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CLINICAL BIOMARKERS IN OLDER PATIENTS WITH AORTIC STENOSIS



THE UNIVERSITY
of EDINBURGH

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To my wife Jin

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DECLARATION

This thesis represents research undertaken in the Centre for Cardiovascular Sciences (University of Edinburgh), the Clinical Research Facility and Edinburgh Heart Centre (Royal Infirmary of Edinburgh). I declare that I have composed this thesis and that the work included is either my own or represents collaborative work in which I have made a substantial contribution. I was personally involved in the conception, initiation, conduct and/or data analysis of all studies presented. The collaborative involvement of others is acknowledged below.

This work was supported by a Clinical Research Fellowship from Chest, Heart and Stroke Scotland (RES/Fell/A163). I collaborated with the Chief Investigators for the SALTIRE trial (Professor David Newby) and an observational study into the role of myocardial fibrosis in aortic stenosis (Dr Calvin Chin and Dr Marc Dweck) who provided blood samples and participant data for Chapter 3. Samples were analysed in collaboration with Professor Michael Marber and team from King's College London. Dr Jacek Kwieceński additionally provided histology data and images. Dr Fiona Strachan, Neil Johnston and Edwin Carter helped with laboratory processing for the variability study in Chapter 4. Dr Catherine Harley and Dr Akila Visvanathan assisted with the systematic review presented in Chapter 5.

Chapters 3 and 5 have been published in peer-reviewed journals. The thesis has not been accepted in any previous applications for a degree and all sources of information have been acknowledged. All clinical studies were undertaken in accordance with the regulations of the South East Scotland Research Ethics Committee and the Declaration of Helsinki.

Atul Anand

24th October 2018

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ABBREVIATIONS

ACC – American College of Cardiology

ACE – Angiotensin Converting Enzyme

AHA – American Heart Association

ANOVA – Analysis of Variance

ARB – Angiotensin II Receptor Blocker

AUC – Area Under the Curve

AV – Aortic Valve

AVR – Aortic Valve Replacement

BAV – Balloon Aortic Valvuloplasty

BMI – Body Mass Index

BNP – Brain-type Natriuretic Peptide

BSA – Body Surface Area

CABG – Coronary Artery Bypass Grafting

CAF – Comprehensive Assessment of Frailty

CES-D – Centre for Epidemiology Studies- Depression

CFS – Clinical Frailty Scale

CHSA – Canadian Study on Health and Ageing

CI – Confidence Interval

CINAHL – Cumulative Index to Nursing and Allied Health Literature

CKD – Chronic Kidney Disease

CMR – Cardiac Magnetic Resonance

cMyC – Cardiac Myosin-Binding Protein C

COMPASS – Cardiac Biomarkers in Older Patients with Aortic Stenosis

COPD – Chronic Obstructive Pulmonary Disease

CORONA – Controlled Rosuvastatin Multinational Trial in Heart Failure

CT – Computed Tomography

CV – Coefficient of Variation

CV_A – Analytical Variability

CV_G – Between-subject Variability

CV_I – Biological Variability

CV_T – Total Subject Variability

DEXA – Dual-energy X-ray Absorptiometry

ECV – Extracellular Volume

EDTA – Ethylenediaminetetraacetic Acid

EDV – End-diastolic Volume

EFS – Edmonton Frail Scale

eGFR – Estimated Glomerular Filtration Rate

EMBASE – Excerpta Medica Database

ESC – European Society of Cardiology

ESV – End-systolic Volume

EWGSOP – European Working Group on Sarcopenia in Older People

HR – Hazard Ratio

IHD – Ischaemic Heart Disease

II – Index of Individuality

ISAR – Identification of Seniors at Risk

LGE – Late Gadolinium Enhancement

LV – Left Ventricle

LVEF – Left Ventricular Ejection Fraction

MACCE – Major Adverse Cardiovascular and Cerebral Events

MCS – Mental Component Score

MPG – Mean Pressure Gradient

MRI – Magnetic Resonance Imaging

NHS – National Health Service

OR – Odds Ratio

PARTNER – Placement of Aortic Transcatheter Valve Trial

PCI – Percutaneous Coronary Intervention

PCS – Physical Component Score

POPS – Proactive Care of Older People Undergoing Surgery

RCV – Reference Change Value

ROC – Receiver Operating Characteristic

SALTIRE – Scottish Aortic Stenosis and Lipid Lowering Trial

SAVR – Surgical Aortic Valve Replacement

SCTS – Society of Cardiothoracic Surgeons of Great Britain and Ireland

SD – Standard Deviation

SF-12 – Short Form Survey- 12 questions

SHERPA – Score Hospitalier d’Evaluation du Risque de Perte d’Autonomie

SPPB – Short Physical Performance Battery

STS – Society of Thoracic Surgeons

SV – Stroke Volume

TAVI – Transcatheter Aortic Valve Implantation

TAVR – Transcatheter Aortic Valve Replacement

TIA – Transient Ischaemic Attack

TUG – Timed Up and Go

UK – United Kingdom

USA – United States of America

VARC – Valve Academic Research Consortium

VISION – Vascular Events in Surgery Patients Cohort Evaluation

WMH – White Matter Hyperintensities

ABSTRACT

The incidence of degenerative aortic stenosis is increasing with an ageing population. Valve replacement is the only proven treatment, but this carries significant procedural risk in older people. Current guidelines advocate intervention in symptomatic severe aortic stenosis, but non-cardiac symptoms and comorbidity may obscure this assessment. Clinical biomarkers offer the potential for objective patient assessment. My aim was to firstly assess the validity and reproducibility of novel blood biomarkers of disease progression in aortic stenosis. Secondly, in older patients considered for valve replacement, my aim was to compare measures of frailty with conventional surgical risk assessment.

In 265 patients with asymptomatic aortic stenosis and 46 healthy controls, I assessed serum concentrations of the sarcomeric protein cardiac myosin binding protein C (cMyC) and objective markers of disease progression and mortality. cMyC concentrations were independently associated with imaging evidence of left ventricular mass, fibrosis volume and extracellular volume. These relationships were not observed in healthy controls. cMyC concentrations were also associated with all-cause mortality over 11 years of follow-up. This suggests a role for cMyC as a novel objective biomarker of aortic stenosis disease severity.

Other blood biomarkers including cardiac troponin, brain-type natriuretic peptide (BNP) and galectin-3 have been suggested as disease biomarkers in aortic stenosis. However, performance and precision of these assays has not been described in older patients. In a study of analytical and biological variability, I undertook repeated hourly and weekly blood sampling for cardiac troponin, BNP and galectin-3 in 14 subjects with severe asymptomatic aortic stenosis. These biomarkers demonstrated low indices of individuality, implying that interpretation requires serial testing for change rather than isolated elevated blood concentrations. The reference change values for weekly fresh sampling were 42% for cardiac

troponin, 55% for BNP and 14% for galectin. These values for cardiac troponin and BNP were lower than equivalent studies in healthy controls and in stable heart failure.

To assess the role of frailty in the assessment of patients for aortic valve replacement, I first performed a systematic review and meta-analysis of studies including frailty assessment before Transcatheter Aortic Valve Implantation (TAVI). This procedure is reserved for patients considered at prohibitive risk of complication from conventional open-heart surgery. Ten cohort studies with 4,592 TAVI patients were included. Frailty was associated with increased risk of early and late mortality, and use of an objective frailty tool rather than subjective assessment identified those at highest risk; these patients experienced greater than double the mortality risk of non-frail individuals.

In 185 patients with severe aortic stenosis, I prospectively assessed frailty using four tools: the Fried phenotype, Edmonton Frail Scale, Short Physical Performance Battery and Clinical Frailty Scale. These measures were compared to surgical risk assessment scores from the Society of Thoracic Surgeons (STS) and EuroSCORE II. Agreement between frailty measures was moderate and unrelated to patient age or the degree of aortic valve severity. Frail patients had poorer physical and mental wellbeing. Frailty increased at higher STS and EuroSCORE estimates, but using principal components analysis I demonstrated divergence between frailty measures and surgical risk estimates. Outcomes after aortic valve replacement are now required to establish if this observation is meaningful for the improved prediction of outcomes after surgery.

My findings suggest that serial testing of blood biomarkers of myocardial injury in patients with aortic stenosis may detect meaningful disease progression prior to decompensation. In patients considered for valve replacement, measurement of frailty differs from existing surgical risk tools and may add to the holistic assessment of older patients.

LAY SUMMARY

Aortic stenosis describes the degeneration of the main valve that controls blood flow out of heart. This more frequently occurs in older people. Without a surgical procedure to replace the valve, aortic stenosis results in thickening and scarring of heart muscle and eventual heart failure. There are no proven alternative treatments to slow or stop progression of disease, and timing valve replacement is challenging. The rate of aortic stenosis progression varies unpredictably between individuals and assessing symptoms of early heart failure can be difficult in older people with other health problems. Further, in older patients undergoing valve replacement, the risks of complications may outweigh potential benefits of surgery. Increasingly, such risk is measured in terms of frailty, which describes individuals at increased risk of dependency or death due to a lack of health reserves. However, frailty is not currently included in the surgical risk calculations that are used to help inform decisions for valve intervention in older patients.

The first aim of this thesis was to test the role and reliability of blood tests of heart damage to identify progression of aortic stenosis. Second, in older patients considered for aortic valve replacement, my aim was to compare measures of frailty with existing surgical risk scores.

In 265 patients with aortic stenosis and 46 healthy controls with normal heart valves, blood levels of a heart muscle protein called cardiac myosin binding protein C (cMyC) were measured. Higher levels of this blood test were independently associated with thickening of heart muscle and scarring on cardiac magnetic resonance imaging in aortic stenosis patients. These relationships were not seen in healthy controls. Patients with higher levels of cMyC were also more likely to die of any cause over 11 years of follow-up. This suggests a possible role for cMyC as a measure to track progression of aortic stenosis using a simple blood test.

Other blood tests may also be useful markers of heart damage in aortic stenosis and in a second experiment I tested the reliability of three different tests in 14 older patients with aortic stenosis. Blood tests for heart muscle damage (cardiac troponin), scarring (galectin-3) and heart failure (BNP) were measured repeatedly at hourly and weekly intervals in the same patients to investigate the stability of these measures. I calculated the percentage change in each blood marker that would signify a meaningful change between tests taken weeks apart, such as between clinic appointments. This was a 42% change for cardiac troponin, 55% for BNP and 14% for galectin-3.

To investigate the role of frailty testing in older patients under consideration for aortic valve replacement, I first systematically looked at previous studies in patients undergoing Transcatheter Aortic Valve Implantation (TAVI). This procedure is reserved for patients considered at prohibitive risk of complication from conventional open-heart surgery. Ten studies with 4,592 TAVI patients were included in this assessment. Frailty was associated with increased risk of early and late death after TAVI, and use of a specific frailty tool rather than end-of-the-bed assessment identified those at highest risk; these patients experienced double the risk of dying early after TAVI when compared to non-frail individuals.

To compare frailty measures with existing surgical risk scores, I assessed 185 patients with severe aortic stenosis using 4 different tools. These measures were compared to surgical risk assessment scores from the Society of Thoracic Surgeons (STS) and EuroSCORE II. Agreement between the frailty measures was moderate, but was unrelated to the age of patients or the severity of their aortic stenosis. Frail patients by all measures had poorer physical and mental wellbeing and generally had higher surgical risk scores. However, using a technique called principal component analysis, which compares the patterns in data between patients, the frailty scores appeared to show consistent differences from the two surgical risk scores. This suggests that frailty adds additional information to what is currently

captured by the STS and EuroSCORE, but collecting outcomes of these patients in the future will help understanding of the value of these extra measures in helping to predict outcomes after surgery.

My findings suggest that blood testing for markers of heart damage in patients with aortic stenosis may be a simple method to detect meaningful progression of disease and identify patients at risk of heart failure without valve replacement. In patients considered for valve replacement, measurement of frailty differs from existing surgical risk tools and may add to the holistic assessment of older patients.

CHAPTER 1

ASSESSMENT AND OUTCOMES FOR OLDER PATIENTS WITH AORTIC STENOSIS

CHAPTER 1: ASSESSMENT AND OUTCOMES FOR OLDER PATIENTS WITH AORTIC STENOSIS

1.1 OVERVIEW

Aortic stenosis is the most common valvular heart disease in the Western World, affecting 1 in every 8 older adults over 75 years old.¹ Ageing population demographics continue to increase the incidence of severe stenosis, at which point the risk of progression to decompensated heart failure or death without replacement surgery rises to 40% at one year.² Current guidelines advocate valve replacement in the event of symptomatic severe aortic stenosis, but the effects of comorbidity in an older population may obscure this assessment.³ Decision-making is therefore complex, with exposure to the risks of major cardiac surgery in the absence of meaningful benefit if symptoms are mistakenly attributed to aortic stenosis.

Clinical biomarkers offer the potential for objective patient assessment. Circulating blood markers of myocardial injury may identify the advanced hypertrophic and fibrotic response of the myocardium to aortic stenosis. However, the degree of change in these markers that would be indicative of significant change is currently unclear in an older population. Similarly, patient assessment including biomarkers of frailty may be an additive marker of risk to improve current surgical assessment tools in older populations. Such clinical biomarkers may help make individualised assessments and reduce the risk of harm, but their potential role in the care of older patients with aortic stenosis requires further evaluation.

