effects of PCI are more critical considerations for patient and health service decision-making than survival – especially in elderly patients where longevity benefits could be limited. However, self-rated QoL has been shown to be of prognostic value for estimating risk of death.<sup>387</sup>

In Chapter 4.3, the overall mean QoL was shown to improve in all participants, irrespective of age, following PCI (e.g. EQ-5D: 0.7 to 0.8,  $p < 0.001$ ; SF-12<sub>v2</sub> PCS and MCS: 39.0 vs 44.6 and 47.8 vs 50.9,  $p < 0.001$ ). While follow up was limited to QoL measurement, data linkage with SMR01 and GROS may be possible to provide data to assess short and long-term clinical outcomes. Similar findings of improved QoL have been found in a large number of other studies which have demonstrated improvements in QoL following PCI<sup>242,243, 307,308</sup>

A number of these studies, including randomised controlled trials, have compared QoL outcomes for OMT, PCI and CABG. QoL outcomes of the Clinical Outcomes Utilizing Revascularization and Aggressive Drug Evaluation (COURAGE) Trial and were published in 2007.<sup>215</sup> In this trial, 2,287 patients with stable angina pectoris were randomly assigned to PCI with optimal medical therapy or to optimal medical therapy alone. A number of issues have been highlighted regarding this study: (i) it did not meet pre-specified assumptions about statistical power despite protocol changes made after the trial was underway that placed PCI at a disadvantage; and (ii) only a small percentage of screened patients were included, revascularization was incomplete, 2/3rds of patients had angina weekly or less frequently, and 32% of the medical therapy group needed revascularization. In contrast, the study presented in Chapter 4.3 recruitment was from routine patients attending for non-emergency PCI and 74% of them had a CCS of 4 or 5.

COURAGE<sup>215</sup> assessed SAQ and SF-36 at baseline and at 3 months, 53% of patients in the PCI group and 42% in the medical-therapy group remained angina-free. By 3 months, SAQ scores had increased in the PCI group as compared with the optimal medical therapy group for: physical limitations ( $p = 0.004$ ); angina stability ( $p = 0.002$ ); angina frequency ( $p < 0.001$ ); treatment satisfaction ( $p < 0.001$ ); and QoL ( $p < 0.001$ ). There was improvement in all domains of the SF-36 in both groups between randomization and follow-up at 1 to 3 months ( $P < 0.001$  for all comparisons). This is in keeping with the findings in this thesis which used the SF-12<sub>v2</sub> because the shortened version SF-12 has been shown to correlate highly with the SF-36 in patients with CAD.<sup>388</sup> While the SF-12 provides less detailed information and may not be appropriate to use as a clinical tool on an individual patient basis, it improves efficiency and lowers costs associated with QoL research. Three QoL tools were used in this thesis to allow comparisons to be made. Using the SF-36 rather than the SF-12<sub>v2</sub> would have resulted in an even longer patient questionnaire.

RITA-2<sup>216</sup> also used the SF-36 and had a 27% crossover rate from OMT to PCI. It demonstrated that greater improvement in QoL was seen in the PCI group compared with the OMT with significantly greater improvements in physical functioning, vitality and general health at both three months and one year, but not at three years. Participants in ReQoL showed similar improvements in QoL at 3 months. Longer follow up of participants was outwith the scope of this thesis.

Following PCI, mean QoL was shown to improve as much as younger patients, even after adjusting for baseline differences. This is similar to the findings in the Trial of Invasive Versus Medical Therapy in Elderly Patients (TIME).<sup>244</sup> This trial compared two strategies for treating symptomatic, stable angina in patients aged  $\geq 75$  years. Results confirmed that an invasive diagnostic angiography approach and, depending on the result, PCI or CABG, significantly improved QoL 6 months after the procedure. This improvement was similar for men and women with CAD despite the lower overall scores for women. However, after 1 year, no significant differences in mortality, MI, or symptom improvement were noted between conservative and invasive strategies. This was primarily because, at this time, 43% of patients who initially qualified for pharmacological treatment underwent revascularization because of recurring angina. The TIME study, which looked at individuals aged  $\geq 75$  years, attained similar results to those in the Randomised Intervention Treatment of Angina (RITA-2) study,<sup>216</sup> which examined younger CAD patients with a mean age of 58 years. In both age groups, early improvement in angina symptoms and QoL after invasive treatment for CAD (by PCI or CABG) disappeared with time, after 1 year. Conversely, younger and older CAD patients treated conservatively were found to: have a greater incidence of non-fatal cardiovascular episodes and hospitalizations; use more anti-anginal drugs; require revascularization more often. Hence, their QoL was poorer.

Theile *et al.*<sup>389</sup> used both SF-36 and MacNew to compare outcomes of minimally invasive direct coronary artery bypass surgery versus sirolimus-eluting stenting in isolated proximal left anterior descending coronary artery stenosis. Baseline MacNew scores in the 65 patients receiving stenting were similar to the population in the study in this thesis for the emotional domain of MacNew (4.9 vs 4.9). However, physical and social domains of the study participants were lower (4.4 vs 4.9 and 4.7 vs 5.2 respectively). This is in keeping with the studies extensive exclusion criteria. There

were no significant differences in SF-36 and MacNew domains between stenting and surgery at follow-up, adjusted for baseline. However, patients after both stenting and surgery showed significant improvements from baseline to follow-up in all domains.

While RCTs provide the highest quality evidence and reduce the effects of bias and confounding. However, they are primarily undertaken to allow comparisons to be made between PCI and alternative management. As discussed, elderly patients are underrepresented in them. Observational studies are more likely to provide a “real world” perspective.

Using SF-36 and SAQ, Seto *et al.*<sup>308</sup> did not find differences based on age in QoL after PCI. At observation times of 6 months and 12 months, they authors found similar levels of QoL in 295 patients aged  $\geq 70$  years as in 1,150 patients aged  $< 70$  years. Li *et al.*<sup>320</sup> observed 624 elderly subjects with ACS admitted to hospital. QoL was assessed at baseline and after 6 months by SF-36. The authors found that QoL at baseline decreased with advancing age. However, even though older patients were less likely to undergo angioplasty (56% of patients aged 60–79 years versus 21% of patients aged  $> 80$  years), subjects from the older group who underwent PCI experienced the most improvement in physical health as compared with younger ones. The investigators suggest that age should not be an argument against coronary revascularization with PCI due to the potential benefits in QoL.

Spertus *et al.*<sup>225</sup> assessed QoL in 1518 consecutive non-acutely presenting patients, attending a single centre in the USA, using the SAQ. This disease specific QoL 19-item instrument includes physical limitation, change in angina symptoms, angina frequency, satisfaction with treatment, and QoL. The QoL scale directly assesses patient’s perceptions of their QoL by measuring enjoyment with life, their satisfaction with current health status and their fear of dying or having a heart attack. Höefer 2003<sup>390</sup> suggests that while both the SAQ and the MacNew questionnaires are valid, reliable, and responsive, the MacNew discriminates better between angina grades. In this thesis it was possible to assess the internal consistency of the MacNew Questionnaire in the study participants. The Cronbach’s alpha of 0.95 suggests that the questionnaire used in Chapter 4.3 had good internal consistency and the Spearman’s correlation coefficient suggests that the physical and emotional domains correlate well with the mental and physical domains of the SF-12<sub>v2</sub>.

Permanyer-Miralda *et al.*<sup>391</sup> found that, in a group of 106 patients, the Nottingham Health Profile and the DASI both statistically significantly improved at 1 month and 3 years following PCI. Baseline characteristics of this cohort shows that a higher proportion had milder symptoms at baseline compared with the study in this thesis (11% vs 4.7% - based on CCS).

Similar results were obtained in the study by de Quadros *et al.*<sup>392</sup> Patients with stable angina (n = 110) were assessed by the SAQ before PCI and followed up for 1 year. Authors demonstrate that there was an improvement in all SAQ scales after 1 year in most patients treated with PCI (68% of patients were free of angina 1 year after PCI). In multivariate analyses, QoL before the procedure was the main positive predictor of improvement in QoL. This confirmed the positive impact of PCI on symptom relief in chronic stable angina in everyday clinical practice.

Nash *et al.*<sup>393</sup> examined the baseline predictors of QoL in 1,182 patients before angioplasty. They found that poor QoL at baseline (low physical component (PCS) and low mental component (MCS) scores on SF-36) acted as an independent determinant of improvement from angioplasty 6 months after the procedure. Suggesting that baseline QoL should be taken into consideration during multivariate analysis. However, there is much debate regarding the use of baseline QoL measures in models.<sup>371</sup> Therefore, the multivariate models in this thesis were run with and without baseline measures with little impact on the overall findings.<sup>342</sup>

With decreased incidence of restenosis associated with drug eluting stents and the consequent decrease in recurring angina symptoms; stents should lead to greater improvements in QoL. Recently published data from the Rapamycin-Eluting Stent Evaluated at Rotterdam Cardiology Hospital (RESEARCH) Registry<sup>394</sup> compared drug eluting and bare metal stents. More than 800 consecutive patients (mean age, 62 years) treated with PCI with deployment of sirolimus eluting stents (SES) or BMS were involved. At inclusion, ~50% of participants suffered from stable angina. Patients were not randomized to stent type and QoL was measured with SF-36. At 1 month 59% of patients had good health status after PCI. This was maintained until 12 months. In addition, they found that poor QoL at baseline was predictive of higher mortality at 6-year follow-up, and this effect was independent of demographic and clinical characteristics. The authors

recommend that patient-reported health status should be adopted in standard clinical practice for identification of high-risk CAD patients, who will be (or already have been) treated with drug eluting stents. In this thesis some 68% of participants had a drug eluting stent rather than a bare metal stent deployed. Including stent type deployed into the multivariate analysis in Chapter 4.3 had little impact on the overall findings and did not improve the fit of the model.

Pedersen *et al.*<sup>395</sup> also assessed QoL using the MacNew questionnaire in 667 consecutive post PCI patients. They used global MacNew score to assess risk of MACE following PCI based on having a good or poor score. A poor score was defined as being in the lowest tertile. similar to the findings of the RESEARCH registry, they found that after a median follow up of 2 years incidence of early MACE was higher in patients with a poor score. While outwith the scope of this thesis, data linkage of ReQoL participants to SMR01 could be undertaken to assess short and long term MACCE outcomes.

Sipötz *et al.*<sup>372</sup> assessed QoL before and after PCI in 163 patients from 7 cardiovascular care units in Austria, using the MacNew questionnaire and HADS (Hospital Anxiety and Depression Scale). Participants were aged between 18 and 80 years and were treated with a drug eluting stent called Xience. Their main aim was to describe changes in HRQOL and mental distress. Improvement was seen in the MacNew score following PCI. They also found a strong correlation between MacNew score and HADS. While this thesis did not use HADS, this finding is in keeping with the strong correlation found between MacNew emotional score and the SF-12<sub>v2</sub> mental component score (0.74), suggesting that the MacNew emotional score is a useful way of assessing emotional status. In addition, the MCS derived from SF-36 has shown significant correlation with the MacNew emotional score in cardiac patients.<sup>390</sup> Sipötz *et al.*<sup>372</sup> also found that 48% of patients achieved or exceeded the MacNew MID of 0.5. In contrast, ReQoL found 73.8% of elderly and 77.9% of younger patients achieved the MID. However, this may be because of differences in baseline characteristics, the wider age range, loss to follow up (which was 53% for the Sipötz *et al.*<sup>343</sup> study), and that the MID was based on 1 months post PCI rather than 3 months.

Only a small number of studies have failed to demonstrate a benefit from PCI.<sup>396,397</sup> Hambrecht *et al.*<sup>396</sup> randomised 101 male patients to receive either 12 months of exercise training or PCI following positive angiography. They demonstrated improvements in CCS class by physician blinded clinical assessment which were similar in both groups. However, no QoL tool was used and most of the patients in the study had only mild exercise-induced clinical symptoms and a preserved left ventricular systolic. The second study, patients who were considered unsuitable for any revascularisation, salvage PCI (defined with advanced disease but faced with “no option” as they were experiencing ongoing intractable symptoms) did not improve QoL but did slightly improve angina status compared with baseline.<sup>397</sup> Given the baseline characteristics of the participants, neither of these studies are directly comparable with the study in this thesis. So far, the MacNew hasn’t been used to compare differences in QoL following PCI in elderly and younger patients or in the UK to assess QoL improvements following PCI.

In keeping with the systematic review of the literature in Chapter 2.3, ReQoL confirms the QoL benefit of PCI for elderly patients. In addition Chapter 4.2, shows that elderly patients are at increased risk of complications following PCI (although absolute risk is small). However, deciding who will most benefit vs risk is challenging. Currently many tools are used to assist with clinical decision making include, chronological age e.g. PTP. There is growing interest in the use of biological markers of ageing (such as LTL) to predict incidence, prognosis, and who would most benefit from invasive investigations and/or management of CAD. The first stage in assessing whether LTL could be used for this purpose is to investigate the association between LTL and the presence and severity of CAD. In Chapter 4.4 no association was found between LTL and CAD in This finding is consistent with a number of other observational studies<sup>343,352, 337</sup> while others have found an inverse association between LTL and CAD.<sup>344,339,373</sup> it has been suggested that telomere shortening might contribute to atherosclerosis through various biological ageing pathways, such as cellular senescence. For example, the accumulation of senescent cells, a prominent feature of atherosclerotic plaques, has reduces the regenerative potential of affected tissues and promotes apoptosis, which can further exacerbate inflammatory reactions, and endothelial dysfunction.

Also consistent with previous studies, an inverse relationship was found between LTL and chronological age<sup>344,339,343</sup>. This is in keeping with what is known about age-related telomere attrition and demonstrates validity of the quantitative real-time PCR undertaken by the Shiels lab. However, debate exists as to whether a variable such as LTL, that is related with age, can be a strong “age-independent” determinant of another variable, such as CAD.<sup>398</sup> Despite this, age explained only 3.3% of the variation in telomere length and age was adjusted for, in the analyses.