1.2 AORTIC STENOSIS IN OLDER ADULTS

Below the age of 60, aortic stenosis necessitating valve replacement is almost exclusively associated with congenitally bicuspid valves.⁴ However, as the population ages, degenerative calcification is the predominant driver of stenosis and is being identified in greater numbers of older individuals.^{5,6} Meta-analysis of seven large population prevalence studies suggests that 3.4% of all individuals over 75 years old have severe aortic stenosis. This equates to approximately 1 million older individuals in Europe alone.¹ Population prevalence appears to increase rapidly after the age of 65.⁷ Systematic community screening in this demographic as part of the OxVALVE study identified 1.3% of the population with aortic stenosis. Projections from this dataset suggest that the burden of valvular disease will double over the next twenty years.⁸

Importantly, outcomes have not significantly improved in this growing population with aortic stenosis. In a large American series of valvular heart disease over the last three decades, mortality from aortic valve disease has risen by an average of 1.56% per year, undoubtedly partly driven by reduced competing mortality risks. However, in contrast to progress in the management of coronary disease over this period, there has been virtually no improvement in the age- and sex-adjusted mortality rates from valvular heart disease between 1979 and 2009.^{9,10} In the developing world, rheumatic heart disease remains an important precipitant of aortic valve disease, where stenosis often occurs concurrently alongside regurgitation and mitral valve dysfunction.¹¹ Within Europe, only one in five cases of valvular disease can still be attributed to rheumatic heart disease, as the burden of age-related degenerative disease increases.⁷

1.2.1 PROGRESSION OF DISEASE

Progression of aortic stenosis is unpredictable. A preclinical phase of aortic sclerosis, namely thickened aortic valve cusps without significant blood flow limitation, occurs prior to the

development of calcific aortic stenosis, but many individuals will never progress. Indeed, population studies have suggested that by the age of 85 years old, half of the population will have developed aortic sclerosis. The conversion from sclerosis to stenosis affects approximately 1.8-1.9% of patients per year, but there are no clear methods for identifying who will progress, nor for predicting the rate of disease advancement in an individual.^{12,13} However, observational data has suggested that individuals with the greatest valve calcification are at higher risk of rapid progression of aortic stenosis. Other suggested factors identified for rapid progression are older age (over 50 years old), coexisting coronary artery disease and diabetes mellitus, although in multivariate modelling including these characteristics, only valve calcification remained an independent predictor of death or requirement of valve replacement.^{2,14}

Once aortic stenosis has developed, the response of the myocardium is critical to determining progression and development of symptomatic disease. Left ventricular hypertrophy develops as a result of increased afterload, which is necessary to preserve ejection fraction and normalise left ventricular wall stress.¹⁵ Hypertrophy is associated with reactive interstitial fibrosis as a result of collagen production from myofibroblasts.¹⁶ This process exhibits wide individual heterogeneity, which may explain the variability in progression to symptomatic decline. Those with maladaptive hypertrophy and inappropriately high left ventricular mass appear at highest risk of poor outcomes.¹⁷ Furthermore, the severity of stenosis across the valve is not well related to the degree of hypertrophy.¹⁸ However, it is clear that development of myocardial fibrosis in response to aortic stenosis is a poor prognostic sign, with an up to 8-fold increased mortality risk compared to those without fibrosis, despite similar degrees of aortic stenosis severity and coronary disease.^{18,19}

In the natural history of untreated aortic stenosis, compensation through hypertrophy and fibrosis is eventually exhausted and heart failure ensues. This transition is characterised by

progressive cardiomyocyte death and replacement fibrosis.²⁰ Reductions in left ventricular ejection fraction or cardiac output are end-stage features of aortic stenosis, usually after symptoms have developed. These include exertional dyspnoea as a result of pulmonary hypertension from raised left ventricular pressures, and anginal chest pain from reduced coronary perfusion secondary to greater vascular resistance and increased demand from hypertrophied left ventricular tissue.^{21,22} Exertional pre-syncope or syncope may occur when peripheral vasodilation cannot be matched by increased stroke volume. These symptoms become more pronounced when overt left ventricular failure and fluid overload develop. Sudden cardiac death is also associated with untreated aortic stenosis, although this is a rare occurrence in the absence warning symptomology.

1.2.2 TREATMENT OPTIONS

There are currently no pharmacological therapies for aortic stenosis. Similarities in the processes of atherosclerosis and valve calcification previously generated interest in statin therapy. However, these drugs have now been proven to not influence disease progression.^{23,24} Purely medical management may be offered to patients who do not wish to undergo a surgical procedure, or in whom the risk of surgery is considered excessive. The mainstay of such an approach is blood pressure control and symptom management, using standard therapies for heart failure when this develops.³

In those suitable for intervention, there are three broad approaches to management which may be considered when symptoms develop or in an asymptomatic patient with evidence of left ventricular impairment.

1.2.2.1 SURGICAL AORTIC VALVE REPLACEMENT (SAVR)

Cardiac surgery via a median sternotomy approach to directly replace the aortic valve remains the mainstay of treatment in the majority of patients considered fit enough for

surgery. In older patients, bioprosthetic valves obviate the need for anticoagulation when compared to mechanical valves, but at the cost of reduced longevity of the prosthesis.²⁵ SAVR typically carries a 4-8% risk of in-hospital mortality in older patients (over 70 years), which may rise with comorbidity.³ A meta-analysis of 8,975 over 80 year olds undergoing combined valve replacement and bypass grafting reported a 30 day mortality rate of 10%.²⁶

1.2.2.2 TRANSCATHETER AORTIC VALVE IMPLANTATION (TAVI)

TAVI has become a widespread and viable alternative for patients considered high-risk for conventional surgery. This is a less invasive approach, usually performed via femoral arterial access. The PARTNER trial randomised patients considered at excessively high-risk from SAVR to either medical management or TAVI. After one year, mortality and hospitalisation was significantly lower in the TAVI group (composite 42.5% vs 71.6% in medically treated group). This was despite higher rates of stroke and bleeding in those undergoing the procedure.²⁷

The subsequent PARTNER II trial assessed those with intermediate surgical risk and suggested that TAVI in this group has similar outcomes to conventional surgery.²⁸ However, meta-analysis of all such studies highlighted potentially poorer outcomes with trans-apical TAVI compared to conventional SAVR, although the inherent perioperative risk of those selected for trans-apical TAVI over SAVR in observational cohorts is likely to be higher.²⁹ In Germany, adoption of TAVI has been rapid, with the number of procedures performed now outstripping surgical valve replacements. However, questions remain over the longevity of the implanted valve and persisting high rates of post-procedure permanent pacemaker implantations (required in 1 in 8 cases in the German TAVI registry).³⁰ Furthermore, patient selection over conventional surgery remains challenging, when the benefits of intervention in the oldest and frailest patients are not always clear.³¹

1.2.2.3 BALLOON AORTIC VALVULOPLASTY (BAV)

This procedure uses the inflation pressure of a balloon across the stenotic aortic valve to temporarily reduce the transvalvular gradient. Effects typically last for weeks to months and must be balanced with risks of periprocedural complications such as bleeding and stroke. In one series of 301 BAV procedures for severe aortic stenosis that was deemed inoperable, serious adverse events occurred in 16% of patients, the most common of which were significant vascular complications (7%).³² Long-term survival after BAV was also noted to be poor, with half of patients dead within 6 months. There are two areas where BAV is advocated. Firstly, as a bridge to definitive therapy in patients suitable for TAVI or SAVR but in whom acutely florid symptoms limit immediate treatment or there is a requirement for urgent major non-cardiac surgery.³ Secondly, where the origin of symptoms cannot be conclusively determined, such as with progressive dyspnoea with evidence of intrinsic lung disease, BAV may offer a therapeutic trial whereby relief of symptoms indicates the presence of truly symptomatic aortic stenosis.³¹

1.2.3 CURRENT GUIDELINES

Current clinical guidance from the European Society of Cardiology (ESC) provide clear advice (class I recommendation) favouring SAVR in patients with severe aortic stenosis who are symptomatic or have provoked symptoms on exercise testing. Severe aortic stenosis should be defined using a combination of measures from doppler echocardiography assessment rather than a single parameter. ESC guidelines recommend severe status is applied where the transvalvular peak jet velocity exceeds 4 metres per second and where valve area is less than 1.0cm². It is also noted that severe aortic stenosis usually occurs with a mean transvalvular pressure gradient greater than 40mmHg.³

ESC guidelines recommend that asymptomatic severe aortic stenosis patients should be considered for SAVR where there is evidence of left ventricular impairment (ejection

fraction <50%), or where the patient is undergoing other major cardiac surgery.³ However, these recommendations are largely based on observational studies of patients with asymptomatic severe aortic stenosis such as that performed by Rosenhek *et al.* in 1994, where the mean age of included participants was just 60 years old.² The ESC recommendations are consistent with those made by the American Heart Association and American College of Cardiology (AHA/ACC).³³

There is lower quality evidence to recommend SAVR in so-called ‘low-flow, low-gradient’ aortic stenosis with preserved ejection fraction, which is more common in older patients. This occurs where valve parameters suggest severe stenosis, but the transvalvular gradient does not meet the criteria for severe disease (i.e. AV_{max} velocity <4m/s and/or mean pressure gradient <40mmHg). However, observational cohort data suggests that these patients have a similar risk of poor outcomes as those with mild-to-moderate aortic stenosis.³⁴

The ESC also provide guidance on patient selection for TAVI. It is recommended that patients are assessed by a “heart team” including cardiologists, cardiac surgeons, imaging specialists and with the potential to include general practitioners, geriatricians and intensive care doctors.³⁵ TAVI may be considered where this team deems conventional SAVR unsuitable due to “severe comorbidities”. Patients selected for TAVI should have a life-expectancy of at least one year and be expected to gain improvement in quality of life after the procedure.³ Interestingly, the one year mortality rate in the PARTNER trial TAVI arm was 30.7%, suggesting that clinician estimation of this outcome is challenging.²⁷ In both European and American guidelines, there is little clarity on assessment of older patients and interpretation of the significance of comorbidity on surgical outcomes. It may be argued that current guideline pathways have failed to keep pace with a fundamental shift in the aortic valve surgical population, from younger patients with failing bicuspid valves, to the elderly with calcific degenerative valve disease and multimorbidity.

1.2.4 NATURAL HISTORY WITHOUT INTERVENTION

When considering complex aortic valve interventions it is important to understand the natural history of medically managed severe aortic stenosis. As previously described, progression of stenosis in an individual patient is unpredictable. Taken in aggregate, valve area decreases on average by 0.1cm^2 per year, while mean transvalvular gradient increases by 7mmHg .^{36,37} Recent studies allocating patients with severe aortic stenosis to either TAVI or medical management have provided relevant data on outcomes for those managed conservatively. In two such series, one-year all-cause mortality rates varied between 40-51%.^{27,38} Rosenhek *et al.* reported event rates in a severely stenotic cohort for a composite endpoint of death or aortic valve replacement necessitated by symptoms as 33% at one year, 44% at two years and 67% by four years.² The hard outcomes for medically managed patients are therefore poor, but this must be balanced against the risks of intervention and quality of life in elderly individuals. It is however possible that clinician perceived risk of cardiac surgery prevents referral of patients who may benefit from valve replacement; a multicentre study has suggested that up to one third of patients with severe aortic stenosis are not considered suitable for assessment referral for valve replacement. In the majority of these cases, conventional risk scores were not significantly higher than in those who received surgery, suggesting either variability of referral or assessment of these patients.³⁹

1.2.5 COMPLICATIONS OF AORTIC VALVE REPLACEMENT

Complication rates from SAVR have been progressively falling with time. Across all age groups, estimates suggest a short-term mortality rate of less than 3% for isolated SAVR procedures, although this increases with age and concomitant cardiac procedures such as coronary bypass grafting.⁴⁰⁻⁴² Between 1995 and 2003, outcomes from 32,839 cardiac operative procedures were recorded by the Society of Cardiothoracic Surgeons of Great Britain and Ireland (SCTS). This revealed an in-hospital mortality rate of 3.3% in those under 50 years old, but a comparable rate of 10.3% in those over 79 years old. In risk

modelling based on large consecutive series of SAVRs, age was a key determinant of short-term mortality risk, surpassed only by a procedure being performed as an emergency.^{43,44}

Patients undergoing TAVI are generally older, but only experienced a 5% mortality rate at 30 days after TAVI in the PARTNER trial cohort.²⁷ However, the intermediate death rates are striking, with 54% and 72% of TAVI patients dead by 3 and 5 years respectively.^{45,46} This compares to a 28% 5-year mortality rate amongst high-risk isolated AVR cases.⁴⁷ Non-mortality complication event rates are also more significant in older populations.

1.2.5.1 STROKE

Calcification of the stenotic aortic valve and associated atherosclerotic changes in the aorta are significant potential foci for embolisation and stroke at the time of aortic valve replacement. This may be a devastating consequence of an elective surgical procedure. During SAVR, immediate stroke events appear related to cannulation of a calcified aorta to initiate cardiopulmonary bypass.⁴⁸ However, late strokes may continue to occur as a result of microembolisation following valve replacement.⁴⁹ Intracardiac surgery such as valve replacement has long been observed to carry a higher stroke risk than extracardiac procedures such as coronary artery bypass grafting.⁵⁰ Overall post-SAVR stroke rates are estimated at 1.5%, but rising to between 2% and 4% in older cohorts.^{47,48}

Periprocedural stroke was initially reported as a more common outcome amongst TAVI patients complicating up to 6% of procedures. Transcranial doppler studies suggest that embolisation occurs during the balloon valvuloplasty and valve deployment phases of the procedure.⁵¹ Stroke event rates are likely to have fallen with technological improvements and increased procedural experience. However, predisposition to delayed strokes remain a significant concern; long-term follow-up of the PARTNER trial TAVI cohort revealed that 15.7% had experienced a major stroke by three years after the procedure, compared to 5.5% in those receiving medical therapy.⁴⁵

1.2.5.2 DELIRIUM AND COGNITIVE DECLINE

Delirium is a common but severe post-operative complication. It is characterised by inattention, altered arousal and disorganised thinking. In some studies, this has been described in up to half of all older cardiac surgery patients, with greater risk in those procedures including valve replacement.⁵²⁻⁵⁵ Across all ages, delirium acts as an independent risk factor for death, even ten years after surgery.⁵⁶ It is also associated with severe patient and carer distress, increased length of hospital stay and loss of functional independence.^{57,58} Saczynski *et al.* assessed cognitive trajectories for one year after cardiac surgery in those who did and did not develop post-operative delirium. Using the mini-mental state examination as a test of global cognitive function, the 46% of all patients who developed delirium had persisting cognitive deficits for at least one month after surgery and a trend towards persisting effects up to one year.⁵⁹ This adds to other research that has established a link between delirium and future cognitive decline, or acceleration of pre-existing dementia.^{60,61} Traditional views of a harmless and transient post-operative confusional state are therefore outdated; delirium represents acute brain dysfunction with poor clinical, functional and pathological outcomes.

It has been postulated that post-operative delirium may be related to cerebral microembolism. However, rates appear lower after TAVI despite microembolic events being a near universal phenomenon.^{54,62-64} The long-term consequences of these microemboli are unclear, but are associated with an increasing burden of cerebral small vessel disease. White matter hyperintensities (WMH) are one form of these lesions and have been strongly related to cognitive decline in longitudinal studies.⁶⁵ Preoperative high WMH burden has been associated with delirium after cardiac surgery, supporting a theory of measurable cerebral vulnerability.⁶⁶ As with delirium in other circumstances, the pathogenesis of post-operative delirium is unclear and is likely to reflect multiple putative causative derangements rather than a final common pathway. This may include dysregulated neuroinflammation, altered

neuroendocrine responses, oxidative stress and disturbed circadian rhythms in response to physiological stressors experienced during surgery and recovery.⁶⁷

1.2.5.3 FUNCTIONAL DECLINE AND QUALITY OF LIFE

A limitation of aortic valve replacement studies has been a focus on mortality, rather than functional and quality of life outcomes, which may be of particular importance in older patients. A systematic review of such outcomes after TAVI by Kim *et al.* concluded that:

*“more comparative studies on functional status and quality of life are needed for informed treatment decision making”*⁶⁸

In one single-centre study of cardiac surgery including SAVR, 10% of patients required ‘institutional’ care in place of discharge home after surgery, defined as rehabilitation or ongoing nursing level care.⁶⁹ Abah *et al.* systematically reviewed the evidence for quality of life changes amongst those over 80 years old undergoing cardiac surgery. The majority appeared to experience improvements, but a significant minority of between 8-19% of all patients experienced declines in quality of life after surgery.⁷⁰

Amongst the PARTNER randomised controlled trial cohort of TAVI patients, function was measured using the New York Heart Association symptom scale. This appeared to improve in the majority of patients with effects sustained for at least three years.⁴⁵ However, assessing quality of life components of the Kansas City Cardiomyopathy Questionnaire performed in the study and subsequent registry revealed that up to 33% of the 2,137 participants undergoing TAVI had a ‘poor’ 6-month outcome.⁷¹ Others have reported smaller proportions of TAVI patients with functional decline, but these individuals remain an important and significant minority.⁷²

Despite the uncertainty of evidence in this area, ESC guidelines do prioritise expected functional outcomes over survival, stating that TAVI is ‘absolutely contraindicated’ in those without an expected improvement in quality of life.³ The optimum method of identifying these vulnerable individuals is unclear, but is of paramount importance to the management of older patients with aortic stenosis.