The comparison group used in Chapter 4.4 comprised of those with normal arteries on angiography or those who had a history of CAD based on having a previous PCI, CABG, or AMI. Angiography is undertaken primarily to investigate symptoms associated with CAD (73.1% of those attending for angiography) or as an investigation of another cardiac problem such as valvular heart disease (21.4%), often prior to surgical intervention. Similarly, Samani *et al.*<sup>341</sup> compared 10 cases with severe CAD and 20 controls, who had angiography undertaken to investigate valvular heart disease or chest pain of uncertain origin. They used Southern Blot with an interassay variation of 0.8%. Unlike the study in this thesis, they found cases had mean terminal restriction fragments 303 bps shorter: equivalent to 8.6 years younger. However, the study in this thesis has much larger numbers and has been adjusted for common confounders such as sex and deprivation.

A similar approach was also used by Carlquist *et al.*<sup>362</sup> who recruiting consecutive patients attending for angiography in Utah, USA. Similar to the population presented in Chapter 4.3, mean age was ~63 years of age and there was a greater proportion of men than women. Sixty-two point two percent of the Utah patients were found to have CAD on angiography compared with 73.1% here. It is unclear from this conference abstract if this was solely based on angiographic appearance or if, like in the thesis, included those with a clear history of CAD based on history of PCI, CABG, or AMI. They found that longer LTL was associated with a decreased risk of CAD only in univariate analysis (OR=0.54,  $p<0.0001$ ) but not in multivariate.

Mukherjee *et al.*<sup>342</sup> recruited 238 consecutive Indian patients admitted to a single centre hospital for coronary investigation or treatment of CAD (74% for diagnostic angiography, 20 for PCI and 6% for CABG). Cases were matched to control for age, sex, BMI, and smoking history, with no statistically significant difference in fasting lipids. Unlike the participants presented in Chapter 4.3 Controls in this study were drawn from the general population rather than those used in Chapter

4.4, who had negative angiographic findings. They were assessed as being free from CAD based on self-reported history, Rose Angina Questionnaire, and medications list. In cases, CAD was defined as the presence of  $\geq 50\%$  stenosis of coronary arteries compared with  $\geq 75\%$  in the study in this thesis. No indication is given if participants could have had a previous history of PCI, CABG, or AMI. Unlike the vast majority of studies, including the study in this thesis, the authors did not find an association with age. The authors suggest this surprising finding may be related to the wide age range and Indian ethnicity of the subjects.

Kurz *et al.*<sup>349</sup> also recruited participants prospectively from patients undergoing elective coronary angiography. Cases ( $n=64$ , mean age  $77.2 \pm 5.2$  years) were those undergoing investigation of CAD prior to valve replacement surgery for critical calcific aortic valve stenosis (CAC). Controls ( $n=129$ ) were those without CAC on direct measurement of the aortic valve pressures during coronary angiography (mean age  $75.9 \pm 4.3$  years). While there was an association between LTL and CAC, no significant association was found between CAD and LTL, in cases or controls, even after “investigating for possible confounding” (results not presented in the paper). LTL was measured using Southern Blot with an interassay variability of  $7.8\% \pm 7\%$ . In addition, to the validation provided through the inverse association with age, the CoV in this thesis was found to be  $0.51\%$ , which shows a high degree of precision for the technique (in comparison to the Kurtz *et al.* study). This is consistent with similar quantitative Real-Time PCR studies from Shields the laboratory.<sup>8,180</sup> It is also much lower than suggested by the literature generally for the for qPCR ( $6.45\%$  suggested by Aviv *et al.*<sup>129</sup>), and even lower than the  $1.74\%$  attributed to Southern Blot. The CoV of other published studies assessing the association between LTL and CAD range from  $1.28\text{-}9\%$ , as shown in Chapter 2.4.

A number of populations based cohort studies have assessed incidence of developing CAD.<sup>344,339,343</sup> These longitudinal studies offer the benefit of assessing the temporal nature of the association thus providing more evidence towards a causal association. However, these studies are heterogeneous in nature with differences in study designs (e.g. retrospective or prospective),

populations (e.g. confined to elderly participants<sup>348</sup>); LTL analysis method; and CAD endpoints. Evidence from large high quality studies with robust adjustments for potential confounders suggest that there is likely to be a modest association between LTL and CAD outcomes.

Weischer *et al.*<sup>337</sup> using participants in the Copenhagen City Heart Study and the Copenhagen General Population Study, demonstrated multifactorially adjusted HR for the shortest versus the longest decile of telomere length of 1.24 95% CI 1.01-1.53 for CAD. Suggesting only modest increased risk of CAD. Adjustment included inflammatory markers and lipids but also physical activity, alcohol intake and, in women, menopausal status and hormone replacement therapy. The CoV of quantitative real-time PCR was 2%. The study population consisted of 19,838 participants followed up for up to 19 years. There were 2,038 who developed CAD within the follow up period.

There is increasing evidence to suggest that potential biomarkers of ageing may be influenced by early life factors<sup>399</sup> (such as intrauterine and early years environment) with telomere length known to undergo rapid loss until ~5 years of age, after which it appears to plateau until gradual loss begins in middle age. Past studies have shown that 15%–25% of screened adult subjects might even increase their telomere length over time,<sup>178</sup> This has been criticized because ageing is known to display a unidirectional progression, and therefore it is thought to be unlikely that LTL increases with age. Apparent lengthening may be a result of changes in peripheral blood stem cells or shifting patterns of leucocyte sub-populations. As a one off LTL measurement was used in this thesis, it is not possible to assess changes over time. In addition, data on early years life experiences were not assessed. Debate exists as to the benefit of measuring telomere length in different subpopulations of leucocyte cells. However, in the study in this thesis, we measured only leucocyte telomere length as an average of total white cells rather than TL in separate leucocyte subpopulations.

A number of studies have shown an association between LTL and adverse MACCE outcomes in those with existing CAD.<sup>356,357,358</sup> Ruff *et al.*<sup>358</sup>. and Perez-Rivera *et al.*<sup>357</sup> used populations who attended with ACS and found that short LTL was strongly associated with subsequent AMI. However, Farzaneh-far *et al.*<sup>356</sup> in the San Francisco based Heart and Soul Study found an increased risk of death and heart failure but not of a subsequent CV event. Exclusions included

those with a previous history of AMI in the last 6 months, those who deemed themselves unable to walk 1 block, or those planning to move out of the area. These exclusions limit the generalisability of the findings to a population of people with CAD attending clinics. Carlquist *et al.*<sup>361</sup> followed up patients who had undergone coronary angiography for a median of 9.2 years and found that, after adjustment for a number of baseline characteristics (including medication use, number of stenosed vessels, and hyperlipidaemia), longer LTL was associated with decreased risk of death (HR=0.51,  $p<0.001$ ) but not with AMI or CVA. It will be possible to link the cohort in this thesis by datalinkage to: SMR01, for hospital discharges; GROS, for death data; and to the SCCRR, for angiography and revascularization procedures. In addition, for those without evidence of CAD on angiography, similar long term outcomes and incidence of CAD can be assessed. It has been hypothesised that, by reducing the proliferative potential of vascular smooth muscle cells, cellular senescence promotes the thinning of fibrous caps and the instability of atherosclerotic plaques, therefore making rupture and subsequent AMI much more likely.<sup>400</sup>

Calvert *et al.*<sup>359</sup> included 170 patients with stable and unstable angina who were referred for PCI and underwent 3-vessel virtual histology intravascular ultrasound. Shorter LTL was found to be associated with high-risk plaque morphology but not total 3-vessel burden; adding to the evidence that leucocyte ageing is involved in vulnerable plaque formation.

The literature suggests that cardiovascular drugs may impact on telomere biology (eg, in relation to treatment by antihypertensive<sup>176,401</sup>). It was not possible to assess the influence of cardiovascular drugs on the association between LTL and CAD in this thesis. It should be possible to review clinical records and elicit such information on medications used by the participants in this study. However, this is outwith the scope of this thesis.

Another limitation is whether using LTL reflects the tissue cell telomere length in coronary arteries. However, there is evidence to suggest that the correlation in telomere length between leucocytes and other tissues (including vascular tissue) in healthy humans is strong.<sup>123,124</sup> In addition, LTL derived from peripheral blood has been associated with the presence of atherosclerotic plaques in the carotid arteries; however, it was not found to be a proxy for local plaque telomere length.<sup>331</sup> Further research is required to assess the correlation between LTL and coronary artery endothelial cells and/plaques, which may show stronger associations with CAD.

Further analysis of the blood collected in this sample could be undertaken to assess inflammatory markers (such as IL6 or CRP). As discussed in the introduction, although inflammatory markers are non-specific, abnormal values are thought to indicate clinical or subclinical levels of chronic inflammation that contribute to the ageing process – possibly through oxidative stress. Senescent cells are associated with a high level of intracellular ROS and accumulation of oxidative damage to DNA and proteins.<sup>78</sup> Telomere shortening is considered to be one of the major cause of replicative cellular senescence and is increased as a result of mild oxidative stress.<sup>46,78,72</sup> They are also thought to contribute to the development and progression of atherosclerosis.<sup>300,301,302,303,304,</sup>

Controversy exists as to how appropriate the use of telomere length is as a biomarker of ageing, particularly as studies move from laboratory based to population based observational studies.<sup>8,96,97</sup> Therefore, exploring the association between alternative biomarkers of ageing and CAD would be useful. Particularly in this population where no association was found between LTL and CAD. In addition to inflammatory markers, blood collected from the participants in this thesis can be used to measure other biomarkers of ageing such as CDKN2a, AEGs, Fetuin A, Sertuins 1-9, homocystine, and DNA methylation. All of which have been associated with ageing and atherosclerosis in humans (see Chapter 1.1.4 for a fuller discussion).<sup>97,98,99,100,101,104,105</sup>

## 6. OVERALL CONCLUSION

Population projections from GROS suggest that the phenomenon of population ageing will continue in Scotland for at least the next 25 years. This demographic trend is likely to result in a rise in the prevalence and complexity of degenerative disorders such as CAD. Therefore, elderly patients are likely to represent an increasing proportion of those presenting for investigation and treatment of CAD, with resultant implications for health services in the future. Chapters 4.1 and 4.2 demonstrated that elderly people do account for an increasing number, and proportion, of patients presenting for the investigation and management of CAD and that over time the baseline risk profile has been increasing. This demographic trend, together with changes in patient selection

and case mix, will likely increase the underlying risk of periprocedural complications. However the time trend data for PCI complications presented in Chapter 4.2 suggests that we have managed to offset this effect, presumably as a result of technical improvements and the adoption of new devices and adjuvant therapies. However, further developments may be required if we are to avoid worsening outcomes in the future.

Chapter 4.1 demonstrated that the threshold for investigation, via coronary angiography, and subsequent intervention, using PCI, was higher among this increasingly large group of patients, even after taking account of co-morbidities. This suggests a chronological age-based inequality in access to investigation and treatment. Inequality might, nonetheless, be justified if the risks were unacceptably high or benefits less among elderly patients. However, this was not found to be the case. Chapters 4.1 and 4.2 demonstrated that elderly patients do have a higher risk of early complications than younger patients, but their absolute risk was, nonetheless, low. This suggests that coronary angiography and PCI are safe procedures to perform in the elderly. These findings have significant implications for the delivery of cardiovascular clinical services to an increasingly important sub-group of patients. Further investigation of referral patterns and apparent thresholds are required, using populations referred for CAD investigation and not receiving diagnostic coronary angiography.

The current ESC guidelines <sup>3</sup> recommend that referral for investigation of CAD by non-invasive techniques is preferred except in those who would most benefit from revascularisation, (for example, high PTP, where it is used to assess the severity of CAD to inform future management options); in patients where the findings of non-invasive testing are inconclusive; in some professions who cannot have stress testing for regulatory reasons; or in those where optimal medical management fails to control symptoms. However, severity of clinical symptoms, the patient's risk of adverse events from coronary angiography, and patient preferences, are also taken into consideration in determining investigation of CAD. These recently changed guidelines are likely to have a significant impact on referral patterns for coronary angiography in the future. It may well off-set the effects of population ageing on the number and proportion of elderly patients

undergoing coronary angiography. However, the PTP is influenced by the prevalence of CAD in the population (as well as clinical features of an individual) and prevalence of CAD is known to increase with chronological age. Further investigation of this guideline change on referral patterns and apparent thresholds in elderly patients using populations referred for CAD investigation and not receiving non-invasive diagnostic testing, or diagnostic coronary angiography, is required.

PCI is generally undertaken in elderly patients to alleviate the signs and symptoms of CAD. Therefore, assessing the benefits of PCI on QoL is as important as investigating the risk of peri-procedural complications and longer term MACCE outcomes (as shown in Chapter 4.2.). QoL is used to measure an individual's experience of his or her health status and health-related well-being. A number of QoL tools have been developed to document experience of illness and healthcare and to measure what effects of treatment are deemed to be of greatest importance to patients. However, there is no consensus on the definition of QoL or what is the most appropriate measurement tool. In keeping with the systematic review of the literature in Chapter 2.3, the findings of ReQoL confirms that QoL (using generic and disease specific QoL measurement tools) following PCI in older patients improves at least as much as in younger patients.

Chapters 4.1, 4.2, 4.3 all used time from birth to define elderly. In contrast to chronological ageing, biological ageing involves variable structural and functional changes that take place at the cellular, tissue and organ level; these ultimately affect the overall performance of the body. Biological ageing is thought to vary between individuals of the same chronological age and increase susceptibility to ill health and disease. There is currently much interest in whether biomarkers of ageing may provide a better measurement of "miles on the clock" and whether biological age contributes to the development and progression of disease, such as CAD, and can explain socioeconomic inequalities in health. However, we found no association between LTL and either the occurrence of CAD, or its severity, on cross-sectional study. While LTL is considered a useful biomarker of ageing, the findings in this thesis suggest that LTL may not be as useful in CAD. Longitudinal studies are required to assess the usefulness of LTL as a biomarker of ageing in CAD and to investigate whether LTL is associated with adverse outcomes in patients diagnosed with CAD.

This thesis has used a systematic review, one near systematic review, two secondary data analyses, and two primary research studies to explore both chronological and biological ageing in CAD. Overall, the findings suggest that: (i) the ageing population is likely to continue to have an impact on cardiovascular services for the near future; (ii) adverse outcomes following angiography and PCI are more common in elderly patients; (iii) there appears to be different thresholds for investigation and management of CAD in elderly patients; (iv) coronary angiography and PCI are safe procedures in elderly patients with PCI improving their QoL; and (v) LTL may not be as useful a biomarker of ageing in CAD.