1.3 CURRENT ASSESSMENT OF OLDER AORTIC STENOSIS PATIENTS

Given the variation in outcomes after aortic valve replacement, objective assessment of procedural risk is essential to guide choice of management. Current assessment is largely based on models constructed from large datasets of observed procedural outcomes, with a focus on early mortality and morbidity.

1.3.1 CARDIAC SURGERY RISK ASSESSMENT TOOLS

The most widely used risk tool is from the Society of Thoracic Surgeons (STS), where the SAVR model is based upon outcomes from 67,292 procedures performed between 2002 and 2006.⁷³ The covariates include age, gender, ethnicity, body mass index, comorbidities and preoperative cardiac status. There is however no inclusion of functional status or dependency in the model. The most commonly cited output is the STS operative mortality, which predicts the percentage risk of death during the initial surgical admission or within 30 days if discharged. In the PARTNER trial of TAVI or medical management for high-risk surgical candidates, a threshold STS score of 10% was chosen to define the group for randomisation.²⁷ Further output scores include estimated risk of stroke, acute renal failure, prolonged ventilation, deep sternal wound infection and the requirement for reoperation.⁷³

Similarly, the EuroSCORE II risk assessment tool was initially based on 6,753 consecutive SAVR procedures across 154 surgical units over 12 weeks in 2010. The model includes similar covariates to the STS score, but does additionally prescribe risk to patients with 'severe mobility impairment secondary to musculoskeletal or neurological dysfunction'.⁷⁴ Due to the challenges of collecting data from disparate centres, the outcome measure is defined as mortality during the initial surgical admission rather than by a fixed follow-up period. The included derivation dataset is also much smaller than for the STS score, which is particularly noteworthy for the estimation of risk in older patients. The calculator carries a warning stating that:

“Of over 20,000 patients in the EuroSCORE database, only 21 patients were aged over 90 - therefore the risk model may not be accurate in these patients. Please exercise clinical discretion in interpreting the score.”

Even without considering holistic markers of risk in older populations such as function and frailty, both scores do not include important conditions such as a history of stroke, liver disease, ‘porcelain’ aorta and prior chest irradiation which may increase surgical risk.⁷⁵ It is also worth acknowledging that these risk scores derived in cardiac surgery populations are not validated for prediction of outcomes after TAVI. Indeed the ESC guidelines acknowledge the deficiencies in this area, stating that:

“In the absence of a perfect quantitative score, the risk assessment should mostly rely on the clinical judgement of the ‘heart team’, in addition to the combination of scores”³

Ongoing registries of TAVI procedures will eventually have sufficient power to model risk in a similar manner to the STS and EuroSCORE tools.

1.3.2 DISCRIMINATION AND CALIBRATION OF EXISTING RISK SCORES

Model accuracy is defined by two complementary measures: discrimination and calibration. Discrimination describes how successfully a model separates cases into groups; in the example of the STS and EuroSCORE tools discrimination for operative risk is generally good. The c-statistic for the discrimination of operative mortality in valve replacement surgery using the STS score is 0.78⁷⁶ and by EuroSCORE II is 0.69.⁷⁷ However, these data are provided as measures of discrimination across the whole adult surgical population; it is unclear if performance is maintained amongst the oldest patients. Calibration is also

important, assessing how well predicted probabilities agree with observed risk.^{78,79} On this measure, both STS and EuroSCORE perform relatively poorly, particularly in older populations. In a study of valvular heart surgery patients, the STS score predicted 40% less mortality than was observed, whilst the logistic EuroSCORE (a predecessor of the EuroSCORE II) expected mortality rate was greater than three-fold higher than actual levels.⁸⁰ The lack of calibration therefore limits the ability of these models to accurately estimate operative risk in an *individual* rather than as part of a *cohort* of patients with similar characteristics.⁸¹ This strongly suggests that existing tools fail to capture critical components of the risks of cardiac surgery in older patients. Calibration of models also vary between populations, reflecting differences in baseline risk including age demographics; models therefore frequently require recalibration when applied to disparate populations.

1.3.3 CHALLENGES OF ASSESSMENT IN OLDER PATIENTS

Guidelines for aortic valve replacement necessitate the presence of symptoms or left ventricular impairment in all but the lowest risk patients. However, in an elderly population with multiple comorbidity, discriminating symptoms from aortic stenosis is challenging. A Scottish population-level epidemiological study of trends in aortic valve disease retrospectively assessed 13,220 individuals with aortic stenosis. Across all ages, 17% of patients had co-existing significant respiratory disease, a further 11% diabetes mellitus, 9% cancer and 8% renal disease.⁸² These figures are likely to be even higher in older populations, where respiratory comorbidity has been noted in between 18%-24% of cases.^{27,47}

It has been suggested that exercise stress testing may unmask true symptoms where there is doubt due to comorbidity.⁸³ However, many older patients are unlikely to tolerate such investigations or gain meaningful results due to the limitations of poor mobility. Similar challenges exist in determining myocardial fibrosis by cardiac magnetic resonance imaging. Many older patients are unable to tolerate prolonged scanning lying flat or the intermittent

breath-holding required for such diagnostic imaging. Others would advocate a trial of therapy for aortic stenosis by means of balloon valvuloplasty, but this exposes the patient to important risks from valve manipulation such as stroke in order to reach a firm diagnosis. In the event of short-lived symptomatic benefit indicating a culprit aortic valve, these risks are effectively duplicated by undertaking definitive valve replacement.

1.4 NOVEL ASSESSMENT TOOLS

The evidence presented shows that objective, non-invasive biomarkers of functionally relevant aortic stenosis are currently lacking. Furthermore in the assessment of patients for valve replacement, existing surgical risk scores lack the calibration to provide reliable individual measures of risk, particularly in an older patient. Assessment and decision-making in this population would be greatly enhanced by objective markers of aortic stenosis progression and surgical risk, acknowledging that outcomes after surgery include quality as well as quantity of life. These may be considered as biomarkers, defined by the Oxford English Dictionary as:

*“a diagnostic indicator of (predisposition to) a medical condition”*⁸⁴

This thesis will consider two broad groups of biomarkers. First, putative biomarkers of the consequences of aortic stenosis, including markers of ventricular wall stress, fibrosis and myocardial injury. These may be considered as candidates for tracking disease progression that could provide early identification of a declining patient in whom aortic valve replacement should be considered. Second, physical performance and frailty measures may act as biomarkers of perioperative risk and long-term mortality. Such measures could be used to inform and modify a decision for aortic valve replacement, potentially by enhancing the prediction of existing surgical risk scores. These measures may not necessarily just influence the binary decision to proceed to valve replacement; increased awareness of perioperative risk may identify a population for enhanced early post-operative care (e.g. a longer monitoring period in an intensive care setting) and rehabilitation programmes after a valve procedure.

These two groups of biomarkers will now be considered in more detail.

1.4.1 BLOOD BIOMARKERS OF MYOCARDIAL INJURY

An objective assessment of the myocardial response to aortic stenosis to detect early decompensation may identify a window of opportunity for effective intervention.

Progression of aortic stenosis is characterised by cardiomyocyte death and replacement fibrosis.²⁰ Circulating biomarkers of myocardial injury and cell death are therefore attractive potential markers of advancing disease.

1.4.1.1 CARDIAC TROPONIN

Cardiac troponin is a structural sarcomeric protein present in cardiac muscle (Figure 1.1), with plasma concentration acting as a highly specific marker for myocardial injury.⁸⁵ It may now be quantified with high precision at extremely low circulating blood concentrations.^{86,87} Chin *et al.* investigated whether plasma cardiac troponin concentration was associated with long-term prognosis in patients with asymptomatic aortic stenosis of moderate severity. Cardiac troponin was quantifiable using a high-sensitivity assay in all cases of aortic stenosis. One in ten patients had concentrations above the upper reference limit that is recommended as the diagnostic threshold for myocardial infarction. Cardiac troponin concentration was an independent predictor of outcomes (valve replacement or cardiovascular death) at 10 years after adjustment for age, sex, symptoms, coronary artery calcium and severity of valvular disease (HR 2.10 per 2-fold increase in cardiac troponin, 95% confidence intervals [CI] 1.22–3.61, $p=0.007$). Interestingly, levels of this biomarker correlated with left ventricular hypertrophy and replacement fibrosis rather than the severity of stenosis.^{88,89}

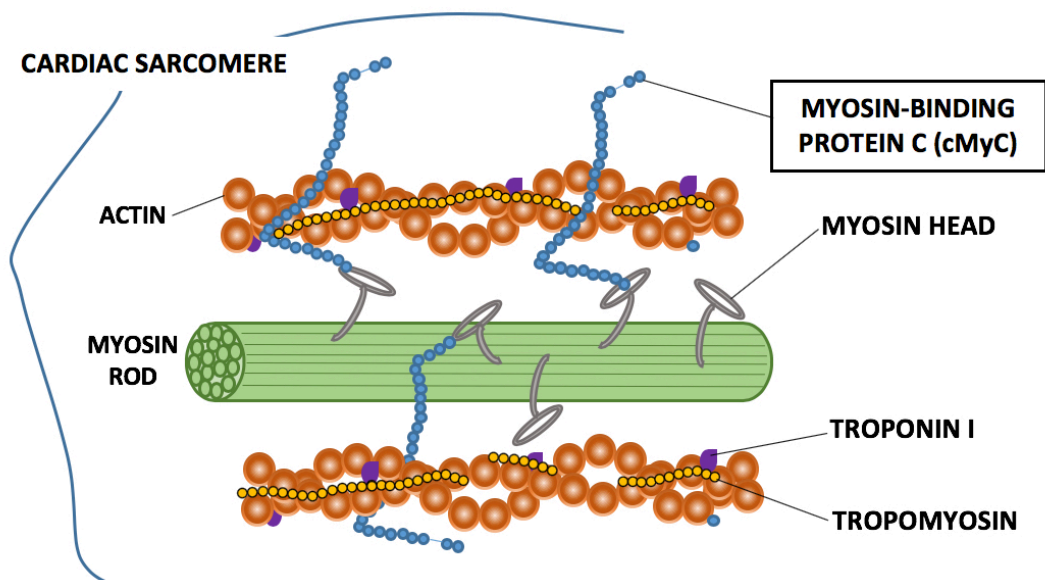


Figure 1.1 – Simplified schematic representation of a cardiac sarcomere. This demonstrates that cardiac troponin I binds to actin in thin myofilaments which hold the troponin-tropomyosin complex in place. Troponin I exists within a troponin complex including troponin T and troponin C (not shown). Cardiac myosin binding protein C is a larger structure that binds along the myosin thick filament.

In a separate analysis cardiac troponin concentrations were 4-fold higher in patients with aortic stenosis and left ventricular strain than in those without hypertrophy or strain (18.6ng/L (IQR 9.0–45.2) with strain vs 4.3ng/L (IQR 2.5–7.3) without, $p < 0.001$), and this relationship persisted after adjustment for age, sex and systolic blood pressure.⁹⁰ This suggests the response to pressure overload may be as important as pressure overload itself, with cardiac troponin proving a candidate biomarker of functionally significant aortic stenosis.

These associations were however based on a single assessment of troponin at baseline in patients with moderate aortic stenosis. The performance of this assay in older patients with severe disease is uncertain. Individual variation of a biomarker around a homeostatic set-point defines the biological variation of a test.⁹¹ Without formal investigation using serial sampling in a controlled experiment, caution must be taken when interpreting single blood results or changes in such markers over time.

1.4.1.2 CARDIAC MYOSIN BINDING PROTEIN C (cMYC)

Like cardiac troponin, cMyC is a sarcomeric protein (Figure 1.1) with a release profile that is exquisitely specific for cardiac tissue. It has an essential role in the structural integrity and function of the sarcomere and is more abundant than troponin.⁹² Studies using models of ischaemic cardiac injury reveal earlier release and decay kinetics compared to troponin, suggesting a possible role as a more dynamic marker of myocardial injury.⁹³ Additionally, cMyC is present in the circulation as two distinct measurable forms: a full-length 149-kDa protein and a cleaved 40-kDa fragment released in times of cardiac stress. This fragment may have a pathogenic role in cardiac tissue by competing for actin and myosin binding sites. Overexpression of this ‘poison peptide’ in mouse models rapidly produces hypertrophic cardiomyopathy and heart failure.⁹⁴

cMyC has never been studied in patients with aortic stenosis, primarily due to insufficient sensitivity of the assay to detect low level release. However, recent technological advances open new avenues to explore the potential of this biomarker of myocardial injury to identify patients with early progressive aortic stenosis.⁹⁵

1.4.2 BLOOD BIOMARKERS OF MYOCARDIAL FIBROSIS AND HEART FAILURE

As cardiomyocyte death progresses, fibrosis and heart failure become predominant features in patients with progressive aortic stenosis. Circulating biomarkers may provide early warning of patients at risk of decompensation.

1.4.2.1 B-TYPE NATRIURETIC PEPTIDE (BNP)

BNP is a well-established marker of heart failure that is widely used in clinical practice. It is a natriuretic and vasodilating hormone, released predominantly by ventricular cardiomyocytes in response to volume overload and stretch.⁹⁶ The inactive N-terminal of the prohormone (termed NT-proBNP) is also quantifiable and may be more stable for delayed laboratory measurement. BNP may have a role in identifying patients with aortic stenosis who are at risk of decompensation. In a study of 126 patients with asymptomatic disease, a BNP value of >61pg/ml successfully predicted the likelihood of symptom development or the requirement for valve replacement.⁹⁷ However, Lim *et al.* reported a different series of aortic stenosis patients where similar BNP concentrations of >66pg/ml identified already *symptomatic* patients with a sensitivity and specificity of 84 and 82% respectively.⁹⁸ This questions the generalisability of single-measure BNP approach. Serial testing in an individual patient may be more informative for detecting disease progression, although as with cardiac troponin, concerns about biological variability in aortic stenosis patients require further evaluation.⁹⁹

1.4.2.2 GALECTIN-3

Galectin-3 is a beta-galactoside-binding lectin protein expressed by activated macrophages. Particularly high concentrations are observed in the lung, spleen, adrenal gland, gastrointestinal tract, ovary and uterus. Cardiac expression is relatively low, but importantly may be upregulated in disease states.¹⁰⁰ Higher circulating concentrations of galectin-3 are noted in heart failure, with a postulated role for the protein in the development of the disease.¹⁰¹ Increased expression of galectin-3 promotes proliferation of cardiac fibroblasts and deposition of type I collagen, which are critical steps in the development of myocardial fibrosis and subsequent failure.¹⁰²

In a study of ventricular remodelling by serial echocardiograms in 240 heart failure patients, rising galectin-3 levels were observed in a group of patients with remodelling compared to static or falling concentrations in the non-remodelled cases.¹⁰³ Interestingly, there were no significant differences in NT-proBNP levels, suggesting a potential additive role for galectin-3 in the identification of early changes on the pathway to decompensated heart failure. In a small study of patients undergoing aortic valve replacement, circulating galectin-3 concentrations correlated with interstitial levels from explanted valve tissue. Preliminary in vitro work demonstrated activation of both fibrotic and osteogenic pathways, perhaps suggesting a direct role for galectin-3 in valve calcification.¹⁰⁴

1.4.3 FRAILITY

Frailty is a multimodal concept describing loss of strength, endurance and physiological reserve across multiple systems that increases vulnerability for developing dependency or death when exposed to a stressor.¹⁰⁵ Common physiological stressors in older adults include infection, trauma and poor control of multiple comorbid diseases. Frailty becomes more common with age, but is a very distinct concept encapsulating biological rather than chronological years; indeed the majority of individuals over 85 years old are not frail. It also captures more than just recognised illness; a quarter of those affected by physical frailty may

have no notable comorbidity or disability.¹⁰⁶ Common frailty models focus on the development of a physical phenotype or the gradual accumulation of deficits over time.^{107,108}

1.4.3.1 FRIED PHENOTYPE

The Fried phenotype considers frailty to be the development of at least three out of five possible traits: weakness, slowness, reduced physical activity, exhaustion and unintentional shrinking (weight loss). It is easy to see how these components may be interlinked in a ‘cycle of frailty’; weakness developing through loss of muscle mass may precipitate slow gait speed and reduced activity. Individuals with one or two traits are said to be ‘pre-frail’ or vulnerable. The phenotype was developed in 5,317 community dwelling participants of the Cardiovascular Health Study aged over 65 years old. Frailty was independently predictive of future hospitalisation, loss of independence with activities of daily living, falls and death.¹⁰⁷

1.4.3.2 ROCKWOOD FRAILTY INDEX

While the frailty phenotype uses physical attributes as a surrogate for loss of physiological reserve, Rockwood’s frailty index takes a different approach by attempting to specifically define the loss of reserve. Frailty is described as part of continuous spectrum of up to 80 accumulated deficits, which may be comorbidities, symptoms, signs or disabilities that do not saturate in the population as part of normal ageing.¹⁰⁹ Each deficit is given equal weighting, with the index simply being the proportion of total possible deficits affecting an individual. Frailty indices have shown excellent discrimination for poor health outcomes in older individuals.^{108,110-112} Such an approach models the *compensation law of mortality* observed in all complex systems; as it ages, the hazard rate of a system changes in relation to its redundancy. This redundancy may be considered in humans as the degree of physiological reserve possessed by an individual. As health deficits accumulate, redundancy becomes exhausted and the system as a whole becomes unreliable and prone to failure (or death).^{113,114} This process is observed across frailty indices, where individuals appear unable

to develop an index value greater than approximately 0.7 – developing any combination of 70% of possible deficits appears to exhaust redundancy and results in death. It is plausible that such a biomarker of robustness and reserve of an individual may improve assessment of older patients under consideration for major surgical procedures.

1.4.3.3 OTHER FRAILITY MEASURES

Despite these two core theories of frailty, there remains a lack of consensus on the optimum assessment method, resulting in a vast array of tools. Many of the instruments described as measuring frailty do not directly relate to the consensus definition described. As this definition includes the risk estimation for future dependency or death, multiple correlates of frailty may demonstrate predictive power for these outcomes while being conceptually very distinct from frailty. This lack of specificity and true consensus around a limited number of frailty measures frequently impairs direct comparison between studies and meta-analysis of results.

It is also recognised that cognitive impairment frequently coexists with physical frailty but is not reflected in the frailty phenotype criteria and has minimal weighting in most frailty indices. International consensus has been reached on the concept of ‘cognitive frailty’,¹¹⁵ and more recent frailty tools do incorporate cognitive testing.¹¹⁶

Some frailty measures have been formally examined in cardiac surgical populations. The Comprehensive Assessment of Frailty (CAF) incorporates additional physical and laboratory tests with the frailty phenotype. Individuals are scored between 0–35 points, with higher values representing greater impairments and those with scores ≥ 10 described as frail. Discrimination for 12-month mortality was good for an older cardiac surgical population ≥ 74 years old (area under the curve [AUC] 0.70, 95% CI 0.60–0.80). Age alone was not predictive of death and the relationship between the CAF and one-year mortality remained significant after adjustment for preoperative EuroSCORE (OR 1.09 per point increase in

CAF, 95% CI 1.05–1.14, $p < 0.001$).^{117,118} In another study by Afilalo *et al.* of 152 patients ≥ 70 years old undergoing cardiac surgery, slow gait speed (≥ 6 seconds to walk 5 metres) as a measure of frailty was associated with increased risk of postoperative mortality or major morbidity (OR 2.63, 95% CI 1.17–5.90, $p < 0.05$). When combined with measures of disability, slow gait speed improved the discrimination of the STS score for a combined mortality and major morbidity outcome (AUC 0.73 vs 0.68 with STS score alone).¹¹⁹ This suggests frailty measures incorporate important risk factors for surgery in elderly cohorts that are not identified by conventional assessment. Similar relationships between frailty and mortality have been observed in TAVI populations, but once more there is a lack of evidence to suggest one tool over another.¹²⁰⁻¹²²

A further limitation of studies in this area has been a focus on mortality, rather than the effect of pre-intervention frailty on subsequent functional and quality of life outcomes, which may be of particular importance in older patients. In an elderly patient with severe aortic stenosis, it is unclear how much frailty is ‘reversible’ by SAVR or TAVI.¹²³ It is plausible that a proportion of patients will have irreversible frailty and will be liable to the complications of cardiac intervention without scope to improve their wellbeing due to overwhelming intrinsic non-cardiac disease driving the frail state. However, even where frailty is a direct consequence of cardiac disease, it does not have to be reversible to be useful as a risk marker to guide decisions for intervention and the intensity of post-operative care. Valve replacement alone may not be sufficient to reverse frailty, particularly if the loss of physical function accrued during progression of disease cannot be recovered through exercise training after intervention. Using objective measures to identify a target population with reversible frailty for valve replacement has potential to improve outcomes for elderly populations with aortic stenosis.

1.5 AIMS AND HYPOTHESES

There are two core aims to this thesis. Firstly, it will seek to address the assessment of older patients with aortic stenosis, by investigating the validity of novel biomarkers of disease progression in situations where traditional symptom-based assessment is challenging.

Secondly, in those considered for aortic valve replacement, this thesis will explore clinical biomarkers of surgical risk and how these compare to existing tools.

The following hypotheses will be addressed:

- i) cMyC as a novel biomarker of myocardial injury will predict progression of aortic stenosis and outcomes (Chapter 3).
- ii) Cardiac troponin, BNP and galectin-3 as markers of myocardial injury, fibrosis and heart failure will demonstrate satisfactory variability in older patients with aortic stenosis to make clinical monitoring by serial sampling feasible (Chapter 4).
- iii) Pre-operative frailty will predict important patient outcomes including mortality after TAVI in an older population with severe aortic stenosis (Chapter 5).
- iv) Measures of frailty will identify patient factors currently not captured by conventional surgical risk scores in an older population with severe aortic stenosis (Chapter 6).

CHAPTER 2

METHODOLOGY

CHAPTER 2: METHODOLOGY

2.1 OVERVIEW

Data for Chapter 3 of this thesis was collected from two aortic stenosis patient studies previously recruited in Edinburgh: the Scottish Aortic Stenosis and Lipid Lowering Trial (SALTIRE) led by Dr Joanna Cowell and an observational cohort study of myocardial fibrosis in aortic stenosis led by Dr Calvin Chin. Additional sample analysis for cardiac myosin-binding protein C (cMyC) was undertaken for this thesis. Data for Chapters 4 and 6 were prospectively collected as part of observational cohort and quality improvement studies of older patients with aortic stenosis including blood biomarker and frailty assessment.

Chapter 5 is a systematic review and meta-analysis of previously published literature in the field of frailty assessment and TAVI.

General methodological processes are presented in this chapter with additional study-specific methodology detailed in each of the chapters that follow.

2.2 COHORTS

Recruitment for the SALTIRE²³ and myocardial fibrosis in aortic stenosis⁸⁹ studies has been previously described in detail. Briefly, the SALTIRE randomised controlled trial recruited adults (≥ 18 years old) between March 2001 and April 2002 with calcific aortic stenosis who were demonstrated by echocardiography to have a minimum peak velocity across the aortic valve of 2.5m/s. The major exclusion criteria included active or chronic liver disease, severe coexisting non-aortic valvular heart disease, intolerance of statin therapy or total cholesterol < 4.0 mmol/L, left ventricular dysfunction (ejection fraction $< 35\%$ on echocardiography), permanent pacemaker or implantable cardioverter-defibrillator device. Of 455 eligible individuals screened for the study, 155 (34%) were randomised to receive either 80mg atorvastatin or placebo once daily in a double-blind manner. Echocardiography and computed tomography (CT) assessment of aortic valve calcium were performed annually for up to three years of study participation (median follow-up 25 months). While atorvastatin reduced serum cholesterol, no difference was observed in the progression of aortic stenosis when compared to those in the placebo arm (adjusted mean difference in annual change in mean aortic valve velocity 0.002m/s, 95% confidence intervals -0.066 to 0.070).²³

Of the 155 participants in SALTIRE, 104 had viable serum samples remaining for analysis of cMyC. These samples had been stored at -80°C . The post-hoc analysis presented in Chapter 3 utilises novel cMyC measurement and additional follow-up data collected from hospital patient records and Scottish national death records for a median of 11.3 years after study recruitment. For the purposes of the analysis the group was considered as a single observational cohort without distinction of the original study arm (atorvastatin or placebo) given the neutral findings of the randomised trial.

The myocardial fibrosis in aortic stenosis observational cohort study recruited patients with all degrees of aortic stenosis from mild to severe determined by routine outpatient clinical care echocardiography. These participants were deemed to be clinically stable by their

reviewing cardiologist. The major exclusion criteria included significant coexisting non-aortic valvular disease (more than moderate severity), contraindication to magnetic resonance imaging, and the presence of acquired or inherited cardiomyopathies. A further group of healthy controls without evidence of significant valve or coronary disease were also recruited. All participants underwent cardiac magnetic resonance (CMR) for markers of disease severity at the time of baseline blood sampling. Of the 161 patients with aortic stenosis included in this study, ten underwent tru-cut myocardial biopsy at the time of subsequent aortic valve replacement.⁸⁸ These samples were used for histological analysis (performed by Dr Jacek Kwieciński) for autophagy and oncosis with detail provided in Chapter 3. Autophagy describes the regulated mechanism of disassembly of unnecessary or dysfunctional components, while oncosis refers to unprogrammed ischaemic cell death.¹²⁴ In contrast, apoptosis is used to describe programmed cell death.

Chapters 4 and 6 include participants with moderate-severe aortic stenosis specifically studied for these analyses of blood biomarkers and frailty. This comprises 80 individuals recruited into an ongoing observational cohort study of older patients with moderate-severe aortic stenosis determined by echocardiography with a minimum peak velocity across the aortic valve of 3.5m/s. All patients underwent comprehensive frailty assessment. Within this group, 14 participants undertook additional blood sampling at hourly and weekly intervals to assess the biological variability of biomarkers of interest in aortic stenosis (Chapter 4). The same frailty assessments were also performed in 105 patients with severe aortic stenosis who were referred to the Transcatheter Aortic Valve Implantation (TAVI) assessment clinic at the Scottish TAVI centre, Royal Infirmary of Edinburgh. These assessments were performed as part of a quality improvement project in this clinic to aid the multidisciplinary team assessment of candidate patients for this procedure. Therefore in total 185 patients with aortic stenosis were assessed for frailty measures across these two cohorts (Chapter 6).

2.3 BLOOD BIOMARKER ASSAYS

Four candidate blood biomarkers of disease progression in aortic stenosis are presented in this thesis. With the exception of cardiac myosin binding protein C (cMyC), these biomarkers are available as part of a high-throughput laboratory platform and are therefore available for clinical use. The cMyC assay was undertaken using a research platform and is currently unavailable for clinical reporting. Precision of each assay is represented by standard performance measures: the Limit of Detection (LoD) which is the lowest concentration of an analyte that can be distinguished from the absence of that substance, and the lower Limit of Quantification (LoQ) which for the purpose of these assays additionally requires the coefficient of variation (CV) to be $\leq 20\%$.¹²⁵ The CV of an assay is a standardised method for expressing the relative variability and is determined by the ratio of the standard deviation to the mean (i.e. $CV = \text{standard deviation}/\text{mean}$).

2.3.1 CARDIAC MYOSIN BINDING PROTEIN C (cMYC)

cMyC was measured in stored serum aliquots that were maintained at -80°C after centrifugation and separation from whole blood samples. Samples were analysed by trained technicians blinded to any participant information. This immunoassay utilises mouse monoclonal antibodies to two cardiac restricted epitopes within the N-terminus of cMyC: IA4 and 3H8. Antibodies to IA4 (the 'capture antibody') were combined with magnetic microparticles (Millipore Sigma, California, USA) before agitating for 2 hours at 25°C with the serum for analysis. The magnetic microparticles were then extracted via a magnetic bed, with any unbound material removed by washing. Fluorescently labelled mouse antibody to 3H8 (the 'detection antibody') was added and then agitated at 25°C for a further 1 hour. The process of separation by magnetic bed and removal of unbound material by washing was repeated. The remaining magnetic microparticles were transferred to a new 384 well plate and exposed to proprietary buffers and fluorescent labelling solution for the Erenna single-molecule counting system (Millipore Sigma, California, USA).

Using this technique, Marjot *et al.* defined this assay LoD at 0.4ng/L and LoQ at 1.2ng/L. The 99th percentile value in healthy individuals without obstructive coronary artery disease was determined at 87ng/L.⁹⁵ As part of the assessment of assay precision and calibration prior to sample analysis for the studies presented in Chapter 3, known concentrations of control cMyC material were tested across a range of 8 concentrations to generate a standard curve (Figure 2.1).