Population projections from GROS suggest that the phenomenon of population ageing will continue in Scotland for at least the next 25 years. This demographic trend is likely to result in a rise in the prevalence and complexity of degenerative disorders such as CAD. Therefore, elderly patients are likely to represent an increasing proportion of those presenting for investigation and treatment of CAD with resultant implications for health services in the future. Chapters 4.1 and 4.2, confirm that elderly people do account for an increasing number and proportion of patients presenting for the investigation and management of CAD and that over time the baseline risk profile has been increasing. The demographic trend, together with changes in patient selection and case mix, will likely increase the underlying risk of periprocedural complications. However the time trend data for PCI complications presented in Chapter 4.2 suggests that we have managed to offset this effect, presumably as a result of technical improvements and the adoption of new devices and adjuvant therapies. However, further developments may be required if we are to avoid worsening outcomes in the future.

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coronary angiography and PCI are safe procedures to perform in the elderly. These findings have significant implications for the delivery of cardiovascular clinical services to an increasingly important sub-group of patients. Further investigation of this referral patterns and apparent threshold are required using populations referred for CAD investigation and not receiving diagnostic coronary angiography, is required.

The current ESC guidelines<sup>139</sup> recommend that referral for investigation of CAD by non-invasive techniques is preferred except in: those those who would be most likely benefit from revascularisation (for example, high PTP, where it is used to assess the severity of CAD (rather than diagnose CAD) to inform future management options); in patients where the findings of non-invasive testing are inconclusive; in some professions who cannot have stress testing for regulatory reasons; or in those where optimal medical management fails to control symptoms. However, severity of clinical symptoms, the patient's risk of adverse events from coronary angiography, and patient preferences, are also taken into consideration in determining investigation of CAD. This change is likely to have a significant impact on referral patterns for coronary angiography in the future. It may well off-set the effects of population ageing on the number and proportion of elderly patients undergoing coronary angiography. However, the PTP is influenced by the prevalence of CAD in the population (as well as clinical features of an individual) and prevalence of CAD is known to increase with chronological age. Further investigation of this guideline change on referral patterns and apparent thresholds in elderly patients using populations referred for CAD investigation and not receiving non-invasive diagnostic testing or diagnostic coronary angiography, is required.

PCI is generally undertaken in elderly patients to alleviate the signs and symptoms of CAD. Therefore, assessing the benefits of PCI on QoL is as important as investigating the risk of peri-procedural complications and longer term MACCE outcomes (as shown in Chapter 4.2.). QoL is used to imply an individual's experience of his or her health status and health-related well-being. A number of QoL tools have been developed to document experience of illness and healthcare and to measure what effects of treatment are deemed to be of greatest importance to patients. However, there is no consensus on the definition of QoL or the most appropriate tool to measure it with. In keeping with the systematic review of the literature in Chapter 2.3, the findings of ReQoL

confirms that QoL (using generic and disease specific QoL measurement tools) following PCI in older patients improves at least as much as younger patients.

Chapters 4.1, 4.2, 4.3 all used time from birth to define elderly. In contrast to chronological ageing, biological ageing involves variable structural and functional changes that take place at the cellular, tissue and organ level; ultimately affecting the overall performance of the body. It is thought to vary between individuals of the same chronological age and increase susceptibility to ill health and disease. There is currently much interest in whether biomarkers of ageing may provide a better measurement of “miles on the clock.” and whether biological age contributes to the development and progression of disease, such as CAD, and can explain socioeconomic inequalities in health. However, we found no association between LTL and either the occurrence of CAD, or its severity, on cross-sectional study. While LTL is considered a useful biomarker of ageing, the findings in this thesis suggest that LTL may not be as useful in CAD. Longitudinal studies are required to assess the usefulness of LTL as a biomarker of ageing in CAD and to investigate whether LTL is associated with adverse outcomes in patients diagnosed with CAD.

This thesis has used: 1 systematic review, 1 near systematic review, 2 secondary data analysis, and 2 primary research projects to explore both chronological and biological ageing in CAD. Overall, the findings suggest that: (i) the ageing population is likely to continue to have an impact on cardiovascular services for the near future; (ii) adverse outcomes following angiography and PCI are more common in elderly patients; (iii) there appears to be different thresholds for investigation and management of CAD in elderly patients; (iv) coronary angiography and PCI are safe procedures in elderly patients and PCI improves their QoL; and (v) LTL may not be as useful a biomarker of ageing in CAD.

## **6.1. *Recommendations for future research***

- Chapters 4.1 and 4.2 investigated the trends, case-mix, and adverse outcomes associated with coronary angiography and PCI. Results suggest that there appears to be

different thresholds for investigation and management of CAD in elderly patients. However, the findings are based on patients having these procedures. To determine the extent to which there are inequalities in referral threshold, as well as procedure threshold, further studies should be undertaken on patients referred to cardiology services for investigation of CAD rather than just those receiving it. In addition, populations attending GPs with symptoms suggestive of CAD should be studied to explore whether inequalities exist in referral to specialist services for CAD investigation.

- The recent ESC guideline on managing stable CAD has updated the recommendation on who should be referred for invasive investigation and management. This change is likely to have a significant impact on referral patterns for coronary angiography and PCI in the future. It may well off-set the effects of population ageing on the number and proportion of elderly patients undergoing coronary angiography. However, further investigation of this guideline change is required on referral patterns and apparent thresholds in elderly patients using populations referred for CAD investigation but not receiving non-invasive diagnostic testing or diagnostic coronary angiography.
- The ReQoL study in Chapter 4.3 assessed QoL outcomes in patients undergoing PCI. However, patients were only followed up to 3 months post procedure, due to the constraints of data collection within a PhD programme. The systematic review in Chapter 2.3 suggests that improvements in quality of life may well extend to at least 3 years. However, this review only included a small number of studies. Further research is required to assess the long term impact of PCI on QoL.

- ReQoL (in Chapter 4.3) assessed QoL outcomes; however, these may well be influenced by adverse clinical outcomes such as MACCE. Additional research should be carried out on this cohort by data linkage with SMR01 to assess short and long-term MACCE outcomes. This would also allow the investigation of the association between baseline QoL and frailty with MACCE outcomes.
- Given the small number of participants included in ReQoL, a larger study would be important to provide more robust evidence of the impact of PCI in elderly patients. Given the heterogeneity of studies included in the systematic review in Chapter 2.3, further research which assesses the most appropriate measurement tool for QoL in this group would be important. This would likely involve comparisons between 2 or more QoL life tools such as in ReQoL. However, given the low recruitment rate in ReQoL, care must be taken that the questionnaire length is not too onerous for participants. In addition, recruitment and consent for such a study would be best undertaken at the time of procedure consent; given the success of recruitment for the study in Chapter 4.4.
- The findings in Chapter 4.4 suggest that LTL may not be as useful a biomarker of ageing in CAD as some studies have suggested. However, the main limitations are the cross-sectional nature of the study and that the comparison group comprised of those undergoing angiography for symptoms suggestive of CAD, rather than using a sample from the general population. Therefore, longitudinal studies are required to assess the usefulness of LTL as a biomarker of ageing in CAD.
- In addition, Chapter 4.4. found no association between the occurrence of in-lab complication and telomere length and in keeping with Chapter 4.1 and 4.3 there were

only a small number of in-lab complications. It was not possible for this thesis to assess the association between LTL and short and longer-term adverse outcomes. Additional research should be undertaken to investigate whether LTL is associated with adverse outcomes in patients diagnosed with CAD. Data linkage with SMR01 would allow this to be undertaken in this cohort.

- There is debate in the literature as to whether or not LTL is an appropriate biomarker of ageing. However, in Chapter 4.4 only LTL was used. Further analysis of the blood taken from participants should be used to investigate emerging biomarkers of ageing, such as those discussed in Chapter 1.1.3. For example, fetuin A, sirtuin 1-7, CDKN2a. In addition, inflammatory markers should be measured in the blood, given the association between inflammation and cellular senescence.
- Chapter 4.4 used a cross-sectional study design to investigate the association between LTL and CAD, rather than longitudinal measurement of telomere length in the same individuals. Serial measurements of LTL in patients with and without CAD would allow assessment of telomere attrition of patients with CAD and the influence of interventions over time.
- LTL is thought to correlate well with telomere length in somatic tissue, such as endothelial cells; however, further laboratory based research is required to confirm this correlation.
- Given the debate as to whether telomere shortening is the primary abnormality, or a result of accelerated attrition of telomeres by cardiovascular risk factors and disease, further research is required to better understand the mechanisms involved. To do this,

research which assesses the lifetime trajectory of telomere length and its association with degenerative diseases, such as CAD, is required. For example, this should include the use of large longitudinal studies such as birth cohorts (which would ideally include an assessment of prenatal factors, given that prenatal factors could programme a foetus for increased risk of CAD) or cohorts comprising of healthy individuals. In addition, this approach is likely to provide a better understanding of the ageing process itself and its relationship to cardiovascular risk factors and disease.

## **6.2. *Lessons learned***

Undertaking this PhD has overall been a challenging and rewarding experience. It has afforded a valuable insight into the different theories and concepts of ageing and quality of life. This knowledge and understanding of these theories and concepts are very useful, and will be particularly as research in this area is likely to continue to be important in the future

This PhD has allowed me the opportunity to apply all of the statistical techniques studied as part of my MPH course and many of those included in the FPH Part A syllabus.

I was able to gain experience in coordinating and conducting two primary research projects. This included gaining NHS ethics, developing protocols, questionnaire design, and actively liaising with the cardiac research nurses at the Golden Jubilee National Hospital. In addition, I learned that undertaking primary research projects is a considerable undertaking and that it can be fraught with difficulties such as slow recruitment.

I've learned that writing up a PhD is exponentially more challenging than an MPH, or even 5 MPH projects (although equivalent in word count!). I also learned that referencing software is amazing useful until it isn't.

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## 8. APPENDICES

### ***8.1. Ethics approval letter for Revascularisation and Quality of Life (ReQoL)***

#### **West of Scotland REC 2**

Western Infirmary  
Ground floor, Tennent Institute  
38 Church Street  
Glasgow G11 6NT  
Telephone: 0141 211 2123  
Facsimile: 0141 211 1847



21 August 2009

Dr Cathy Johnman  
Clinical Lecturer Public Health  
University of Glasgow  
1 Lilybank gardens  
Glasgow G12 8RZ

Dear Dr Johnman **Study Title:**

**ReQoL: Revascularisation: Quality of life and clinical outcomes following revascularisation procedures.**

**REC reference number:**

**09/S0709/58**

**Protocol number:**

**1**

The Research Ethics Committee reviewed the above application at the meeting held on 18 August 2009. Thank you for attending to discuss the study

#### **Ethical opinion**

The members of the Committee present gave a favourable ethical opinion of the above research on the basis described in the application form, protocol and supporting documentation, subject to the conditions specified below. As discussed at the meeting the following points require to be clarified through the Committee Co-ordinator before the favourable opinion is valid.

#### **1) Participant Information Sheet**

- The Researcher confirm that the study was looking at the quality of life in the over 75's population in comparison to the under 75's but this was not reflected in the Participant Information Sheet. This should be made clear in the Participant Information Sheet.

- The final sentence at 'What will happen if I take part?' should be changed to read 'The Research Nurse will contact you only as a reminder or if we require further information about your health'.
- At 'Contact for further information' as well as contact details for the Researcher, there should be contact details for someone independent from the study who could speak to participants.

A revised Participant Information Sheet taking account of the above comments should be submitted.

## 2) **Consent Form**

- Each statement starts with 'I agree'. Each statement should begin with 'I understand'.
- The final statement should be reworded to read 'I understand that the Research Team may wish to access my notes to provide additional follow-up information about my health'.

A revised Consent Form taking account of the above comments should be submitted.

## 3) **Questionnaire**

- It is preferred that the section on 'Questions About You' at the end of the questionnaire should be on a separate page as these details are only required once and not at each follow up visit.

A revised Questionnaire taking account of this comment should be submitted.

## **Ethical review of research sites**

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

### **Conditions of the favourable opinion**

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

### **After ethical review**

Now that you have completed the application process please visit the National Research Ethics Service website > After Review

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.

The attached document "After ethical review – guidance for researchers" gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators

- Progress and safety reports
- Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

We would also like to inform you that we consult regularly with stakeholders to improve our service. If you would like to join our Reference Group please email [referencegroup@nres.npsa.nhs.uk](mailto:referencegroup@nres.npsa.nhs.uk).

**09/S0709/58**

**Please quote this number on all correspondence**

With the Committee's best wishes for the success of this project

Yours sincerely

**Liz Jamieson**

**Committee Co-ordinator on behalf of Dr S Langridge, Chair**

Email: [Liz.Jamieson@ggc.scot.nhs.uk](mailto:Liz.Jamieson@ggc.scot.nhs.uk)

Enclosures:

List of names and professions of members who were present at the meeting and those who submitted written comments  
"After ethical review – guidance for researchers"

## **8.2. Participant information leaflet for Revascularisation and Quality of Life (ReQoL)**

Version 1 31/08/09 Code: AB/147118/1

**GOLDEN JUBILEE NATIONAL HOSPITAL**

NHS National Waiting Times Centre



### **PARTICIPANT INFORMATION LEAFLET** **Revascularisation and Quality of Life Study (ReQoL)**

#### **Introduction**

You are being invited to take part in a research study. Before you take part it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and to discuss it with others if you wish. Ask us if there is anything that is not clear or if you would more information (contact details are at the end of this leaflet)

#### **What is the purpose of the study?**

We would like to collect information on the impact of this procedure on your general health and quality of life. We are particularly interested in how the health and quality of life of older patients are affected in comparison to younger patients, undergoing this procedure. We also want to determine which types of questions are most helpful in assessing your health and quality of life.

#### **Why have I been chosen?**

You have been chosen because you are having a cardiac procedure to treat the narrowing of the blood vessels of the heart in the Golden jubilee hospital.

#### **Do I have to take part?**

It is up to you to decide whether or not to take part. If you decide to take part you will be given the opportunity to discuss your involvement with the research nurse at the time of your admission where you will be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time without having to give a reason. Whether you take part or not, will not affect the type or the standard of care you receive.