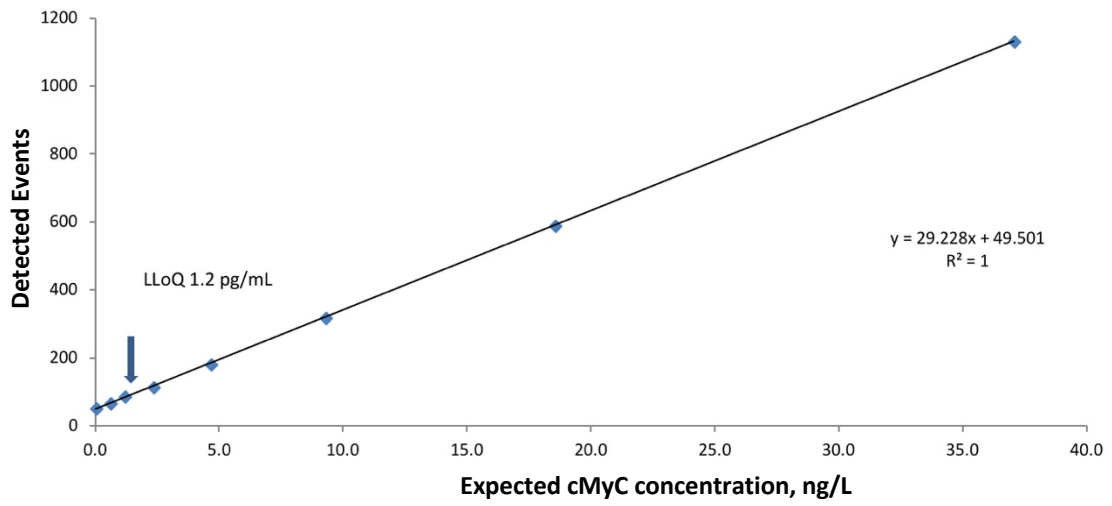


Figure 2.1 – Standard Curve for cMyC. This demonstrates high precision of the assay across the low-range tested.

2.3.2 CARDIAC TROPONIN

Cardiac troponin was measured using the ARCHITECT *STAT* high-sensitive troponin I assay (Abbott Diagnostics, Illinois, USA). This two-step immunoassay is widely used in clinical laboratories and has been well established for quantification of low circulating troponin concentrations. It is a rapid test, allowing reporting of results within 30 minutes of sample collection. The manufacturer reported LoD ranges between 1.1–1.9ng/L and based on prior work the LoQ is 1.5g/L to satisfy a $\leq 20\%$ CV criterion.^{88,126} The 99th centile in healthy reference populations has been demonstrated to be sex-dependent at 34ng/L for men and 16ng/L for women.⁸⁷ Blood samples were drawn into ethylenediaminetetraacetic acid (EDTA) tubes, held on ice and centrifuged within 30 minutes to derive a fresh plasma sample for analysis within 60 minutes of blood collection. Remaining plasma was frozen in aliquots for later retesting to allow fresh-frozen comparisons.

2.3.3 B-TYPE NATRIURETIC PEPTIDE (BNP)

Whole BNP was measured using the ARCHITECT *STAT* two-step sandwich immunoassay (Abbott Diagnostics, Illinois, USA). This required a plasma sample, obtained from the centrifugation of EDTA whole blood. As previous work using alternative but similar assays had demonstrated non-linear degradation of BNP after freezing, analysis was performed on fresh samples within 60 minutes of sampling as described for cardiac troponin above.¹²⁷ The ARCHITECT *STAT* assay uses monoclonal antibodies specific for human BNP, with detection by chemiluminescence. The manufacturer stated LoD is 10pg/mL.

2.3.4 GALECTIN-3

Galectin-3 was measured using the ARCHITECT *STAT* two-step sandwich immunoassay (Abbott Diagnostics, Illinois, USA) with labelling by M3/M28 anti-galectin-3 coated microparticles and detection by chemiluminescence. Analysis was performed on EDTA-derived plasma samples within 60 minutes of sampling and later repeated on samples frozen

at -80°C for fresh-frozen comparison. Existing performance data for this assay suggests a LoD between 0.5–1.7ng/mL and LoQ between 2.1–4.0ng/mL.¹²⁸

2.4 FRAILITY AND QUALITY OF LIFE MEASURES

Frailty assessments were performed in an identical manner in 186 participants across the two cohorts described above. Physical measurements required for the Fried phenotype, Edmonton Frail Scale (EFS) and Short Physical Performance Battery (SPPB) were assessed concurrently to minimise burden to the participant. Similarly, components of the Fried phenotype, EFS and the 12-item Short Form Survey (SF-12) assessed by participant response were combined into a single questionnaire (see Appendix I). The frailty measurements were deliberately chosen for their speed of completion, to be feasible candidates for inclusion into a busy clinic environment. In the current TAVI assessment clinic, patients are allocated approximately 30 minutes for full surgical and nursing assessment for aortic valve replacement suitability. The complete frailty assessment required approximately 15 minutes to complete.

2.4.1 FRIED FRAILITY PHENOTYPE

The Fried phenotype was obtained using two physical measures and three questionnaire responses to ascertain five components.

Grip strength was assessed by three trials (two dominant hand, one non-dominant hand) of maximal unsupported hand grip strength assessed using a Jamar digital dynamometer. This machine provides a maximal strength measure obtained on each trial to the nearest 0.1kg. The associated phenotypic trait of weakness was allocated if participants achieved lower than the 20th percentile derived from population data adjusted for gender and body mass index (see Table 2.1).¹⁰⁷ If a participant was unable to complete the grip strength assessment they were allocated a mark for weakness.

Gait speed was assessed using an unobstructed 5 metre track marked by cones, with a clear 1 metre runoff at each end. Participants started in the runoff area and were asked to walk at their normal pace past the farthest away cone using their own walking aids if required.

Timing was taken by stopwatch for the period the participant was travelling between the two cone markers. Three trials were undertaken with each participant, with the mean of these three recordings used to calculate a gait speed in metres per second. The associated phenotypic trait of slowness was allocated if participants achieved lower than the 20th percentile gait speed derived from population data adjusted for gender and height (see Table 2.1).¹⁰⁷ If a participant was unable to complete all three trials they were allocated the slowness marker.

Shrinking was assessed by a positive questionnaire response for self-reported weight loss:

“Have you unintentionally lost more than 10lbs (4.5kg) in weight in the last year?”

Exhaustion was assessed using responses to two statements from the Centre for Epidemiology Studies Depression (CES-D) scale¹²⁹:

“I felt that everything I did was an effort in the last week” and

“I could not get going in the last week”

Participants were provided with four options for each statement and asked to pick the single response that best fitted their experience. A response of “moderate amount of the time (3-4 days per week)” or more frequent for either question resulted in allocation of an exhaustion trait.

For determining **low physical activity**, participants were asked if, in the last 3 months, they either (a) did not perform any weight bearing physical activity, (b) spent more than 4 hours continuously sitting per day, or (c) went for a short walk once per month or less. The presence of any of these markers were used to allocate the low physical activity trait to a participant. This is a modification of definition used by Fried *et al.* in the original description of the phenotype, but is consistent with use in large frailty studies such as the Invecchiare in Chianti study¹³⁰ and the Frailty Intervention Trial.¹³¹ The original Fried phenotype used the

Minnesota leisure time physical activity questionnaire.¹³² This content was considered less relevant for non-American respondents and the length of the questionnaire was also prohibitive.

A final Fried phenotype frailty status was then determined by the presence of any three or more of these five traits in an individual participant. The pre-frail phenotype was considered in those with one or two traits.¹⁰⁷

2.4.2 EDMONTON FRAIL SCALE (EFS)

The EFS is a multidimensional assessment across the domains of prior hospitalisations, self-reported health, functional dependence, social support, polypharmacy, nutrition, mood, continence and cognitive function.¹¹⁶ It is frequently considered as a frailty tool although it does include some measures of disability in keeping with many similar measures. These assessments are largely completed by questionnaire (see Appendix I), but include a simple clock-drawing test for cognition and Timed Up and Go (TUG) as a measure of mobility and balance. In the clock-drawing test, the participant was asked to draw a standard clock-face, to write on all the numbers and place the hands to show the time of ten-past-eleven. The TUG was measured by stopwatch as the time taken to rise from an armchair independently, walk 3 metres to a marker (using any aids required), turn around and then return back to a seated position in the chair. For consistency, the same style of armchair was used for all participants.

Participants were scored using criteria shown in Table 2.2. The total scale score reflects increasing frailty from 0 points up to a maximum of 17 points. The original authors of the EFS did not set cut-offs with which to dichotomise a population by frailty, although others have proposed this at a scale score ≥ 8 , with pre-frailty or vulnerability at 6–7 points.¹³³

Table 2.1 – Scoring thresholds for grip strength and gait speed used to derive weakness and slowness criteria of the Fried phenotype. Thresholds are determined from Fried *et al.*¹⁰⁷ with conversion of walk times over 15 feet into gait speed cutoffs suitable for a 5 metre timed walk.

Domain	Gender	BMI (kg/m ²)	Height (cm)	Threshold
Grip Strength	Male	≤24		≤29kg
		24.1–26		≤30kg
		26.1–28		≤31kg
		>28		≤32kg
	Female	≤23		≤17kg
		23.1–26		≤17.3kg
		26.1–29		≤18kg
		>29		≤21kg
Gait Speed	Male		≤173	≥0.65m/s
			>173	≥0.76m/s
	Female		≤159	≥0.65m/s
			>159	≥0.76m/s

Table 2.2 – Scoring of the Edmonton Frail Scale. The questionnaire used to collect patient reported components is shown in Appendix I.

Domain	0 Points	1 Point	2 Points
Number of hospital admissions in the last year	No admissions	1-2	≥ 3
Self-rated health	Excellent/Very Good/Good	Fair	Poor
Number of activities of daily living requiring assistance	0-1	2-4	5-8
Availability of social support when needed	Always	Sometimes	Never
Use of ≥ 5 regular prescribed medications	No	Yes	
Forgetting to take medications	No	Yes	
Recent loss of weight such that clothing is looser	No	Yes	
Often feeling sad or depressed	No	Yes	
Urinary incontinence	No	Yes	
Clock-drawing task	No errors	Minor spacing error	Any other error
Timed Up and Go	0-10 seconds	11-20s	>20s or unable

2.4.3 SHORT PHYSICAL PERFORMANCE BATTERY (SPPB)

The SPPB comprises three tests of lower extremity function: gait speed, standing balance and chair rises. Although frequently described as a frailty measure, the SPPB is best described as a measure of physical performance, although the components are clearly related to the frailty construct. Each element scores a maximum of 4 points, with a lower score indicating greater impairment and 0 points an inability to complete the task. The gait speed scoring allocation in the original description of the SPPB was based on the time taken to walk an 8 foot track.^{134,135} To reduce repetitive testing, these cut-off times were converted into a speed in metres per second and points were allocated using the mean measure of gait speed obtained from the Fried phenotype testing across three trials of a 5 metre walk. Although the distance covered by this gait assessment varies from the original description of the SPPB across a 4 metre track, multiple walking tests across marginally different lengths could introduce a bias from participant fatigue, and so a pragmatic decision was made to perform a single walking test and convert thresholds into true gait speeds (in metres per second).

For standing balance, participants were sequentially asked to maintain their feet in side-by-side, semi-tandem and tandem positions for 10 seconds each without the use of external balancing aids. The balance positions and allocation of points are shown in Figure 2.2. Assessment was stopped at the point at which a participant was unable to complete a full 10 second balance. Participant safety was a priority and testing was performed with staff in close proximity to the participant for support in case of imbalance. As with all elements of assessment, the activity was demonstrated to participants, who only undertook the balance testing if they were agreeable. Testing was also rarely terminated where research staff, on observing an individual during the research visit, felt they were at excessive risk of falling by attempting a balance posture.

For the final component of the SPPB, participants were asked to rise from a chair with their arms folded across their chest, so as not to use their upper limbs or chair arms for propulsion. If a participant was able to complete this task, they were timed standing up and sitting down in this manner 5 times as quickly as possible. Scores between 1–4 were allocated according to cut-offs defined by the study authors¹³⁵, with 0 points for those unable to complete all 5 chair rises.

The sum total of these three components was used to produce a SPPB score between 0 and 12 points. To dichotomise the population for frailty, a cut-off of ≤ 5 points was used in keeping with the work of others, although there is a lack of consensus in this area and different thresholds have been suggested.¹²³



Side-by-Side
(10 seconds = 1 point)



Semi-Tandem
(10 seconds = 1 point)



Tandem
(3–9 seconds = 1 point;
10 seconds = 2 points)

Figure 2.2 – Feet position and scoring for standing balance component of the SPPB. The test is completed from left to right and terminated when a participant is unable to complete 10 seconds at any level. Scoring for this component is between 0 points (unable to start or manage 10 seconds side-by-side) and 4 points (manages 10 seconds in each of the three postures).

2.4.4 CLINICAL FRAILTY SCALE (CFS)

The CFS is a structured scale of descriptors to guide selection between nine levels ranging from “very fit: 1” to “terminally ill: 9”. It requires no physical measures or formal questionnaire responses, but knowledge of the participant is required. Assessment criteria include activity, symptoms and assistance usually required with personal activities of daily living (e.g. washing and toileting), and instrumental tasks necessary for independent community living (e.g. managing finances and medications). Frailty may be assessed as a continuum, but is considered present at a score ≥ 5 , with vulnerability or pre-frailty at 4 points (see Figure 2.3).¹¹⁰ Clinic nursing staff or research nurses completed the CFS based on their professional assessment, discussion with the participant or family and any documentation of premorbid functional status.

As frailty measures were designed to score participants as having a frailty marker when unable to complete an assessment (e.g. gait speed), complete frailty data was available in all participants included in the study.

Clinical Frailty Scale*



1 Very Fit – People who are robust, active, energetic and motivated. These people commonly exercise regularly. They are among the fittest for their age.



2 Well – People who have **no active disease symptoms** but are less fit than category 1. Often, they exercise or are very **active occasionally**, e.g. seasonally.



3 Managing Well – People whose **medical problems are well controlled**, but are **not regularly active** beyond routine walking.



4 Vulnerable – While **not dependent** on others for daily help, often **symptoms limit activities**. A common complaint is being “slowed up”, and/or being tired during the day.



5 Mildly Frail – These people often have **more evident slowing**, and need help in **high order IADLs** (finances, transportation, heavy housework, medications). Typically, mild frailty progressively impairs shopping and walking outside alone, meal preparation and housework.



6 Moderately Frail – People need help with **all outside activities** and with **keeping house**. Inside, they often have problems with stairs and need **help with bathing** and might need minimal assistance (cuing, standby) with dressing.



7 Severely Frail – **Completely dependent for personal care**, from whatever cause (physical or cognitive). Even so, they seem stable and not at high risk of dying (within ~ 6 months).



8 Very Severely Frail – Completely dependent, approaching the end of life. Typically, they could not recover even from a minor illness.



9. Terminally Ill - Approaching the end of life. This category applies to people with a **life expectancy <6 months**, who are **not otherwise evidently frail**.

Scoring frailty in people with dementia

The degree of frailty corresponds to the degree of dementia. Common **symptoms in mild dementia** include forgetting the details of a recent event, though still remembering the event itself, repeating the same question/story and social withdrawal.

In **moderate dementia**, recent memory is very impaired, even though they seemingly can remember their past life events well. They can do personal care with prompting.