#### **What will happen if I take part?**

On admission, one of the research nurses will ask you some questions about your health and how your health affects your usual activities. We will then post out a similar questionnaire at 3 months, 6 months and 12 months after you procedure (this should only take around 20 minutes to complete). The research nurse will contact you only as a reminder or if we require further information about your health. Version 1 31/08/09 Code: AB/147118/1

What are the possible benefits of taking part?

Taking part in the study will be of no direct benefit to you but will be very useful in assessing the overall effect of cardiac procedures for narrowing of the blood vessels of the heart on your health and quality of life. It will give us valuable information which will be helpful in caring for future patients.

Will my taking part in the study be kept confidential?

All information which is collected about you during the course of the research will be kept strictly confidential.

What will happen to the results of the research study?

Only those directly involved in the study will have access to any information which has your name and address on it and then, only for the purposes of arranging the study follow up.

Your name and other details will be removed from the questionnaire and will be kept separate from your answers.

Any information which is then examined for this study will have your name and address removed so that you cannot be recognised from it.

Who has funded the research?

The research is jointly funded by the NHS and by the Alexander and Margaret Hutchison Trust (associated with the University of Glasgow) which was set up to help and support research which is likely to be of benefit to the health and welfare of the residents of the West of Scotland.

Who has reviewed the study?

The study was reviewed and approved by the West of Glasgow Ethics Committee.

For independent advice on this study, if required please contact:

Dr Hany Eteiba MD FRCP, FACC, FSCAI  
National Waiting Times Centre Board  
Golden Jubilee National Hospital  
Beardmore Street  
Clydebank  
G81 4HX

Direct Study Contacts:

Research Nurses:

**Joanne Kelly, Maureen Mason and Elizabeth Boyd**  
**Golden Jubilee National Hospital**  
**Beardmore Street**  
**Clydebank**  
**G81 4HX**  
**Tel: 0141 951 5256**  
**Email: joanne.kelly@gjnh.scot.nhs.uk**

### **8.3. Participant consent form for Revascularisation and Quality of Life (ReQoL)**

**GOLDEN JUBILEE NATIONAL HOSPITAL**

NHS National Waiting Times Centre



## **CONSENT FORM**

**ReQoL (Revascularisation and Quality of Life)**

**Study number:** \_\_\_\_\_

**Name:** \_\_\_\_\_

**Hospital: Golden Jubilee National Hospital**

**CHI Number:**  
(Community Health Index)

--	--	--	--	--	--	--	--	--	--

**Please put your  
initials in boxes**

I confirm that I have read and understood the information leaflet

☐

I understand that I will be asked to complete an initial questionnaire on admission and that additional questionnaires will be sent to me at 3, 6 and 12 months after my procedure

☐

I understand that the research team may wish to contact me for follow up should it be required

☐

I understand that my GP will be informed of my involvement in this study

☐

I understand that the research team may wish to access my notes to provide additional follow-up information about my health.

--	--	--

Name of participant

Date

Signature

--	--	--

Name and designation of person taking consent

Date

Signature

#### **8.4. Participant questionnaire for Revascularisation and Quality of Life (ReQoL)**

ReQoL Study number: XXXX

Date:.....

Study number:.....

CHI number:.....

Hospital number:.....

Procedure date: .....

Name:.....

Date of Birth:.....

Address:

Contact telephone number:

GP details:

We would be grateful if you could help us by filling out this questionnaire about your health and quality of life. Any information will have your name and address removed so that you cannot be recognised from it.

Please try and answer all the questions.

### **Overall general health**

**By placing a tick in one box in each group below, please indicate which statements best describe your own health state TODAY**

#### **1. Mobility**

**TICK ONE**

I have no problems in walking about

☐

I have some problems in walking about

☐

I am confined to bed

☐

#### **2. Self-Care**

I have no problems with self-care

☐

I have some problems washing or dressing myself

☐

I am unable to wash or dress myself

☐

#### **3. Usual Activities (e.g. work, study, housework, family or leisure activities)**

I have no problems with performing my usual activities

☐

I have some problems with performing my usual activities

☐

I am unable to perform my usual activities

☐

#### **4. Pain/Discomfort**

I have no pain or discomfort

☐

I have moderate pain or discomfort

☐

I have extreme pain or discomfort

☐

### 5. Anxiety/Depression

I am not anxious or depressed

I am moderately anxious or depressed

I am extremely anxious or depressed

**TICK ONE**

☐☐☐

We would like you to indicate on this scale how good or bad your own health is **TODAY**, in your opinion. Please do this by drawing a line from the box below to whichever point on the scale indicates how good or bad your health state is today.

**Your own  
health state today**

Best  
imaginable  
health state

100

90

80

70

60

50

40

30

20

10

0

Worst  
imaginable  
health state

## Detailed general health

1. In general, would you say your health is: **(TICK ONE BOX ONLY)**

Excellent

Very good

Good

Fair

Poor

☐☐☐☐☐

The following two questions are about activities you might do during a typical day. Does **YOUR HEALTH NOW LIMIT YOU** in these activities? If so, how much?

2. MODERATE ACTIVITIES, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf: **(TICK ONE BOX ONLY)**

Yes, limited a lot

Yes, limited a little

No, not limited at all

☐☐☐

3. Climbing SEVERAL flights of stairs: **(TICK ONE BOX ONLY)**

Yes, limited a lot

Yes, limited a little

No, not limited at all

☐☐☐

During the PAST 4 WEEKS have you had any of the following problems with your work or other regular activities AS A RESULT OF YOUR PHYSICAL HEALTH? **(Please tick one box per question)**

**(TICK ONE BOX ONLY)**

All of  
the time

A good bit  
of the time

Some  
of the  
time

A little  
of the  
time

None of  
the time

(TICK ONE BOX PER QUESTION)

4. ACCOMPLISHED LESS than you would like ☐ ☐ ☐ ☐ ☐

5. Were limited in the KIND of work or other activities ☐ ☐ ☐ ☐ ☐

During the PAST 4 WEEKS, were you limited in the kind of work you do or other regular activities AS A RESULT OF ANY EMOTIONAL PROBLEMS (such as feeling depressed or anxious)?

All of the time    A good bit of the time    Some of the time    A little of the time    None of the time

6. ACCOMPLISHED LESS than you would like ☐ ☐ ☐ ☐ ☐

7. Did work or activities less carefully than usual ☐ ☐ ☐ ☐ ☐

8. During the PAST 4 WEEKS, how much did PAIN interfere with your normal work (including both work outside the home and housework)? (TICK ONE BOX ONLY)

Not at all    A little bit    Moderately    Quite a bit    Extremely

☐ ☐ ☐ ☐ ☐

The next three questions are about how you feel and how things have been DURING THE PAST 4 WEEKS.

For each question, please give the one answer that comes closest to the way you have been feeling. How much of the time during the PAST 4 WEEKS –

**(TICK ONE BOX PER QUESTION)**

	All of the time	A good bit of the time	Some of the time	A little of the time	None of the time
9. Have you felt calm and peaceful?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
10. Did you have a lot of energy?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
11. Have you felt downhearted and depressed?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

12. During the PAST 4 WEEKS, how much of the time has your PHYSICAL HEALTH OR EMOTIONAL PROBLEMS interfered with your social activities (like visiting with friends, relatives, etc.)? **(TICK ONE BOX ONLY)**

All of the time	A good bit of the time	Some of the time	A little of the time	None of the time
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

### **Heart Health questions**

Please be sure to answer all questions.

1. During the past 4 weeks, how much were you bothered by each of the following problems related to your **heart condition**?

**(TICK ONE BOX PER QUESTION)**

	A lot	Quite a bit	Moderately	A little	Not at all
Chest pain due to angina	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Discomfort in your chest due to angina	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Shortness of breath	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
---------------------	--------------------------	--------------------------	--------------------------	--------------------------	--------------------------

Angina pain that radiates to other parts of your body (eg arms, shoulders, hands, neck, throat, jaw, back)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
--	--------------------------	--------------------------	--------------------------	--------------------------	--------------------------

Palpitations (strong or irregular heart beat)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
---	--------------------------	--------------------------	--------------------------	--------------------------	--------------------------

2. Over the past 4 weeks, on average, how many times have you had chest pain, chest tightness, or angina? **(TICK ONE BOX ONLY)**

	4 or more times per day	1-3 times per day	3 or more times per week but not every day	1-2 times per week	Less than once per week	None over the past 4 weeks
I get chest pain, chest tightness, or angina...	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

3. During the past 4 weeks, have you had **chest pain, chest tightness or angina**: (TICK ONE BOX ONLY)

At rest	On exertion	At rest and on exertion	Not at all
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

4. During the past 4 weeks, how much trouble has your **heart condition** caused you?  
(TICK ONE BOX ONLY)

A lot	Quite a bit	Some	A little	None
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

1. The following questions ask about activities which you might do during a typical day. During the past 4 weeks, has your **heart condition** limited you in your usual daily activities? Please indicate whether your heart condition limits you a lot, limits you a little, or does not limit you at all in the activities listed below.

(TICK ONE BOX PER QUESTION)

All of the time	A good bit of the time	Some of the time	A little of the time	None of the time
--------------------	------------------------------	---------------------	-------------------------	---------------------

1. Moderate activities, such as moving a table, pushing a vacuum cleaner, bowling or playing golf	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
2. Lifting or carrying groceries	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
3. Climbing several flights of stairs	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
4. Climbing one flight of stairs	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
5. Bending, kneeling or stooping	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
6. Walking half a mile	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
7. Walking one hundred yards	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
8. Bathing or dressing yourself	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

### Heart disease quality of life

We would like to ask you some more questions about how you have been feeling **DURING THE LAST 2 WEEKS (rather than 4 weeks)**

☐

**Please tick the box that matches your answer**

1. In general, how much of the time during the last 2 weeks have you felt frustrated, impatient or angry?

- |   |                          |                        |
|---|--------------------------|------------------------|
| 1 | <input type="checkbox"/> | ALL OF THE TIME        |
| 2 | <input type="checkbox"/> | MOST OF THE TIME       |
| 3 | <input type="checkbox"/> | A GOOD BIT OF THE TIME |
| 4 | <input type="checkbox"/> | SOME OF THE TIME       |
| 5 | <input type="checkbox"/> | A LITTLE OF THE TIME   |
| 6 | <input type="checkbox"/> | HARDLY ANY OF THE TIME |
| 7 | <input type="checkbox"/> | NONE OF THE TIME       |

2. How often during the last 2 weeks have you felt worthless or inadequate?

- |   |                          |                        |
|---|--------------------------|------------------------|
| 1 | <input type="checkbox"/> | ALL OF THE TIME        |
| 2 | <input type="checkbox"/> | MOST OF THE TIME       |
| 3 | <input type="checkbox"/> | A GOOD BIT OF THE TIME |
| 4 | <input type="checkbox"/> | SOME OF THE TIME       |
| 5 | <input type="checkbox"/> | A LITTLE OF THE TIME   |
| 6 | <input type="checkbox"/> | HARDLY ANY OF THE TIME |
| 7 | <input type="checkbox"/> | NONE OF THE TIME       |

3. In the last 2 weeks, how much of the time did you feel very confident and sure that you could deal with your heart problem?

- |   |                          |                        |
|---|--------------------------|------------------------|
| 1 | <input type="checkbox"/> | NONE OF THE TIME       |
| 2 | <input type="checkbox"/> | A LITTLE OF THE TIME   |
| 3 | <input type="checkbox"/> | SOME OF THE TIME       |
| 4 | <input type="checkbox"/> | A GOOD BIT OF THE TIME |
| 5 | <input type="checkbox"/> | MOST OF THE TIME       |
| 6 | <input type="checkbox"/> | ALMOST ALL OF THE TIME |
| 7 | <input type="checkbox"/> | ALL OF THE TIME        |

4. In general how much of the time did you feel discouraged or down in the dumps during the last 2 weeks?

- |   |                          |                        |
|---|--------------------------|------------------------|
| 1 | <input type="checkbox"/> | ALL OF THE TIME        |
| 2 | <input type="checkbox"/> | MOST OF THE TIME       |
| 3 | <input type="checkbox"/> | A GOOD BIT OF THE TIME |
| 4 | <input type="checkbox"/> | SOME OF THE TIME       |
| 5 | <input type="checkbox"/> | A LITTLE OF THE TIME   |
| 6 | <input type="checkbox"/> | HARDLY ANY OF THE TIME |
| 7 | <input type="checkbox"/> | NONE OF THE TIME       |

5. How much of the time during the past 2 weeks did you feel relaxed and free of tension?

- |   |                          |                        |
|---|--------------------------|------------------------|
| 1 | <input type="checkbox"/> | NONE OF THE TIME       |
| 2 | <input type="checkbox"/> | A LITTLE OF THE TIME   |
| 3 | <input type="checkbox"/> | SOME OF THE TIME       |
| 4 | <input type="checkbox"/> | A GOOD BIT OF THE TIME |
| 5 | <input type="checkbox"/> | MOST OF THE TIME       |
| 6 | <input type="checkbox"/> | ALMOST ALL OF THE TIME |
| 7 | <input type="checkbox"/> | ALL OF THE TIME        |

6. How often during the last 2 weeks have you felt worn out or low in energy?

- |   |                          |                        |
|---|--------------------------|------------------------|
| 1 | <input type="checkbox"/> | ALL OF THE TIME        |
| 2 | <input type="checkbox"/> | MOST OF THE TIME       |
| 3 | <input type="checkbox"/> | A GOOD BIT OF THE TIME |
| 4 | <input type="checkbox"/> | SOME OF THE TIME       |
| 5 | <input type="checkbox"/> | A LITTLE OF THE TIME   |
| 6 | <input type="checkbox"/> | HARDLY ANY OF THE TIME |
| 7 | <input type="checkbox"/> | NONE OF THE TIME       |

7. How happy, satisfied, or pleased have you been with your personal life during the last 2 weeks?

- |   |                          |  |
|---|--------------------------|--|
| 1 | <input type="checkbox"/> | VERY DISSATISFIED, UNHAPPY MOST OF THE TIME                    |
| 2 | <input type="checkbox"/> | GENERALLY DISSATISFIED, UNHAPPY                                |
| 3 | <input type="checkbox"/> | SOMEWHAT DISSATISFIED, UNHAPPY                                 |
| 4 | <input type="checkbox"/> | GENERALLY SATISFIED, PLEASED                                   |
| 5 | <input type="checkbox"/> | HAPPY MOST OF THE TIME   |
| 6 | <input type="checkbox"/> | VERY HAPPY MOST OF THE TIME                                    |
| 7 | <input type="checkbox"/> | EXTREMELY HAPPY, COULD NOT HAVE BEEN MORE SATISFIED OR PLEASED |

8. In general, how often during the last 2 weeks have you felt restless, or as if you were having difficulty trying to calm down?