In **severe dementia**, they cannot do personal care without help.

* 1. Canadian Study on Health & Aging, Revised 2008.
2. K. Rockwood et al. A global clinical measure of fitness and frailty in elderly people. CMAJ 2005;173:489-495.

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Figure 2.3 – The Clinical Frailty Scale. This is reproduced from the Geriatric Medicine Research Department of Dalhousie University with permission for use for research and educational purposes.¹¹⁰

2.4.5 SHORT FORM SURVEY (SF-12)

This 12-item questionnaire tests self-rated health, physical function, health expectations and emotional wellbeing. The output provides separate physical and mental component summary scores, which are standardised on a scale from 0 to 100, where increasing values represent higher levels of health. The calculation of these summary scores is independent of age. The SF-12 was developed as a short-form of a longer and more intensive 36-item questionnaire (SF-36) that was derived from the Medical Outcomes Study.¹³⁶ Interpretation of question responses in psychometric testing may be subject to geographical and cultural variation. Using data from over 8,000 participants in the Oxford Health and Lifestyle Survey, the median physical component score (PCS) and mental component score (MCS) in a United Kingdom population was shown to be 53.2.¹³⁷ The SF-12 summary scores were calculated under license (Optum SF, Minnesota, USA) in all participants.

2.5 ETHICAL CONSIDERATIONS

Written informed consent was obtained from all participants recruited across the studies included in Chapters 3, 4 and 6. All studies were reviewed by the South East Scotland Research Ethics Committee and procedures were conducted in accordance with the Declaration of Helsinki.

2.6 DATA ANALYSIS AND STATISTICS

Detailed descriptions of statistical methods are presented in each of the following Chapters. Wherever possible, analysis was performed on continuous data without dichotomisation, except where it was considered relevant for clinical interpretation (e.g. defining frailty). Parametric data are summarised using mean \pm standard deviation and non-parametric data by median \pm interquartile range. Blood biomarker concentrations are frequently positively skewed and where this was observed log-transformation was undertaken prior to further testing. All analyses were performed using R (versions 3.1.3 to 3.4.1; <http://www.r-project.org>). Two-sided tests were performed throughout, with statistical significance assigned at $p < 0.05$.

CHAPTER 3

CARDIAC MYOSIN-BINDING PROTEIN C AS A NOVEL MARKER OF MYOCARDIAL INJURY AND FIBROSIS IN AORTIC STENOSIS

Contents based on the following published material with minor changes:
Anand A, Chin C, Shah ASV, Kwiecinski J, Vesey A, Cowell J, Weber E, Kaier T,
Newby DE, Dweck M, Marber MS, Mills NL. Cardiac myosin-binding protein C is a
novel marker of myocardial injury and fibrosis in aortic stenosis.
Heart. 2018;104:1101–1108.

CHAPTER 3: CARDIAC MYOSIN-BINDING PROTEIN C AS A NOVEL MARKER OF MYOCARDIAL INJURY AND FIBROSIS IN AORTIC STENOSIS

3.1 OVERVIEW

Cardiac myosin binding protein C (cMyC) is an abundant sarcomeric protein and novel highly specific marker of myocardial injury. Myocyte death characterises the transition from hypertrophy to replacement myocardial fibrosis in advanced aortic stenosis. We hypothesised that serum cMyC concentrations would be associated with cardiac structure and outcomes in patients with aortic stenosis.

cMyC was measured in two cohorts in which serum had previously been prospectively collected: a *mechanism* cohort of patients with aortic stenosis (n=161) and healthy controls (n=46) who underwent cardiac magnetic resonance imaging, and an *outcomes* cohort with aortic stenosis (n=104) followed for a median of 11.3 years.

In the mechanism cohort, cMyC concentration correlated with left ventricular mass (adjusted $\beta=11.0\text{g/m}^2$ per log unit increase in cMyC, $p<0.001$), fibrosis volume (adjusted $\beta=8.0\text{g}$, $p<0.001$) and extracellular volume (adjusted $\beta=1.3\%$, $p=0.01$) in patients with aortic stenosis but not in controls. In those with late gadolinium enhancement (LGE) indicative of myocardial fibrosis, cMyC concentrations were higher (32 [21–56] ng/L vs 17 [12–24] ng/L without LGE, $p<0.001$). cMyC was unrelated to coronary calcium scores. Unadjusted Cox proportional hazards analysis in the outcomes cohort showed greater all-cause mortality (HR 1.49 per unit increase in log cMyC, 95% CI 1.11–2.01, $p=0.009$).

Serum cMyC concentration is associated with myocardial hypertrophy, fibrosis and an increased risk of mortality in aortic stenosis. The quantification of serum sarcomeric protein concentrations provide objective measures of disease severity and their clinical utility to monitor the progression of aortic stenosis merits further study.

3.2 INTRODUCTION

Aortic stenosis is the most common valvular disease in the Western World and the incidence is rising in keeping with an ageing population.¹³⁸ The response of the myocardium to aortic stenosis is variable, with heterogeneity in the development of ventricular hypertrophy and in how this process ultimately decompensates.¹⁷⁻¹⁹ This results in a poor correlation between the severity of stenosis and the development of symptoms. Decompensation of the hypertrophic response in aortic stenosis is driven by two processes: progressive myocyte cell death and myocardial fibrosis.²⁰ Biomarkers of myocardial injury are therefore an attractive addition to current imaging markers of disease progression, perhaps providing critical evidence of early decompensation that may identify patients who would benefit from early valve replacement. We have previously demonstrated that cardiac troponin I concentration is associated with advanced hypertrophy, replacement fibrosis and poor long-term outcomes in aortic stenosis patients,⁸⁸ suggesting that myocardial injury in advanced aortic stenosis is common, detectable and of prognostic importance.

Cardiac myosin binding protein C (cMyC) is a cardiac-restricted sarcomeric protein located on the thick filament.^{93,139} Recent advances in assay technology have allowed measurement of cMyC with high precision at extremely low concentrations.⁹⁵ It is more abundant in myocardial tissue and the circulation than cardiac troponins and has an important role in the assembly and function of the cardiac sarcomere.^{92,140,141} Indeed, mutations in the *MYBPC3* gene encoding cMyC are the most common known genetic cause of hypertrophic cardiomyopathy.¹⁴² Furthermore, the proteolytic cleavage of cMyC is highly regulated by a variety of myocardial kinases¹⁴³ and the resultant peptide is cardiotoxic.⁹⁴ Hence, there is growing interest in the protein as both a biomarker and a determinant of myocardial injury.¹⁴⁴ Given myocyte death characterises the transition from hypertrophy to replacement myocardial fibrosis in advanced aortic stenosis, we hypothesise that serum cMyC concentrations would be associated with cardiac structure and outcomes in patients with aortic stenosis.

3.3 METHODS

We evaluated cMyC in two cohorts: a *mechanism* cohort of aortic stenosis patients and healthy controls with cardiac magnetic resonance (CMR) imaging, and an *outcomes* cohort of aortic stenosis patients with greater than ten years of clinical follow-up. These groups were derived from existing studies in patients with stable aortic stenosis recruited from cardiology clinics across the South East of Scotland, where serum had been prospectively collected and frozen at the time of study inclusion. Additionally a sub-group of patients from the mechanism cohort underwent myocardial biopsy at the time of subsequent aortic valve replacement (AVR), providing exploratory histological correlation with cMyC concentrations. The studies were conducted in accordance with the Declaration of Helsinki and approved by the local research ethics committee. Written informed consent was obtained from all participants.

3.3.1 MECHANISM COHORT

The mechanism cohort consisted of 161 patients with mild to severe aortic stenosis and 46 healthy volunteers without evidence of significant valvular heart disease enrolled in an observational study assessing the role of myocardial fibrosis in aortic stenosis (NCT:01755936). Exclusion criteria comprised significant (moderate or severe) non-aortic valvular disease or any cardiomyopathy (acquired or inherited). The imaging protocol undertaken in all participants has been described in detail previously.^{88-90,145} Briefly, CMR was performed using a 3T scanner (MAGNETOM Verio, Siemens AG, Healthcare Sector, Erlangen, Germany). Dedicated software was used to assess left ventricular (LV) volume and mass indexed to body surface area and to calculate ejection fraction. Diffuse myocardial fibrosis was determined by fibrosis volume and the extracellular volume (ECV) fraction in keeping with current evidence of reproducibility from T1 mapping.^{145,146} Focal myocardial replacement fibrosis was determined by the late gadolinium enhancement (LGE) technique, with its presence determined visually and independently by two experienced assessors.

Comprehensive echocardiography was performed on all subjects to classify markers of aortic stenosis severity according to European Association of Echocardiography/American Society of Echocardiography guidelines.¹⁴⁷

3.3.2 OUTCOMES COHORT

This cohort was derived from the Scottish Aortic stenosis and Lipid lowering Trial, Impact of REgression (SALTIRE) study. The study design, recruitment and findings have been reported previously.^{23,148} Briefly, between March 2001 and April 2002 a total of 155 patients with asymptomatic moderate to severe aortic stenosis were randomised to receive either atorvastatin or placebo. Sufficient stored sample remained for cMyC analysis in 104 patients. In addition to comprehensive echocardiography, computed tomography (CT) calcium scoring of the coronary arteries was performed (Twin II Flash, Philips Medical Systems).

Outcomes data was obtained by two independent investigators who were blinded to cMyC results. The General Register of Scotland was searched for all deaths. The cause of death was adjudicated for a cardiac cause from the issued death certification, using additional information from electronic health records if necessary. This included summaries of a patient's final hospital admission resulting in death, where the cause was uncertain from the death certificate. Disagreements were resolved by consensus. Electronic health records were also reviewed in all cases for evidence of surgical AVR.

3.3.3 BLOOD SAMPLING AND ANALYSIS

Serum cMyC concentrations were measured in duplicate on the single molecule counting Erenna platform (Singulex/Merck Millipore, CA, USA). The assay has a lower limit of detection (LoD) of 0.4 ng/L, a lower limit of quantification (LoQ) of 1.2 ng/L (at 20% coefficient of variation, CV) and reasonable recovery ($107.1 \pm 3.7\%$; mean \pm -SD), dilutional linearity ($101.0 \pm 7.7\%$) and intra- (CV $11 \pm 3\%$) and inter- (CV $13 \pm 3\%$) series precision. Cardiac troponin was determined using a high-sensitivity assay (Abbott ARCHITECT

STAT, Abbott Diagnostics, IL, USA) as previously described. This assay has a LoD of 1.2 ng/L and based on our previous work a LoQ of 1.5 ng/L (at 20% CV).⁸⁸

3.3.4 MYOCARDIAL BIOPSY AND HISTOLOGICAL ANALYSIS

Tru cut myocardial biopsies were obtained from the left ventricles of 10 patients in the mechanism cohort who underwent AVR and also had cMyC measured. For autophagy and oncosis assessment formalin-fixed, paraffin-embedded 4µm thick tissue sections were cut and dehydrated. Further details of the histological analysis are provided in Appendix II.

3.3.5 STATISTICAL ANALYSIS

Statistical analysis was performed using the statistical software R version 3.3.2 (<http://www.r-project.org>). Continuous variables are presented as mean (standard deviation) or median [interquartile range] for non-parametric data. We used analysis-of-variance (ANOVA) to compare continuous parametric data and the Kruskal–Wallis test for non-parametric data across tertiles of cMyC. Categorical variables are presented as absolute numbers (percentage) and were compared using a Chi-squared test. Due to the positive skewing in the sample, cMyC concentrations were log transformed prior to inclusion in modelling, with the normality of the distribution tested by the Shapiro Wilk test ($p=0.22$ across the population). Multivariate linear regression modelling was used to assess the change in markers of aortic stenosis disease progression with serum cMyC concentration. Receiver operating characteristics (ROC) curve analysis and multivariate logistic regression modelling were used to assess the relationship between cMyC concentrations and late gadolinium enhancement. Survival analysis was performed with a Kaplan-Meier analysis using time to any cause of death, with significance calculated by log-rank test. This relationship was also assessed using Cox proportional hazard modelling. AVR was included as a time-varying covariate as survival could be expected to improve in those who received surgery during the follow-up period. A value of $p<0.05$ was considered statistically significant.

3.4 RESULTS

The mechanism cohort consisted of 161 individuals with aortic stenosis (mean age 69 years, AV_{\max} 3.8 ± 0.9 m/s, 70% male) and 46 healthy control participants (mean age 58 years, AV_{\max} 1.4 ± 0.2 m/s, 63% male). The outcomes cohort consisted of 104 patients (mean age 68 years, AV_{\max} 3.4 ± 0.7 m/s, 68% male) with a median follow-up period of just greater than 11 years (4,067 [3,882–4,161] days).

Baseline characteristics by tertile of cMyC are presented in Tables 3.1 and 3.2 for the mechanism and outcome cohorts respectively. cMyC was measurable above the lower limit of quantification (LoQ 1.2 ng/L) in all but one subject, giving an overall detection rate of 99.7%, including all healthy controls (Figure 3.1). Similar to observations with cardiac troponin, cMyC concentrations were positively skewed across all cohorts (Figure 3.2) and weakly correlated with renal function (Figure 3.3). The median cMyC concentration was similar amongst aortic stenosis patients in the mechanism (20.5 [13.7–33.2] ng/L) and outcome (18.2 [12.2–30.1] ng/L) cohorts ($p=0.21$), with both being higher than in controls (9.5 [7.6–15.1] ng/L, $p<0.001$ for both). Samples were tested in duplicate, with a CV between repeated measurements of $5.9 \pm 5.1\%$ in the mechanism cohort, $6.1 \pm 4.9\%$ across the outcome cohort and $8.5 \pm 4.5\%$ in controls.

Cardiac troponin concentrations were above the LoQ in 92.3% of subjects, but in only 65.2% of healthy controls. A strong correlation was observed between cMyC and cardiac troponin across all samples tested ($r=0.74$, 95% CI 0.69–0.79, $p<0.0001$, Figure 3.1).

Table 3.1 – Baseline characteristics of the mechanism cohort by tertile of cMyC and controls.