- |   |                          |                        |
|---|--------------------------|------------------------|
| 1 | <input type="checkbox"/> | ALL OF THE TIME        |
| 2 | <input type="checkbox"/> | MOST OF THE TIME       |
| 3 | <input type="checkbox"/> | A GOOD BIT OF THE TIME |
| 4 | <input type="checkbox"/> | SOME OF THE TIME       |
| 5 | <input type="checkbox"/> | A LITTLE OF THE TIME   |
| 6 | <input type="checkbox"/> | HARDLY ANY OF THE TIME |
| 7 | <input type="checkbox"/> | NONE OF THE TIME       |

9. How much shortness of breath have you experienced during the last 2 weeks while doing your day-to-day physical activities?

- |   |                          |                                    |
|---|--------------------------|------------------------------------|
| 1 | <input type="checkbox"/> | EXTREME SHORTNESS OF BREATH        |
| 2 | <input type="checkbox"/> | VERY SHORT OF BREATH               |
| 3 | <input type="checkbox"/> | QUITE A BIT OF SHORTNESS OF BREATH |
| 4 | <input type="checkbox"/> | MODERATE SHORTNESS OF BREATH       |
| 5 | <input type="checkbox"/> | SOME SHORTNESS OF BREATH           |
| 6 | <input type="checkbox"/> | A LITTLE SHORTNESS OF BREATH       |
| 7 | <input type="checkbox"/> | NO SHORTNESS OF BREATH             |

10. How often during the last 2 weeks have you felt tearful, or like crying?

- |   |                          |                        |
|---|--------------------------|------------------------|
| 1 | <input type="checkbox"/> | ALL OF THE TIME        |
| 2 | <input type="checkbox"/> | MOST OF THE TIME       |
| 3 | <input type="checkbox"/> | A GOOD BIT OF THE TIME |
| 4 | <input type="checkbox"/> | SOME OF THE TIME       |
| 5 | <input type="checkbox"/> | A LITTLE OF THE TIME   |
| 6 | <input type="checkbox"/> | HARDLY ANY OF THE TIME |
| 7 | <input type="checkbox"/> | NONE OF THE TIME       |

11. How often during the last 2 weeks have you felt as if you are more dependent than you were before your heart problem?

- |   |                          |                        |
|---|--------------------------|------------------------|
| 1 | <input type="checkbox"/> | ALL OF THE TIME        |
| 2 | <input type="checkbox"/> | MOST OF THE TIME       |
| 3 | <input type="checkbox"/> | A GOOD BIT OF THE TIME |
| 4 | <input type="checkbox"/> | SOME OF THE TIME       |
| 5 | <input type="checkbox"/> | A LITTLE OF THE TIME   |
| 6 | <input type="checkbox"/> | HARDLY ANY OF THE TIME |
| 7 | <input type="checkbox"/> | NONE OF THE TIME       |

12. How often during the last 2 weeks have you felt you were unable to do your usual social activities, or social activities with your family?

- |   |                          |                        |
|---|--------------------------|------------------------|
| 1 | <input type="checkbox"/> | ALL OF THE TIME        |
| 2 | <input type="checkbox"/> | MOST OF THE TIME       |
| 3 | <input type="checkbox"/> | A GOOD BIT OF THE TIME |
| 4 | <input type="checkbox"/> | SOME OF THE TIME       |
| 5 | <input type="checkbox"/> | A LITTLE OF THE TIME   |
| 6 | <input type="checkbox"/> | HARDLY ANY OF THE TIME |
| 7 | <input type="checkbox"/> | NONE OF THE TIME       |

13. How often during the last 2 weeks have you felt as if others no longer have the same confidence in you as they did before your heart problem?

1	<input type="checkbox"/>	ALL OF THE TIME
2	<input type="checkbox"/>	MOST OF THE TIME
3	<input type="checkbox"/>	A GOOD BIT OF THE TIME
4	<input type="checkbox"/>	SOME OF THE TIME
5	<input type="checkbox"/>	A LITTLE OF THE TIME
6	<input type="checkbox"/>	HARDLY ANY OF THE TIME
7	<input type="checkbox"/>	NONE OF THE TIME

14. How often during the last 2 weeks have you experienced chest pain while doing your day-to-day activities?

1	<input type="checkbox"/>	ALL OF THE TIME
2	<input type="checkbox"/>	MOST OF THE TIME
3	<input type="checkbox"/>	A GOOD BIT OF THE TIME
4	<input type="checkbox"/>	SOME OF THE TIME
5	<input type="checkbox"/>	A LITTLE OF THE TIME
6	<input type="checkbox"/>	HARDLY ANY OF THE TIME
7	<input type="checkbox"/>	NONE OF THE TIME

15. How often during the last 2 weeks have you felt unsure of yourself or lacking in self-confidence?

1	<input type="checkbox"/>	ALL OF THE TIME
2	<input type="checkbox"/>	MOST OF THE TIME
3	<input type="checkbox"/>	A GOOD BIT OF THE TIME
4	<input type="checkbox"/>	SOME OF THE TIME
5	<input type="checkbox"/>	A LITTLE OF THE TIME
6	<input type="checkbox"/>	HARDLY ANY OF THE TIME
7	<input type="checkbox"/>	NONE OF THE TIME

16. How often during the last 2 weeks have you been bothered by aching or tired legs?

1	<input type="checkbox"/>	ALL OF THE TIME
2	<input type="checkbox"/>	MOST OF THE TIME
3	<input type="checkbox"/>	A GOOD BIT OF THE TIME
4	<input type="checkbox"/>	SOME OF THE TIME
5	<input type="checkbox"/>	A LITTLE OF THE TIME
6	<input type="checkbox"/>	HARDLY ANY OF THE TIME
7	<input type="checkbox"/>	NONE OF THE TIME

17. During the last 2 weeks, how much have you been limited in doing sports or exercise as a result of your heart problem?

- |   |                          |                     |
|---|--------------------------|---------------------|
| 1 | <input type="checkbox"/> | EXTREMELY LIMITED   |
| 2 | <input type="checkbox"/> | VERY LIMITED        |
| 3 | <input type="checkbox"/> | LIMITED QUITE A BIT |
| 4 | <input type="checkbox"/> | MODERATELY LIMITED  |
| 5 | <input type="checkbox"/> | SOMEWHAT LIMITED    |
| 6 | <input type="checkbox"/> | LIMITED A LITTLE    |
| 7 | <input type="checkbox"/> | NOT LIMITED AT ALL  |

18. How often during the last 2 weeks have you felt apprehensive or frightened?

- |   |                          |                        |
|---|--------------------------|------------------------|
| 1 | <input type="checkbox"/> | ALL OF THE TIME        |
| 2 | <input type="checkbox"/> | MOST OF THE TIME       |
| 3 | <input type="checkbox"/> | A GOOD BIT OF THE TIME |
| 4 | <input type="checkbox"/> | SOME OF THE TIME       |
| 5 | <input type="checkbox"/> | A LITTLE OF THE TIME   |
| 6 | <input type="checkbox"/> | HARDLY ANY OF THE TIME |
| 7 | <input type="checkbox"/> | NONE OF THE TIME       |

19. How often during the last 2 weeks have you felt dizzy or lightheaded?

- |   |                          |                        |
|---|--------------------------|------------------------|
| 1 | <input type="checkbox"/> | ALL OF THE TIME        |
| 2 | <input type="checkbox"/> | MOST OF THE TIME       |
| 3 | <input type="checkbox"/> | A GOOD BIT OF THE TIME |
| 4 | <input type="checkbox"/> | SOME OF THE TIME       |
| 5 | <input type="checkbox"/> | A LITTLE OF THE TIME   |
| 6 | <input type="checkbox"/> | HARDLY ANY OF THE TIME |
| 7 | <input type="checkbox"/> | NONE OF THE TIME       |

20. In general during the last 2 weeks, how much have you been restricted or limited as a result of your heart problem?

- |   |                          |                     |
|---|--------------------------|---------------------|
| 1 | <input type="checkbox"/> | EXTREMELY LIMITED   |
| 2 | <input type="checkbox"/> | VERY LIMITED        |
| 3 | <input type="checkbox"/> | LIMITED QUITE A BIT |
| 4 | <input type="checkbox"/> | MODERATELY LIMITED  |
| 5 | <input type="checkbox"/> | SOMEWHAT LIMITED    |
| 6 | <input type="checkbox"/> | LIMITED A LITTLE    |
| 7 | <input type="checkbox"/> | NOT LIMITED AT ALL  |

21. How often during the last 2 weeks have you felt unsure as to how much exercise or physical activity you should be doing?

1	<input type="checkbox"/>	ALL OF THE TIME
2	<input type="checkbox"/>	MOST OF THE TIME
3	<input type="checkbox"/>	A GOOD BIT OF THE TIME
4	<input type="checkbox"/>	SOME OF THE TIME
5	<input type="checkbox"/>	A LITTLE OF THE TIME
6	<input type="checkbox"/>	HARDLY ANY OF THE TIME
7	<input type="checkbox"/>	NONE OF THE TIME

22. How often during the last 2 weeks have you felt as if your family is being over-protective toward you?

1	<input type="checkbox"/>	ALL OF THE TIME
2	<input type="checkbox"/>	MOST OF THE TIME
3	<input type="checkbox"/>	A GOOD BIT OF THE TIME
4	<input type="checkbox"/>	SOME OF THE TIME
5	<input type="checkbox"/>	A LITTLE OF THE TIME
6	<input type="checkbox"/>	HARDLY ANY OF THE TIME
7	<input type="checkbox"/>	NONE OF THE TIME

23. How often during the past 2 weeks have you felt as if you were a burden on others?

1	<input type="checkbox"/>	ALL OF THE TIME
2	<input type="checkbox"/>	MOST OF THE TIME
3	<input type="checkbox"/>	A GOOD BIT OF THE TIME
4	<input type="checkbox"/>	SOME OF THE TIME
5	<input type="checkbox"/>	A LITTLE OF THE TIME
6	<input type="checkbox"/>	HARDLY ANY OF THE TIME
7	<input type="checkbox"/>	NONE OF THE TIME

24. How often during the past 2 weeks have you felt excluded from doing things with other people because of your heart problem?

1	<input type="checkbox"/>	ALL OF THE TIME
2	<input type="checkbox"/>	MOST OF THE TIME
3	<input type="checkbox"/>	A GOOD BIT OF THE TIME
4	<input type="checkbox"/>	SOME OF THE TIME
5	<input type="checkbox"/>	A LITTLE OF THE TIME
6	<input type="checkbox"/>	HARDLY ANY OF THE TIME
7	<input type="checkbox"/>	NONE OF THE TIME

25. How often during the past 2 weeks have you felt unable to socialize because of your heart problem?

- 1 ☐ ALL OF THE TIME
- 2 ☐ MOST OF THE TIME
- 3 ☐ A GOOD BIT OF THE TIME
- 4 ☐ SOME OF THE TIME
- 5 ☐ A LITTLE OF THE TIME
- 6 ☐ HARDLY ANY OF THE TIME
- 7 ☐ NONE OF THE TIME

26. In general, during the last 2 weeks how much have you been physically restricted or limited as a result of your heart problem?

- 1 ☐ EXTREMELY LIMITED
- 2 ☐ VERY LIMITED
- 3 ☐ LIMITED QUITE A BIT
- 4 ☐ MODERATELY LIMITED
- 5 ☐ SOMEWHAT LIMITED
- 6 ☐ LIMITED A LITTLE
- 7 ☐ NOT LIMITED AT ALL

27. How often during the last 2 weeks have you felt your heart problem limited or interfered with sexual intercourse?

- 1 ☐ ALL OF THE TIME
- 2 ☐ MOST OF THE TIME
- 3 ☐ A GOOD BIT OF THE TIME
- 4 ☐ SOME OF THE TIME
- 5 ☐ A LITTLE OF THE TIME
- 6 ☐ HARDLY ANY OF THE TIME
- 7 ☐ NONE OF THE TIME
- ☐ NOT APPLICABLE

Leave blank if you do not wish to answer this question

## Additional questions about your health

1. During the past 2 weeks, how much of the time did you:

**(TICK ONE BOX PER QUESTION)**

All of the time    A good bit of the time    Some of the time    A little of the time    None of the time

Have difficulty reasoning and solving problems, for example making plans, making decisions, learning new things?

☐
☐
☐
☐
☐

Forget, for example things that happened recently, where you put things or appointments?

☐
☐
☐
☐
☐

Have difficulty doing activities involving concentration and thinking?

☐
☐
☐
☐
☐

2. Have you ever been diagnosed with dementia? **(TICK ONE BOX ONLY)**

Yes

☐

No

☐

Not sure

☐

3. How would you describe your current living arrangements?

**(TICK ONE BOX ONLY)**

Living alone	Living with partner/spouse	Living with family	Living in a residential home	Living in a nursing home	Living in supported accommodation (e.g. sheltered housing)
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

4. When you need help with tasks, can you count on someone who is willing and able to meet your demands?

**(TICK ONE BOX ONLY)**

Always	Sometimes	Never
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

If yes who do you receive this support from? **(TICK ANY THAT APPLY)**

☐  
Partner

Other member of the family ☐

Friends ☐

☐

Neighbours

Statutory Organisation ☐

Voluntary Organisation ☐

5. Have you fallen in the last 3 months?  
**(TICK ONE BOX ONLY)**

Yes

☐

No

☐

Not sure

☐

6. Do you have a problem with losing control of urine or your bowel when you don't want to?

**(TICK ONE BOX ONLY)**

Yes

☐

No

☐

Not sure

☐

7. Have you recently lost weight such that your clothing has become looser?

**(TICK ONE BOX ONLY)**

Yes

☐

No

☐

Not sure

☐

8. At times, do you forget to take your prescription medications?

**(TICK ONE BOX ONLY)**

Yes

☐

No

☐

Not sure

☐

9. What medications are you currently taking?

Type	Dose	How often do you take it?


**Please check that you have answered all the questions on each page.**

## **THANK YOU FOR YOUR HELP**

### **Questions about you.**

1. a) To which Ethnic Origin group would you say you belong? :

**(TICK ONE BOX ONLY)**

Black (Caribbean) ☐

Bangladeshi ☐

Black (British) ☐

Black (African) ☐

Chinese ☐

Indian ☐

Pakistani ☐

White (British) ☐

White (European – non UK) ☐

White (European) ☐

Prefer not to say ☐

Other (please state) .....

b) Nationality .....

2. Highest qualification achieved: **(TICK ONE BOX ONLY)**

None ☐

GCSE / 'O' Level/grade / Standard grade/ (G)SVQ Level 2 ☐

'A' Level / Higher/ (G)SVQ Level 3 ☐

Diploma / SVQ Level 4 / HND ☐

Degree ☐

Post-Graduate Qualification ☐

Other (please state) .....

3. Employment:

**Please tick** which category reflects most closely your employment status:

Full-time – paid ☐

Part-time – paid ☐

Full-time – voluntary ☐

☐

Part-time – voluntary

Unemployed due to age (retired) ☐

Never worked do to disability ☐

No longer work due to disability ☐

Training programme ☐

Student

☐

House wife/home maker/house husband

☐

Unemployed

☐

If you work (or have worked), please state the type of work you do (or did) (be as precise as possible, e.g. teacher in primary school, clerk in accounts office)

.....

**Please check that you have answered all the questions on each page.**

**THANK YOU FOR YOUR HELP**

## **8.5. *Shiels lab protocol for telomere length analysis***

### **Telomere Assay Protocol**

**This protocol is designed for use with the Roche Light Cycler 480.**

#### **1.1.1.1 Reagents Needed**

- DNA
- Nuclease free H<sub>2</sub>O
- SYBR Green I Master – 2x SYBR Green I Master Mix  
H<sub>2</sub>O PCR Grade

(ROCHE # 04 887 352 001)

- Telomere Primers
- 36B4 Primers

#### **2.1.1.1 Sample DNA Preparation**

For this assay **35ng** of DNA is required per well. Each sample is assayed in triplicate in both the telomere and 36B4 plates. To each well **5μl** of DNA is added (**7ngDNA/μl**). In total make **35μl (245ng DNA)** of each sample (7x5μl 6 wells for two plates plus 1 well extra). DNA is diluted in nuclease free H<sub>2</sub>O.

- For each sample calculate the volume of DNA and the volume of H<sub>2</sub>O required:

Volume of DNA reqd (μl) = **245/Concentration (ng/μl) of DNA sample**

Volume of H<sub>2</sub>O reqd (μl) = **35 – Volume of DNA reqd**

e.g If your stock concentration of DNA is 18ng/μl, you need 13.61μl of DNA (245/18) and 21.39μl of H<sub>2</sub>O (35-13.61).

- Label eppendorfs with sample number and aliquot the required volume of H<sub>2</sub>O.
- Before aliquoting the DNA, heat to **65oC** for **10 minutes** then vortex and centrifuge briefly to ensure DNA is thoroughly resuspended.
- Aliquot the required volume of DNA, then vortex and centrifuge sample briefly.
- Store samples at 4oC until required.

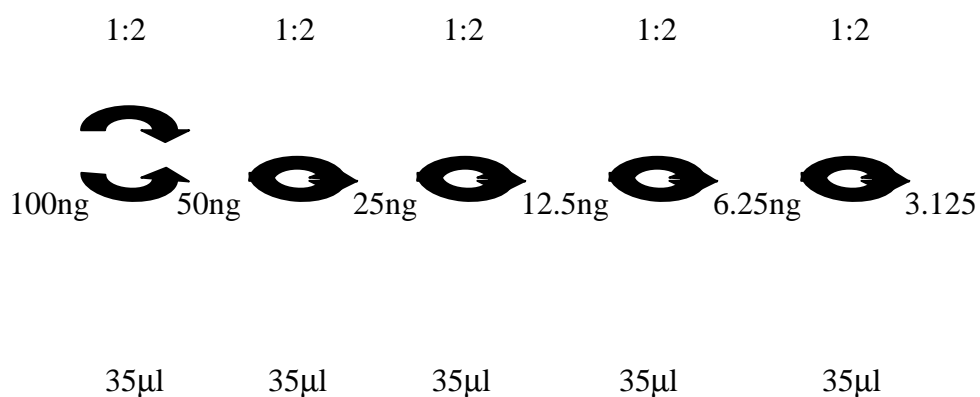
### **3.1.1.1 Standards & Positive Control Preparation**

For absolute quantification analyses, serial dilutions of a standard with known concentration are used to create a standard curve. The standard curve is then used to determine the concentration of the the unknown samples. The **crossing point (CP – the point at which the fluorescence of a sample rises above the background fluorescence)** of the standards (with known concentration) and the samples are used to calculate the concentration of the sample DNA.

As with the samples, each standard is assayed in triplicate in both the telomere and 36B4 plates. A reference sample with known DNA concentration is used to construct the standard curve. The DNA concentration of the standards for this assay range from 100ng to 3.125ng, they are prepared

by serial dilution (1:2). To each well **5 $\mu$ l** of standard DNA is added. Therefore need **35 $\mu$ l** of each standard (7x5 $\mu$ l 6 wells for two plates plus 1 well extra). DNA is diluted in nuclease free H<sub>2</sub>O.

If running a lot of plates within a short space of time try if possible to prepare a stock of standards for more uniformity between different plates.



e.g For a reference sample with concentration 40ng/ $\mu$ l. Begin by preparing 100ng (20ng/ $\mu$ l) standard (**remember 35 $\mu$ l is needed for each standard but for 100ng need to make double the volume (70 $\mu$ l) since this is the starting concentration**)

**100ng standard SD1:** Volume SD1 reqd = 70 $\mu$ l (1400ng DNA)

$$\begin{aligned} \text{Volume reference DNA reqd } (\mu\text{l}) &= \text{Amt of DNA reqd/Conc of reference sample} \\ &= 1400/40 = 35\mu\text{l} \end{aligned}$$

Volume of H<sub>2</sub>O reqd (μl) = **Vol SD1 reqd – Volume of DNA reqd**

$$= 70-35 = 35\mu\text{l}$$

**100ng standard SD1 = 35μl reference DNA + 35μl H<sub>2</sub>O**

**50ng standard SD2 = 35μl SD1 + 35μl H<sub>2</sub>O**

**25ng standard SD3 = 35μl SD2 + 35μl H<sub>2</sub>O**

**12.5ng standard SD4 = 35μl SD3 + 35μl H<sub>2</sub>O**

**6.25ng standard SD5 = 35μl SD1 + 35μl H<sub>2</sub>O**

**3.13ng standard SD6 = 35μl SD1 + 35μl H<sub>2</sub>O**

- Label eppendorfs with standard number and aliquot the required volume of H<sub>2</sub>O.
- Before aliquoting the DNA, heat to **65oC** for **10 minutes** then vortex and centrifuge briefly to ensure DNA is thoroughly resuspended.
- Aliquot the required volume of DNA to SD1, then vortex and centrifuge sample briefly.
- Aliquot the required volume of SD1 and add to SD2, then vortex and centrifuge sample briefly.
- Add SD2 to SD3, SD3 to SD4, SD4 to SD5, SD5 to SD6. Ensure that each standard is vortexed and centrifuged before preparing the next one.
- Store samples at 4oC until required.

A reference positive control (calibrator) is required in each plate. Use the reference DNA that was used to create the standard curve. The concentration of this sample should fall within the range of

your standard curve (approximately half way). Therefore for this assay **50ng** of DNA is required per well for the reference positive control. As with the samples, the reference sample is assayed in triplicate in both the telomere and 36B4 plates. To each well **5µl (10ng/µl)** of reference DNA is added. Therefore need **35µl (350ng)** of reference sample (7x5µl 6 wells for two plates plus 1 well extra). DNA is diluted in nuclease free H<sub>2</sub>O. If running a lot of plates within a short space of time try if possible to prepare a stock of reference DNA sample for more uniformity between different plates.

Volume of DNA reqd (µl) = **350/Concentration (ng/µl) of DNA sample**

Volume of H<sub>2</sub>O reqd (µl) = **35 – Volume of DNA reqd**

e.g If your stock concentration of reference DNA is 40ng/µl, you need 8.75µl of DNA (350/40) and 26.25µl of H<sub>2</sub>O (35-8.75).

- Label eppendorfs with sample number and aliquot the required volume of H<sub>2</sub>O.
- Before aliquoting the DNA, heat to **65oC** for **10 minutes** then vortex and centrifuge briefly to ensure DNA is thoroughly resuspended.
- Aliquot the required volume of DNA, then vortex and centrifuge sample briefly.
- Store sample at 4oC until required.

#### **4.1.1.1 Primer Preparation**

##### **Telomere Primers**

**Telo 1 Sequence (5' to 3')**

**CGG TTT GTT TGG GTT TGG GTT TGG GTT TGG GTT TGG GTT**

**Telo 2 Sequence (5' to 3')**

**GGC TTG CCT TAC CCT TAC CCT TAC CCT TAC CCT TAC CCT**

Always prepare a **100μM** main stock of the primers (in the tube that they are supplied) as per Invitrogen instructions (depends on the nanomole quantity of the primers supplied).

- From the 100μM main stock of primers prepare an **8μM** stock (**1 in 12.5** dilution). (Both telomere primers are used at a final concentration of 300nM. 300μls of 8μM primers is enough for 4 plates).

**8μM Telo 1: Add 25μls of primer to 287.5μls of H<sub>2</sub>O**

**8μM Telo 2: Add 25μls of primer to 287.5μls of H<sub>2</sub>O**

Vortex and centrifuge aliquots to ensure primers thoroughly mixed. Store aliquots at –20oC.

*36B4 Primers*

**36B4d Sequence (5' to 3')**

**CCC ATT CTA TCA TCA ACG GGT ACA A**

### **36b4u Sequence (5' to 3')**

**CAG CAA GTG GGA AGG TGT AAT CC**

Always prepare a **100μM** main stock of the primers (in the tube that they are supplied) as per Invitrogen instructions (depends on the nanomole quantity of the primers supplied).

- 36B4d primer is used at a final concentration of 500nM (in 20μl). Prepare a **25μM** stock from the main 100μM stock. (300μls of 25μM primer is enough for 4 plates).

**25μM 36B4d: Add 80μls of primer to 240μls of H<sub>2</sub>O**

Vortex and centrifuge aliquots to ensure primers thoroughly mixed. Store aliquots at –20oC.

- 36B4u primer is used at a final concentration of 300nM (in 20μl). Prepare an **15μM** stock from the main 100μM stock. (300μls of 15μM primer is enough for 4 plates).

**15μM 36B4u: Add 48μls of primer to 272μls of H<sub>2</sub>O**

Vortex and centrifuge aliquots to ensure primers thoroughly mixed. Store aliquots at –20oC.

### 5.1.1.1 Master Mix Preparation

This assay now uses the SYBR Green I Master from Roche. The kit contains 2x SYBR Green I Master Mix and H<sub>2</sub>O PCR Grade. The only other reagents that need to be added to the master mix are the primers. The total reaction volume per well is **20µl**; **5µl** of this is the sample/standard DNA therefore the remaining **15µl** is comprised of the master mix. Consequently the total volume of master mix required is **1500µl** (96 wells x 15µl plus 4 extra wells). The master mix is prepared as follow

Per Well Total Reaction Volume = **20µl**

2x SYBR Green I Master Mix

$$= 20/2 = \mathbf{10\mu l \text{ per well}}$$

Telo1/Telo2 Primers (8µM stock – need final conc to be 300nM)

$$= (300/8000) \times 20 = \mathbf{0.75\mu l \text{ per well}}$$

36B4d Primer (25µM stock – need final conc to be 500nM)

$$= (500/25000) \times 20 = \mathbf{0.4\mu l \text{ per well}}$$

36B4u Primer (15µM stock – need final conc to be 300nM)

$$= (300/15000) \times 20 = \mathbf{0.4\mu l \text{ per well}}$$

H<sub>2</sub>O = **Total reaction volume – Volume of Master Mix - Volume of primers**

Master mix			TEL	Master mix			36 B4
Vol reqd			Vol Reqd	Vol reqd			Vol Reqd
Reagent	per well (μl)	for 100 wells (μl)		Reagent	per well (μl)	for 100 wells (μl)	
							100
2x master mix	10	1000		2x master mix	10	1000	
Telo 1	0.75	75		36B4d	0.4	40	
Telo 2	0.75	75		36B4u	0.4	40	
H2O	3.5	350		H2O	4.2	420	
total	15	1500		total	15	1500	

- Defrost all reagents on ice. SYBR Green I Master is light sensitive therefore make sure that it is protected from light at all times.
- Prepare Telomere Master Mix. Label 1.5ml eppendorf tube and wrap in foil, aliquot required volume of H<sub>2</sub>O.
- Add Telo 1 and Telo 2 primers to the H<sub>2</sub>O.
- Add the correct volume of SYBR Green I Master. Thoroughly mix the master mix by pipetting up and down. **DO NOT VORTEX OR CENTRIFUGE THE MASTER MIX!!**
- Keep the telomere master mix on ice until ready to use.
- Prepare 36B4 Master Mix. Label 1.5ml eppendorf tube and wrap in foil, aliquot required volume of H<sub>2</sub>O.
- Add 36B4d and 36B4u primers to the H<sub>2</sub>O.
- Add the correct volume of SYBR Green I Master. Thoroughly mix the master mix by pipetting up and down. **DO NOT VORTEX OR CENTRIFUGE THE MASTER MIX!!**
- Keep the 36B4 master mix on ice until ready to use