	All <i>(n=161)</i>	Tertile 1 3-16 ng/L <i>(n=54)</i>	Tertile 2 17-28 ng/L <i>(n=55)</i>	Tertile 3 29-171 ng/L <i>(n=52)</i>	p-value	Controls <i>(n=46)</i>
Age, years	68.5 (11.4)	64.3 (13.0)	69.4 (9.3)	71.8 (10.5)	0.002	57.9 (20.5)
Sex (male), n (%)	112 (69.6)	29 (53.7)	42 (76.4)	41 (78.8)	0.008	29 (63.0)
BMI, kg/m ²	28.9 (4.8)	29.1 (5.2)	28.7 (4.0)	29.0 (5.1)	0.88	26.8 (3.8)
BSA, m ²	1.9 (0.2)	1.9 (0.2)	1.9 (0.2)	1.9 (0.2)	0.71	1.9 (0.2)
Comorbidity						
Diabetes mellitus, n (%)	24 (14.9)	9 (16.7)	6 (10.9)	9 (17.3)	0.59	0 (0.0)
Hypertension, n (%)	109 (67.7)	30 (55.6)	42 (76.4)	37 (71.2)	0.06	12 (26.1)
Hyperlipidemia, n (%)	72 (44.7)	20 (37.0)	22 (40.0)	30 (57.7)	0.07	8 (17.4)
IHD, n (%)	61 (37.9)	12 (22.2)	23 (41.8)	26 (50.0)	0.01	2 (4.3)
Previous PCI, n (%)	25 (15.5)	6 (11.1)	8 (14.5)	11 (21.2)	0.35	2 (4.3)
Previous CABG, n (%)	7 (4.4)	2 (3.7)	1 (1.9)	4 (7.7)	0.33	0 (0.0)
Medications						
Antiplatelet, n (%)	79 (49.1)	17 (31.5)	29 (52.7)	33 (63.5)	0.004	7 (15.2)
ACE-I/ARB, n (%)	67 (41.6)	16 (29.6)	28 (50.9)	23 (44.2)	0.07	9 (19.6)
Beta-blocker, n (%)	55 (34.2)	16 (29.6)	17 (30.9)	22 (42.3)	0.32	5 (10.9)
Diuretic, n (%)	52 (32.3)	16 (29.6)	21 (38.2)	15 (28.8)	0.51	4 (8.7)
Blood Tests						
Creatinine, μmol/L	79.1 (17.1)	73.5 (11.1)	78.6 (15.5)	85.2 (21.5)	0.002	72.5 (15.3)
eGFR, mL/min/1.73m ²	86.3 (18.5)	89.0 (15.9)	88.0 (18.8)	81.7 (20.1)	0.09	97.7 (19.8)
Troponin I, ng/L	6.6 [3.8-12.3]	3.3 [2.3-4.5]	6.7 [4.8-9.5]	15.5 [10-30.2]	<0.001	2.7 [1.1-5.4]
Echo Parameters						
AVmax, m/s	3.8 (0.9)	3.4 (0.8)	3.9 (0.8)	4.3 (0.9)	<0.001	1.4 (0.2)
AV area, cm ²	1.0 (0.4)	1.1 (0.4)	0.9 (0.3)	0.9 (0.4)	0.006	2.4 (0.6)
AV MPG, mmHg	34.4 (18.6)	25.9 (13.5)	34.5 (16.6)	43.2 (21.3)	<0.001	4.2 (1.4)
Indexed LV mass, g/m ²	122.7 (32.0)	102.3 (22.8)	127.6 (27.3)	139.4 (33.7)	<0.001	93.2 (24.4)
E/e'	14.6 (7.6)	12.4 (4.8)	13.1 (5.0)	18.3 (10.4)	<0.001	8.6 (2.5)
MRI Parameters						
Indexed LV mass, g/m ²	88.8 (21.5)	74.2 (15.2)	90.1 (16.6)	102.5 (22.1)	<0.001	65.8 (13.7)
Ejection fraction, %	66.8 (7.3)	67.5 (5.6)	65.8 (8.3)	67.2 (7.8)	0.44	64.4 (4.4)
Indexed SV, ml/m ²	47.6 (10.2)	44.3 (8.4)	47.1 (9.6)	51.4 (11.2)	0.001	46.1 (8.2)
Indexed ESV, ml/m ²	24.2 (9.8)	21.6 (6.6)	25.5 (11.5)	25.6 (10.2)	0.055	26.0 (7.4)
Indexed EDV, ml/m ²	71.8 (16.9)	65.9 (13.3)	72.6 (17.8)	77.0 (17.8)	0.003	72.1 (14.6)
ECV, %	27.8 (2.6)	27.1 (2.2)	27.4 (2.1)	28.9 (3.2)	0.001	26.6 (1.7)
Fibrosis volume, g	44.6 (15.3)	36.0 (8.9)	44.2 (11.1)	54.5 (18.5)	<0.001	31.0 (7.6)
Outcomes						
AVR, n (%)	41 (25.5)	8 (14.8)	20 (36.4)	13 (25.0)	0.04	0 (0.0)

Cardiac death, n (%)	1 (0.6)	0 (0.0)	0 (0.0)	1 (1.9)	0.35	0 (0.0)
All cause death, n (%)	6 (3.7)	1 (1.9)	1 (1.8)	4 (7.7)	0.19	0 (0.0)

Values are number (%), mean (SD) or median [interquartile range]. P-value represents the difference between tertiles of cMyC by ANOVA.

Abbreviations: BMI = body mass index; BSA = body surface area; IHD = ischemic heart disease; ACE-I = angiotensin converting enzyme- inhibitor; ARB = angiotensin receptor blockers; eGFR = estimated glomerular filtration rate; AV = aortic valve; MPG = mean pressure gradient; LV = left ventricle; SV = stroke volume; ESV = end-systolic volume; EDV = end-diastolic volume; ECV = extracellular volume fraction; AVR = aortic valve replacement.

Table 3.2 – Baseline characteristics of the outcome cohort by tertile of cMyC

	All Patients <i>(n=104)</i>	Tertile 1 5-14 ng/L <i>(n=35)</i>	Tertile 2 15–26 ng/L <i>(n=35)</i>	Tertile 3 27–132 ng/L <i>(n=34)</i>	p-value
Age, years	68.2 (9.8)	64.4 (10.5)	70.1 (9.0)	70.0 (9.1)	0.02
Sex (male), n (%)	71 (68.3)	21 (60.0)	19 (54.3)	31 (91.2)	0.002
BMI, kg/m ²	27.9 (4.5)	27.6 (4.1)	28.1 (4.9)	27.8 (4.7)	0.91
BSA, m ²	1.9 (0.2)	1.9 (0.2)	1.9 (0.2)	2.0 (0.2)	0.19
Comorbidity					
Diabetes mellitus, n (%)	2 (1.9)	1 (2.9)	0 (0.0)	1 (2.9)	0.60
Hypertension, n (%)	57 (54.8)	16 (45.7)	24 (68.6)	17 (50.0)	0.13
Hyperlipidaemia, n (%)	7 (6.7)	0 (0.0)	5 (14.3)	2 (5.9)	0.06
IHD, n (%)	17 (16.3)	2 (5.7)	10 (28.6)	5 (14.7)	0.03
Medications					
Aspirin, n (%)	42 (40.4)	13 (37.1)	16 (45.7)	13 (38.2)	0.74
Anticoagulant, n (%)	10 (9.6)	1 (2.9)	4 (11.4)	5 (14.7)	0.23
ACE-I /ARB, n (%)	20 (19.2)	5 (14.3)	7 (20)	8 (23.5)	0.63
Beta-blocker, n (%)	23 (22.1)	8 (22.9)	11 (31.4)	4 (11.8)	0.14
Blood Tests					
Creatinine, µmol/L	91.3 (21.3)	85.3 (18.1)	89.3 (17.9)	100.1 (25.6)	0.01
eGFR, mL/min/1.73m ²	73.5 (17.4)	77.2 (13.9)	71.7 (16.4)	71.6 (21.3)	0.31
Troponin I, ng/L	7.2 [5.4-12.6]	4.9 [3.9-6.0]	8.3 [6.7-10.6]	14.3 [12.1-17.9]	<0.001
Echo Parameters					
AVmax, m/s	3.4 (0.7)	3.4 (0.7)	3.4 (0.8)	3.5 (0.6)	0.90
AV area, cm ²	1.0 (0.4)	1.0 (0.4)	1.0 (0.4)	1.0 (0.4)	0.89
AV PG, mmHg	48.8 (19.2)	47.6 (18.7)	49.7 (21.8)	49.2 (17.1)	0.89
Indexed LV mass, g/m ²	184.4 (52.2)	170.0 (56.8)	175.7 (38.8)	208.7 (52.0)	0.004
Ejection fraction, %	66 (31)	69 (22)	66 (25)	61 (44)	0.64
CT coronary calcium score	382.6 (611.1)	370.3 (608.3)	491.0 (783.2)	283.6 (360.2)	0.37
Outcomes					
AVR, n (%)	48 (46.2)	19 (54.3)	15 (42.9)	14 (41.2)	0.49
Cardiac death, n (%)	16 (15.4)	4 (11.4)	7 (20.0)	5 (14.7)	0.61
All cause death, n (%)	36 (34.6)	7 (20.0)	14 (40.0)	15 (44.1)	0.08

Values are number (%), mean (SD) or median [interquartile range]. P-value represents the difference between tertiles of cMyC by ANOVA.

Abbreviations: BMI = body mass index; BSA = body surface area; IHD = ischemic heart disease; ACE-I = angiotensin converting enzyme- inhibitor; ARB = angiotensin receptor blockers; eGFR = estimated glomerular filtration rate; AV = aortic valve; MPG = mean pressure gradient; LV = left ventricle; AVR = aortic valve replacement.

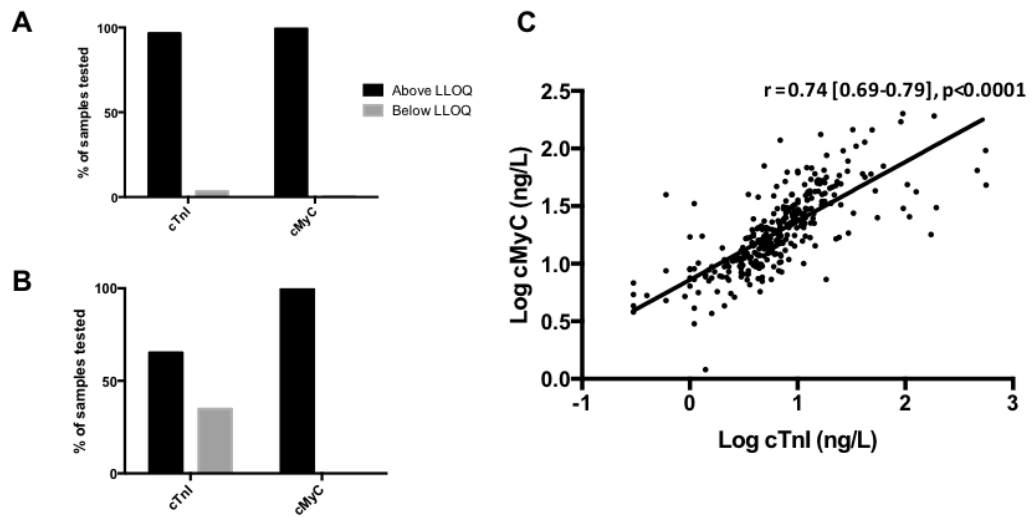


Figure 3.1 – Assay performance of the cMyC assay against high-sensitivity cardiac troponin I. (A) Proportion of patients with aortic stenosis (mechanism and outcome cohorts) and (B) controls (mechanism cohort) in whom cardiac troponin and cMyC concentrations were above the lower limit of quantification (20% CV) of 1.5 ng/L and 1.2 ng/L respectively. (C) Correlation between cardiac troponin I and cMyC concentrations across all cohorts (aortic stenosis and control patients).

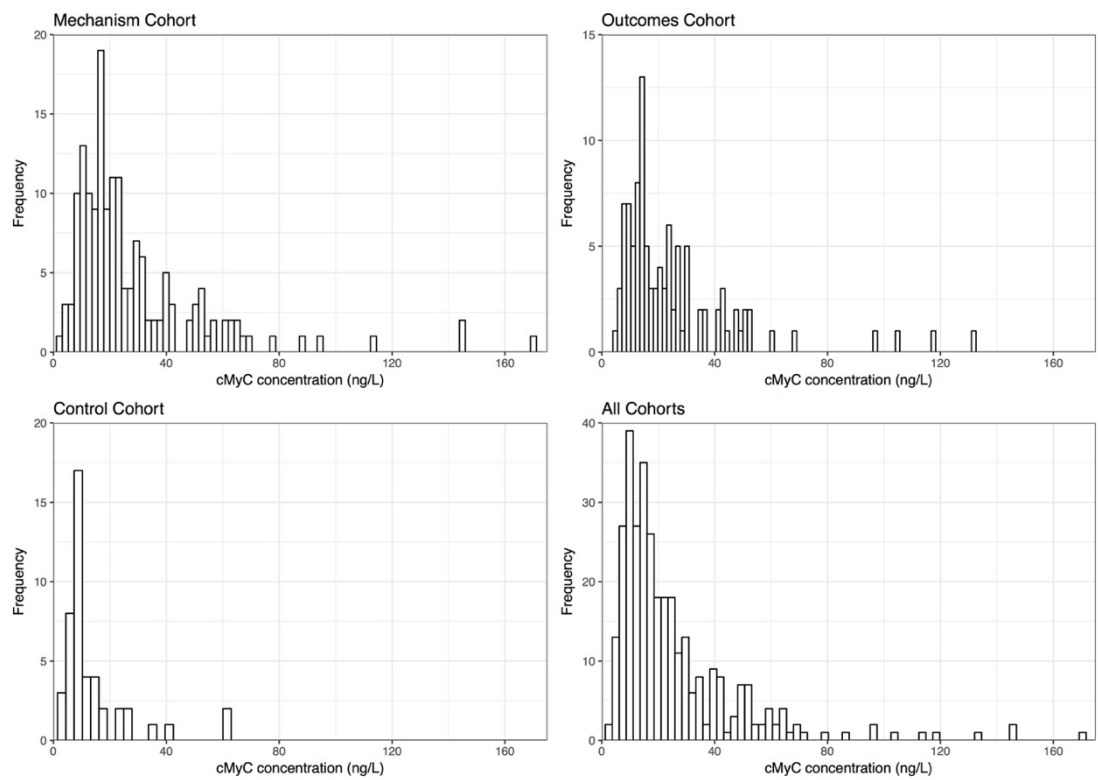


Figure 3.2 – Frequency histograms of cMyC concentrations measured in each cohort and all samples tested. This demonstrated similarly positively skewed distributions in each case. cMyC was detectable above the lower limit of quantification in 99.7% of samples tested.

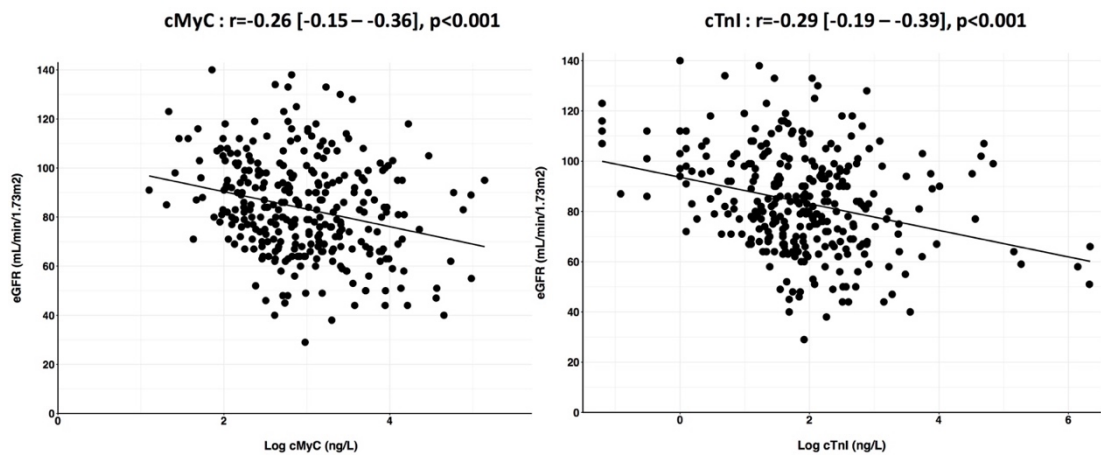


Figure 3.3 – Relationship between renal function and sarcomeric proteins. The correlation between cMyC (left) and cTnI (right) with estimated glomerular filtration rate across all patients with aortic stenosis and controls. Pearson correlation coefficient (r) presented with 95% confidence interval.