#### 6.1.1.1 Plate Construction

Telomere and 36B4 plates should both be prepared on the same day and preferably on the day they are going to be run. Prepare one plate at a time, generally telomere plate first then 36B4 plate. This prevents confusion and also allows you to run the first plate whilst preparing the second plate. Both plates should have the same layout ie all samples should have the same well position in both plates. In each plate there will be 6 standards, a reference sample (SC positive control), a no template control (negative control – no DNA is added to this well only master mix. The purpose of this is to make sure that there is no contamination of any reagents) and 24 unknown samples. Plate layout is normally as follows:

		1	2	3	4	5	6	7	8	9	10	11	12
telomere & 36B4	A	SD1	SD1	SD1	1	1	1	9	9	9	17	17	17
	B	SD2	SD2	SD2	2	2	2	10	10	10	18	18	18

C	SD3	SD3	SD3	3	3	3	11	11	11	19	19	19
D	SD4	SD4	SD4	4	4	4	12	12	12	20	20	20
E	SD5	SD5	SD5	5	5	5	13	13	13	21	21	21
F	SD6	SD6	SD6	6	6	6	14	14	14	22	22	22
G	SC	SC	SC	7	7	7	15	15	15	23	23	23
H	ntc	ntc	ntc	8	8	8	16	16	16	24	24	24

- Label the plate, with Telomere/36B4, study, date and your name. Take note of the plate barcode.
- Set the 20µl pipette to 15µls and the 10µl pipette to 5µls, to prevent the set volumes from changing wrap the pipette dials in tape.
- Carefully pipette 15µls of Telomere Master Mix into each of the wells. Change pipette tip after each well, ensures accuracy and also acts as a check that master mix is added to each individual well. Once master mix is added to all wells and you are preparing your standards/sample, remember to protect the plate from light by covering with foil.
- Vortex and centrifuge briefly all standards and samples.
- Heat all standards/samples to 95°C for 5 minutes, then transfer to ice.
- Beginning with columns 1-3, carefully pipette 5µls of DNA to the triplicate wells (A1, then A2, then A3, then B1, B2, B3 etc etc etc). As with the master mix change pipette tip after each well. **Remember to add only H<sub>2</sub>O to the NTC well.** Once DNA has been added to the 3 columns cover them to prevent confusion and pipetting into the wrong well.
- Continue adding DNA to the columns in triplicate 4-6, 7-9 and 10-12.
- Once all the wells have both master mix and DNA added cover the plate with the sealing film, making sure that is properly sealed to prevent loss of samples.
- Wrap the completed plate in foil to protect it from light.
- Centrifuge the plate at **~2000rpm** for **2 minutes**.
- At this stage either run plate on Light Cycler 480 or store at 4°C until ready to use.
- Once the telomere plate is finished begin the 36B4 plate, preparing it in the exact same way as the telomere plate.

### 7.1.1.1 Running Plates on LightCycler 480

#### Defining Experimental Conditions

- Once your plate is ready to be run on the Light Cycler 480, switch on the computer and machine.
- Double click on the LightCycler 480 Software icon, login to the appropriate database.
- Starting from the Overview window you can create a new experiment or open an existing file.
- To start a new experiment click on the “new experiment” icon, this will take you to the Main Window, where you input your experimental conditions, plate layout, sample names etc.
- The default opening screen is “Experiment” – input experimental conditions here. For the Telomere and 36B4 runs the conditions are as follows:

#### TELOMERE RUNNING CONDITIONS

<i>Program Name</i>	Heat Start (HS)				
<i>Cycles</i>	1	<i>Analysis Mode</i>		None	
<b>Target (oC)</b>	<b>Acquisition Mode</b>	<b>Hold (hh:mm:ss)</b>	<b>Ramp (oC/s)</b>	<b>Rate</b>	<b>Acquisitions (per oC)</b>
95	None	00:10:00	4.4		

<i>Program Name</i>	Amplification (Amp)				
<i>Cycles</i>	30	<i>Analysis Mode</i>		Quantification	
<b>Target (oC)</b>	<b>Acquisition</b>	<b>Hold</b>	<b>Ramp</b>	<b>Rate</b>	<b>Acquisitions</b>

	<b>Mode</b>	<b>(hh:mm:ss)</b>	<b>(oC/s)</b>	<b>(per oC)</b>
95	None	00:00:05	4.4	
59	None	00:00:10	2.2	
72	Single	00:02:00	4.4	

<i>Program Name</i>		Melt			
<i>Cycles</i>		1	<i>Analysis Mode</i>		Melting Curves
<b>Target (oC)</b>	<b>Acquisition Mode</b>	<b>Hold (hh:mm:ss)</b>	<b>Ramp (oC/s)</b>	<b>Rate</b>	<b>Acquisitions (per oC)</b>
95	None	00:00:30	4.4		
65	None	00:00:30	2.2		
95	Continuous	00:02:00	0.29		2

<i>Program Name</i>		Cool			
<i>Cycles</i>		1	<i>Analysis Mode</i>		None
<b>Target (oC)</b>	<b>Acquisition Mode</b>	<b>Hold (hh:mm:ss)</b>	<b>Ramp (oC/s)</b>	<b>Rate</b>	<b>Acquisitions (per oC)</b>
40	None	00:00:10	2.2		

### 36B4 RUNNING CONDITIONS

<i>Program Name</i>	Heat Start (HS)				
<i>Cycles</i>	1	<i>Analysis Mode</i>	None		
<b>Target (oC)</b>	<b>Acquisition Mode</b>	<b>Hold (hh:mm:ss)</b>	<b>Ramp (oC/s)</b>	<b>Rate</b>	<b>Acquisitions (per oC)</b>
95	None	00:05:00	4.4		

<i>Program Name</i>	Amplification (Amp)				
<i>Cycles</i>	35	<i>Analysis Mode</i>	Quantification		
<b>Target (oC)</b>	<b>Acquisition Mode</b>	<b>Hold (hh:mm:ss)</b>	<b>Ramp (oC/s)</b>	<b>Rate</b>	<b>Acquisitions (per oC)</b>
95	None	00:00:05	4.4		
58	None	00:00:15	2.2		
72	Single	00:00:15	4.4		

<i>Program Name</i>	Melt				
<i>Cycles</i>	1	<i>Analysis Mode</i>	Melting Curves		
<b>Target (oC)</b>	<b>Acquisition Mode</b>	<b>Hold (hh:mm:ss)</b>	<b>Ramp (oC/s)</b>	<b>Rate</b>	<b>Acquisitions (per oC)</b>

95	None	00:00:30	4.4	
65	None	00:00:30	2.2	
95	Continuous	00:02:00	0.29	2

<i>Program Name</i>		Cool			
<i>Cycles</i>		1	<i>Analysis Mode</i>		None
<b>Target (oC)</b>	<b>Acquisition Mode</b>	<b>Hold (hh:mm:ss)</b>	<b>Ramp (oC/s)</b>	<b>Rate</b>	<b>Acquisitions (per oC)</b>
40	None	00:00:10	2.2		

- **For both telomere and 36B4 plates check that the reaction volume is 20µl**

*Having set the experimental conditions these can be saved as templates to prevent having to input the conditions each time an experiment is run. To create a template, click “Save as Template” and select the location to save the template and enter a name for the template. To use this template in future runs, start a new experiment and click “Apply Template” icon and then select the required template. The template experimental conditions are then applied to the new experiment.*

## **Entering Sample Information**

- *The next step is to input your sample information, depending on the number and type of samples (eg normal vs tumour) you may want to create subsets within the plate layout. This*

*is useful if you are not running a full plate but also allows you to analyse the plate as a whole and by individual subsets. To do this click on the “Subset Editor” icon, highlight the areas of the plate to be in a subset, then name this subset.*

- To input sample information click on the “Sample Editor” icon. At this stage you can either select to input data into the whole plate or into subsets, if they have been created.
- Select either Abs Quant or Rel Quant depending on what type of analysis is required.
- Highlight the triplicate wells, input the sample name, select the sample type (Unknown, Standard, Negative Control) and click “Make Replicate” Icon. **(It is important to make the samples replicates so that when the analysis is performed, the average of all three triplicates is taken into account).** For standards enter the known concentration into the table.

As with the experimental conditions you can save sample information as a template to prevent having to input the information each time. Follow the same steps as described for experimental templates.

## Running Experiment

- *Once all sample information is entered, return to the experiment window (click on the “Experiment” icon).*
- *Load the prepared plate into the machine. Press the push button on the front of the machine (located next to the instrument status LEDs). Place the plate into the loading arm of the loader with the flat edge pointing towards the instrument). Press the plate loading push button again to retract the loader with plate into the machine. You are now ready to start the run.*
- *Click the “Start Run” icon, you will then be asked to save your run (it wont start until you have saved the experiment). Once saved the run begins.*
- *A status bar on the “Data” tab indicates the progress of the running experiment.*
- *Once the run is finished press the plate loading push button and remove the plate, press the button again to close the loader. You are now ready to analyse your experiment or to begin a new experiment.*

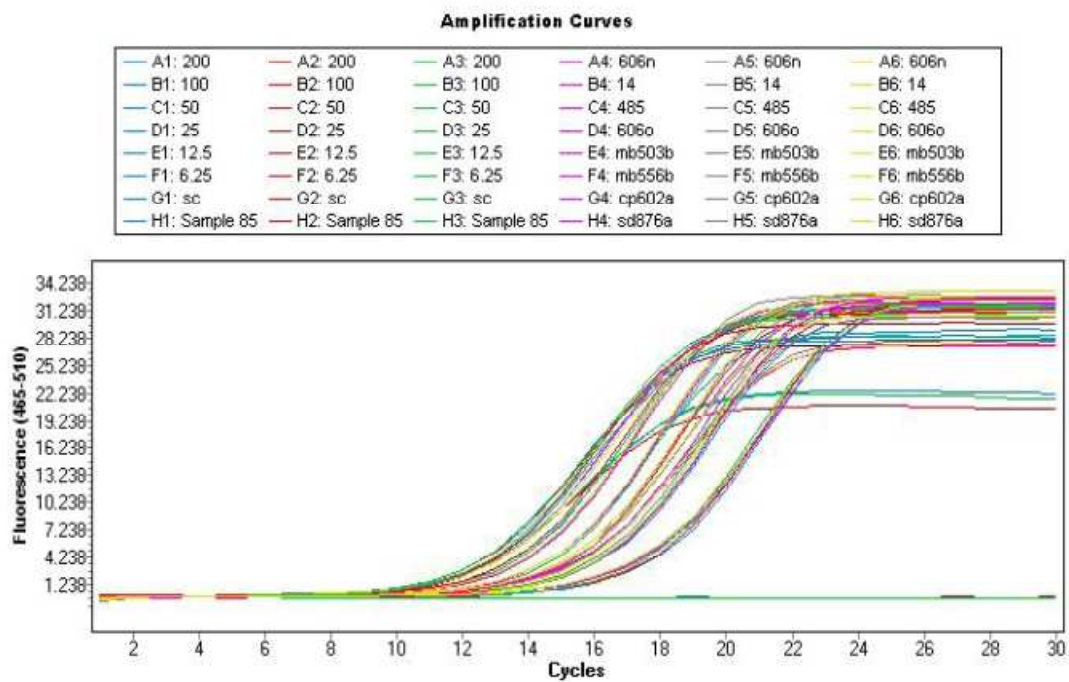
## Absolute Quantification

*Absolute quantification enables you to quantify a single target sequence and express the final result as an absolute value. For absolute quantification analyses, serially diluted standards with known concentration are used to generate a standard curve.*

*A perfect amplification reaction has an efficiency of 2 (every PCR product is replicated once in every cycle). The LightCycler 480 software automatically calculates the efficiency. An efficiency of 2 corresponds to a standard curve slope = -3.3. For accurate analysis and calculation of telomere length (Relative T/S) it is necessary to ensure that telomere and 36B4 plates have similar efficiencies, slopes between -2.9 and -3.8 are generally deemed acceptable.*

- *To perform absolute quantification analysis, click on the “Analysis” icon. In the Create New Analysis box select “Abs Quant/2<sup>nd</sup> Derivative Max” or “Abs Quant/Fit Points”. (See the Roche LightCycler 480 Manual for more details on the differences between these two methods).*
- *Click Calculate – the standard curve is generated.*

As well as the standard curve, the error, efficiency and slope of the reaction will be displayed. The error is a measure of the accuracy of the quantification result based on the standard curve, this value should be less than 0.2. The efficiency should be as close to 2 and the slope close to -3.3 as possible.



Error: 0.0369

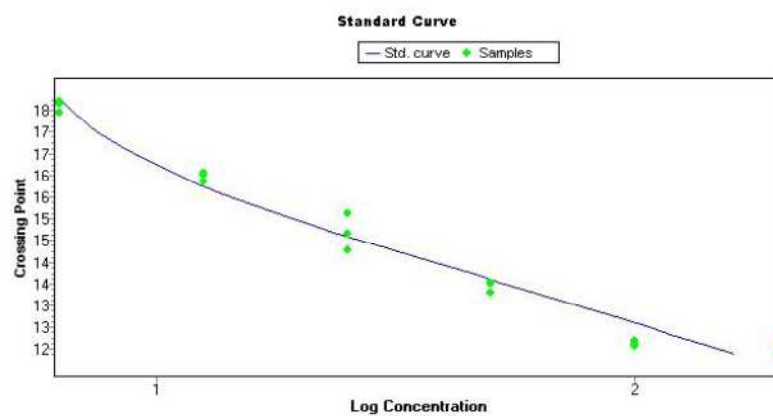
Efficiency: 1.991

Slope: -3.343

YIntercept:

20.29

Link: 0.000



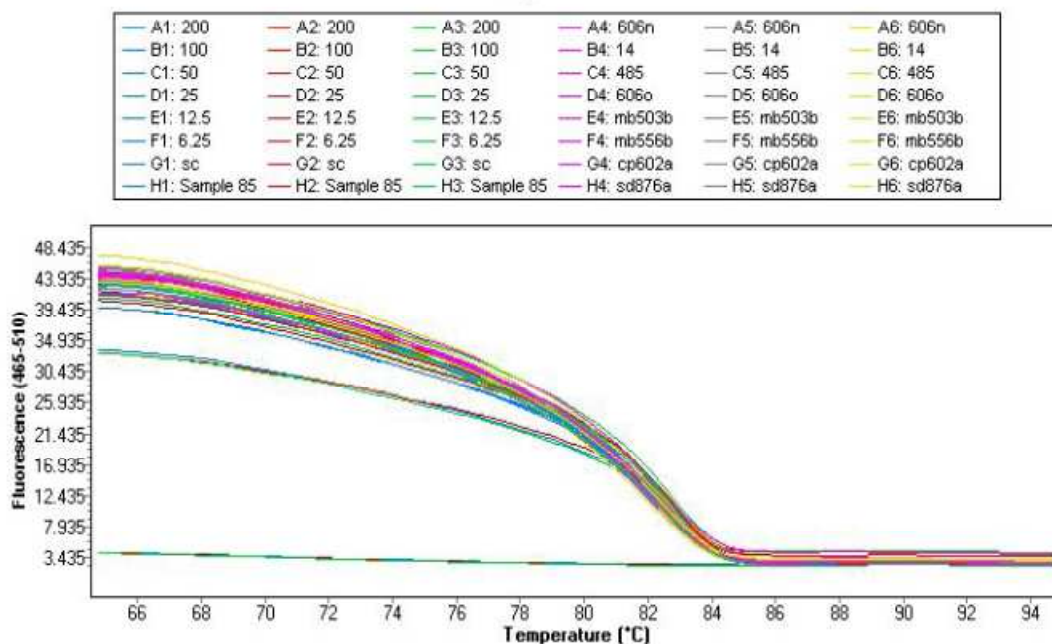
- *Having generated the curve, the software then determines the DNA concentration of the unknown samples, by using the point at which the unknown sample Cp value falls on the standard curve. Cp data from the standards is used to convert the Cp data from the unknown samples into DNA concentrations.*
- *Check the DNA concentration of your reference sample (SC), the concentration should be approximately 50ng in both the telomere and 36B4 plates.*
- *Having ensured both telomere and 36B4 plates have worked, proceed to calculating the T/S and relative T/S of all samples.*

## **Melting Curve Analysis**

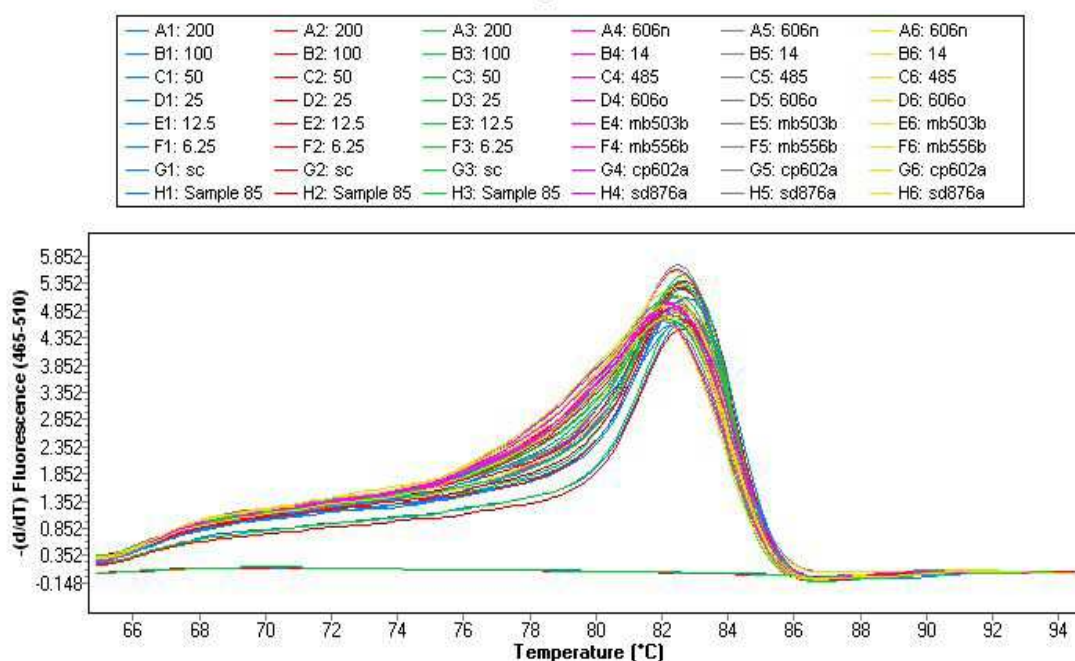
*The purpose of melting curve analysis is to determine the characteristic melting temperature of the target DNA. It is also useful for checking your no template control (negative control) for contamination and for the formation of primer-dimers.*

- *Click the “Analysis” icon*
- *In the Create New Analysis box select “Tm Calling”*
- *Click Calculate, a melting curve chart and a melting peak chart will be generated. The Tm for the telomere products is ~83oC and for the 36B4 products is ~82.5oC.*

**Melting Curves**



**Melting Peaks**



## **8.6. Ethics approval letter for biological ageing and coronary artery disease**

**WoSRES**

**West of Scotland Research Ethics Service**



Dr Cathy Johnman  
Public Health and Health Policy  
University of Glasgow  
1 Lilybank Gardens  
G12 8RZ

### **West of Scotland REC 5**

Ground Floor - Tennent Building  
Western Infirmary  
38 Church Street  
Glasgow  
G11 6NT

Date 1 June 2011

Direct line 0141 211 2482  
Fax 0141 211 1847  
E-mail WoSREC5@ggc.scot.nhs.uk

Dear Dr Johnman

**Study title:** Biomarkers of ageing in coronary heart disease.

**REC reference:** 11/AL/0307

The Research Ethics Committee reviewed the above application at the meeting held on 18 May 2011.  
Thank you for attending to discuss the study.

### **Ethical opinion**

To summarise your discussion with the Committee, you confirmed the following main points:

- You plan to include all cardiologists and make the procedure routine. This should therefore ensure that the patient numbers should be achieved.
- The storage procedure of the patient top sheets, blood sheets and blood samples and that they will all be kept separately. Permission has been obtained from the Data Protection Officer.
- You expect to publish the results when the linkage data becomes available, which is usually at the beginning of each summer.
- Some of the blood will be used for testing with the remainder retained for future research subject to consent by the participant. It was also noted that future research on the retained blood would be subject to ethical approval.
- Although follow up was planned for one year, this could be longer due to when linkage data became available.

The members of the Committee present gave a favourable ethical opinion of the above research on the basis described in the application form, protocol and supporting documentation, subject to the conditions specified below.

#### **Ethical review of research sites**

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see “Conditions of the favourable opinion” below).

#### **Conditions of the favourable opinion**

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

*Management permission (“R&D approval”) should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements.*

Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at <http://www.rdforum.nhs.uk>.

*Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.*

*For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.*

*Sponsors are not required to notify the Committee of approvals from host organisations*

**1) The Consent Form requires to be amended as follows:**

- a) The standard footer should be added stating 1 copy to the participant and the other retained for the Research File.**
- b) An additional clause is required for the DNA analysis to be carried out on the blood samples.**

**2) The GP Letter should have the name of the Researcher at the bottom.**

**3) The Participant Information Sheet requires to be amended as follows:**

- a) The standard statement regarding the complaints procedure should be added.**
- b) In the section "Who has reviewed the study?", the name of the Ethics Committee should be changed to West of Scotland Research Ethics Committee 5.**

**4) All documentation should have the official logo and contact details.**

It is responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

You should notify the REC in writing once all conditions have been met (except for site approvals from host organisations) and provide copies of any revised documentation with updated version numbers. Confirmation should also be provided to host organisations together with relevant documentation

### **Approved documents**

The documents reviewed and approved at the meeting were:

<i>Document</i>	<i>Version</i>	<i>Date</i>
Covering Letter		28 April 2011
GP/Consultant Information Sheets		13 April 2011
Investigator CV		
Other: CV - Supervisor -		
Other: CV - Supervisor		
Participant Consent Form	1.0	13 April 2011
Participant Information Sheet	1.0	14 April 2011
Protocol	1	13 April 2011
Questionnaire	1.0	13 April 2011
REC application		28 April 2011

### **Membership of the Committee**

The members of the Ethics Committee who were present at the meeting are listed on the attached sheet.

### **Statement of compliance**

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

### **After ethical review**

Now that you have completed the application process please visit the National Research Ethics Service website > After Review

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.

The attached document “After ethical review – guidance for researchers” gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Progress and safety reports
- Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

We would also like to inform you that we consult regularly with stakeholders to improve our service. If you would like to join our Reference Group please email [referencegroup@nres.npsa.nhs.uk](mailto:referencegroup@nres.npsa.nhs.uk).

With the Committee's best wishes for the success of this project

Yours sincerely

A handwritten signature in black ink that reads "R Galbcher". The signature is written in a cursive style with a large, stylized 'R'.

On behalf of

**Mrs Liz Tregonning**

**Vice-Chair**

Enclosures:                      List of names and professions of members who were present at the meeting and those who submitted written comments

“After ethical review – guidance for researchers”

Copy to:                              Dr Catherine Sinclair, National waiting Times Centre, Golden Jubilee National Hospital

## **8.7. Participant information leaflet for biological ageing and coronary artery disease**



University  
of Glasgow

Golden Jubilee National Hospital  
NHS national waiting times centre



### **PARTICIPANT INFORMATION LEAFLET**

#### **Biological ageing and coronary heart disease**

##### **Introduction**

You are being invited to take part in a research study. Before you take part it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and to discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information (contact details are at the end of this leaflet or ask the cardiac research nurse)

##### **What is the purpose of the study?**

Physical ageing (also known as biological ageing) is faster in some people than others. People of the same age since birth can have different biological ages.

We want to compare people with heart disease with people of the same age who do not have heart disease to see if they have a different biological age and, if so, why. In particular, we wish to investigate whether biological ageing is related to inflammation which can be more common in patients with heart disease.

To do this, we want to take a small blood sample to measure markers of biological ageing (such as telomere length which is the length of the protective cap at the end of chromosomes) and

markers of inflammation. Our aim is to use the findings of this study to benefit patients in the future by providing a better understanding of what causes heart disease and how we might prevent it.

### **Why have I been chosen?**

You have been chosen because you are having a cardiac test at the Golden Jubilee to investigate whether or not you have coronary heart disease or you are having a procedure to treat narrowing of the blood vessels of the heart.

### **Do I have to take part?**

It is up to you to decide whether or not to take part. If you decide to take part you will be given the opportunity to discuss your involvement with your cardiologist or one of the cardiac research nurse at the time of your admission where you will be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time without having to give a reason. Whether you take part or not, will not affect the type or the standard of care you receive.

### **What will happen if I take part?**

We will take a small amount of blood (10millilitres - about 2 teaspoonfuls) to be tested for biomarkers of biological ageing. The doctor will take this through the same needle that he/she will be using for your cardiac test therefore no additional jags are required. We will not feedback any information regarding blood results to participants as they will be analysed in an anonymised manner.

We will ask you to complete a very short quality of life questionnaire which should take less than 5 minutes to complete.

We will use routine clinical information to follow up you up for 1 year after your test. We can do this without having to contact you again.

### **What are the possible benefits of taking part?**

Taking part in the study will be of no direct benefit to you but will be very useful in providing valuable information which could be helpful in caring for future patients.

**Will my taking part in the study be kept confidential?**

All information which is collected about you during the course of the research will be kept strictly confidential. If you agree, we will contact your GP to inform them that you are taking part in this study.

Your information will be held within a secure environment both here and in the University of Glasgow Research Office.

**What will happen to the results of the research study?**

Only those directly involved in the study will have access to any information which has your name and address on it and then, only for the purposes of arranging the study follow up.

Information will be transported to the University of Glasgow Research Office within a secure bag, which is locked, and taken by NHS transport services.

Your name and other details will be removed from the questionnaire and will be kept separate from your answers.

Any information which is then examined for this study will have your name and address removed so that you cannot be recognised from it.

Results of the study will be published in medical journals and will form part of the Chief Investigator's PhD thesis.

**Who has funded the research?**

The research is jointly funded by the NHS and by the University of Glasgow Patterson Bequest Fund.

**Who has reviewed the study?**

The study was reviewed and approved by the West of Scotland Ethics Committee 5.

**Direct Study Contacts:**

**Research Nurses:**

Joanne Kelly, Maureen Mason and Elizabeth Boyd

Golden Jubilee National Hospital

Beardmore Street

Clydebank

G81 4HX

Tel: 0141 951 5256

Email: joanne.kelly@gjnh.scot.nhs.uk

**Chief Investigator:**

Dr Cathy Johnman

Hutchison Research Fellow/Clinical Lecturer/SpR Public Health

Centre for Population and Health Based Sciences

Public Health and Health Policy

University of Glasgow

1 Lilybank Gardens

Glasgow

G12 8RZ

0141 330 8547

Email: Cathy.Johnman@glasgow.ac.uk

**For independent advice on this study, if required, please contact:**

Dr Roy Gardener, Consultant Cardiologist

National Waiting Times Centre Board

Golden Jubilee National Hospital

Beardmore Street

Clydebank

G81 4HX

**What if I have a complaint about this study?**

If you have any concerns or complaints about any aspect of this study you should ask to speak to Dr Cathy Johnman, the Chief investigator of this study or one of the Cardiac Research Nurses at the Golden Jubilee National Hospital. If you remain unhappy and wish to complain formally you can do this through the usual NHS complaints procedure.

## 8.8. Participant consent form for biological ageing and coronary artery disease



University  
of Glasgow

Golden Jubilee National Hospital  
NHS national waiting times centre



### CONSENT FORM

#### Biological ageing and coronary heart disease

Study number: .....

Name: .....

Hospital: Golden Jubilee Hospital

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**CHI Number:**  
(Community Health Index)

**Please put your  
initials in boxes**

I confirm that I have read and understood the information leaflet

☐

I understand that I will be asked to complete an initial questionnaire on admission and that additional questionnaires will be sent to me at 3, 6 and 12 months after my procedure

☐

I understand that the research team may wish to contact me for follow up should it be required

☐

I understand that my GP will be informed of my involvement in this study

☐

I understand that the research team may wish to access my notes to provide additional follow-up information about my health.

☐

Name of participant	Date	Signature

Name and designation of person taking consent	Date	Signature

Version 2.0 03/06/2011