3.4.1 cMYC AND MECHANISTIC IMAGING MARKERS

In adjusted multiple regression analyses, cMyC concentration was associated with indexed LV mass in those with aortic stenosis who underwent CMR imaging ($\beta=11.0\text{g/m}^2$ per log unit increase in cMyC after adjustment for age, sex, renal function, AV_{\max} , cardiac troponin and comorbidity; 95% CI 4.7–17.3, $p<0.001$, Figure 3.4a). This relationship was numerically positive, but did not approach statistical significance amongst healthy controls ($\beta=2.7\text{g/m}^2$, 95% CI -4.8–10.1, $p=0.47$, Figure 3.5a). Similarly, fibrosis volume was related to cMyC in those with aortic stenosis (adjusted $\beta=8.0\text{g}$ per log unit increase in cMyC; 95% CI 3.5–12.6, $p<0.001$, Figure 3.4b) but not in controls ($\beta=1.8\text{g}$, 95% CI -1.6–5.2, $p=0.28$, Figure 3.5b). ECV as a marker of diffuse myocardial fibrosis was associated with cMyC in aortic stenosis patients (adjusted $\beta=1.3\%$, 95% CI 0.3–2.3%, $p=0.01$), but not in healthy controls (adjusted $\beta=0.5\%$, 95% CI -0.5–1.5%, $p=0.32$). Detailed modelling is shown in Tables 3.3 (LV mass), 3.4 (fibrosis volume) and 3.5 (ECV %).

To ensure collinearity between cMyC and cardiac troponin was not affecting these results, the final fully adjusted models were repeated without inclusion of cardiac troponin in those with aortic stenosis. The relationship between cMyC and LV mass remained ($\beta=14.1\text{g/m}^2$ per log unit increase in cMyC after adjustment; 95% CI 9.8–18.5, $p<0.001$), and this was also seen with fibrosis volume (adjusted $\beta=9.8\text{g}$; 95% CI 6.7–12.9, $p<0.001$) and ECV (adjusted $\beta=1.5\%$, 95% CI 0.8–2.2%, $p<0.001$).

cMyC concentrations were related to severity of aortic stenosis across the range of AV_{\max} measures in the mechanism cohort (adjusted $\beta=0.80\text{m/s}$, 95% CI 0.50–1.10, $p<0.001$, Figure 3.4c), although this was not observed in the narrower range included in the outcome cohort (Figure 3.5c). An association was further noted between cMyC and diastolic function, measured by echocardiography E/e' in the mechanism cohort (adjusted $\beta=3.76$, 95% CI 1.21–6.32, $p=0.004$, Figure 3.4d). There was no relationship between cMyC and objective

measures of coronary disease by CT calcium scoring (adjusted $\beta=0.03$, 95% CI -0.17-0.23, $p=0.76$, Figure 3.5d).

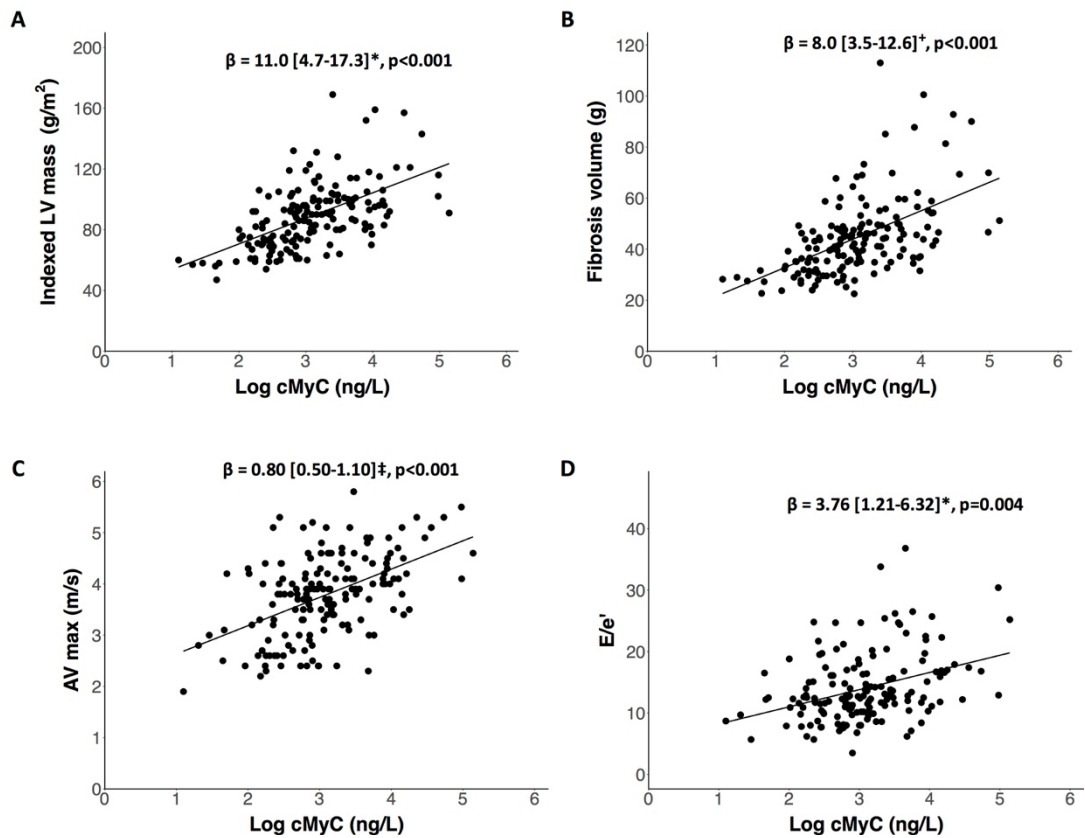


Figure 3.4 – Relationships between cMyC and markers of disease severity in the mechanism cohort. β values [95% confidence interval] represent change in the progression variable for each log unit change in cMyC concentration after adjustment in multivariate modelling. Markers of disease severity are: (A) Indexed LV mass (grams/m²) determined by MRI; (B) Fibrosis volume (grams) by MRI; (C) AVmax (metres/second) determined by echocardiography; (D) Diastolic function from echocardiographic measures of E/e' ratio. *Adjusted for age, sex, glomerular filtration rate, AV_{max}, cardiac troponin, history of ischaemic heart disease, diabetes and hypertension; [†]As above plus body surface area; [‡]As above excluding AV_{max}.

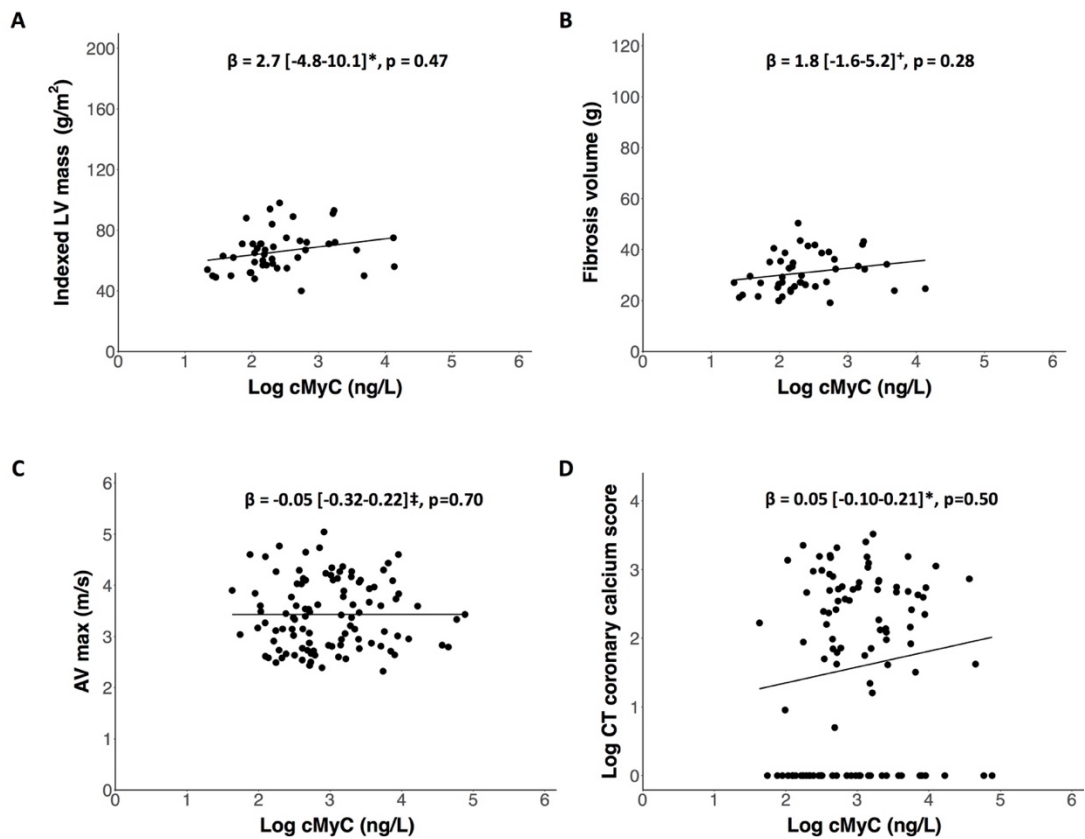


Figure 3.5 – Relationships between cMyC and markers of disease progression in control patients and the outcome cohort. Linear regression correlations cMyC concentration with (A) Indexed LV mass (grams/m²) in control patients determined by MRI (mechanism cohort); (B) Fibrosis volume (g) in control patients by MRI (mechanism cohort); (C) AV Max (metres/second) determined by echocardiography (outcome cohort); (D) CT coronary calcium score (outcome cohort). β values [95% confidence interval] represent change in the progression variable for each log unit change in cMyC concentration after adjustment in multivariate modelling (see Supplementary Tables S1-S3). *Adjusted for age, sex, glomerular filtration rate, AV_{max}, cardiac troponin, history of ischaemic heart disease, diabetes and hypertension; †As above plus body surface area. ‡As above excluding AV_{max}.

Table 3.3 – Linear regression modelling for predictors of change in LV mass in (A) aortic stenosis patients and (B) controls. Model 1 unadjusted; Model 2 adjusted for age and sex; Model 3 additionally adjusted for glomerular filtration rate and AV_{max}; Model 4 additionally adjusted for cardiac troponin; Model 5 additionally adjusted for comorbidity (ischaemic heart disease, diabetes mellitus and hypertension). β values represent change in LV mass in univariate and multivariate analyses. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

(A) Aortic Stenosis Patients

Variable	Model 1 β	Model 2 β	Model 3 β	Model 4 β	Model 5 β
Log cMyC	16.8*** (13.0 – 20.7)	17.4*** (13.5 – 21.3)	14.0*** (9.7 – 18.3)	10.9*** (4.7 – 17.1)	11.0*** (4.7 – 17.3)
Age, per 10 years		-4.3*** (-1.9 – -6.7)	-3.3** (-0.8 – -5.7)	-3.3** (-0.9 – -5.7)	-3.0* (-0.3 – -5.6)
Male sex		10.4*** (4.6 – 16.2)	10.2*** (4.6 – 15.9)	10.6*** (5.0 – 16.2)	11.4*** (5.4 – 17.4)
eGFR, per fall of 10mL/min/1.73m ²			-1.4 (-2.9 – 0.1)	-1.3 (-2.8 – 0.1)	-1.3 (-2.8 – 0.2)
AV _{max} , per 1m ²			6.4*** (3.3 – 9.5)	6.6*** (3.5 – 9.7)	6.6*** (3.5 – 9.8)
Log cTnI				2.5 (-1.1 – 6.1)	2.5 (-1.2 – 6.2)
Ischaemic heart disease					-2.3 (-8.1 – 3.4)
Diabetes mellitus					-1.1 (-8.3 – 6.1)
Hypertension					0.0 (-6.2 – 6.2)
Adjusted r ²	0.32	0.42	0.48	0.49	0.48

*eGFR – estimated glomerular filtration rate. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.*

(B) Controls

Variable	Model 1 β	Model 2 β	Model 3 β	Model 4 β	Model 5 β
Log cMyC	5.3 (-0.9 – 11.5)	4.2 (-1.9 – 10.2)	6.8* (0.3 – 13.4)	3.1 (-4.4 – 10.6)	2.7 (-4.8 – 10.1)
Age, per 10 years		-2.0* (-0.2 – -3.9)	-0.5 (-2.6 – 1.7)	-1.1 (-3.3 – 1.1)	-1.8 (-4.1 – 0.6)
Male sex		12.3** (4.5 – 20.1)	9.5* (1.7 – 17.2)	9.5* (2.0 – 17.0)	8.9* (1.4 – 16.4)
eGFR, per fall of 10mL/min/1.73m ²			-3.1** (-0.8 – -5.4)	-3.0* (-0.8 – -5.3)	-2.4* (-0.1 – -4.8)
AV _{max} , per 1m ²			1.9 (-12.6 – 16.4)	-0.1 (-14.3 – 14.2)	-7.8 (-24.7 – 9.1)
Log cTnI				3.9 (-0.2 – 8.1)	4.6* (0.4 – 8.8)
Ischaemic heart disease					12.3 (-7.2 – 31.7)
Hypertension					4.0 (-5.1 – 13.1)
Adjusted r ²	0.04	0.25	0.33	0.37	0.39

eGFR – estimated glomerular filtration rate. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$. Diabetes excluded from modelling as there were no positive cases within the control group.

Table 3.4 – Linear regression modelling for predictors of change in fibrosis volume in (A) aortic stenosis patients and (B) controls. Model 1 unadjusted; Model 2 adjusted for age and sex; Model 3 additionally adjusted for glomerular filtration rate and AV_{max} ; Model 4 additionally adjusted for body surface area; Model 5 additionally adjusted for cardiac troponin; Model 6 additionally adjusted for comorbidity (ischaemic heart disease, diabetes mellitus and hypertension). β values represent change in fibrosis volume in univariate and multivariate analyses. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

(A) Aortic Stenosis Patients

Variable	Model 1 β	Model 2 β	Model 3 β	Model 4 β	Model 5 β	Model 6 β
Log cMyC	11.2*** (8.4 – 14.0)	11.7*** (8.9 – 14.6)	9.7*** (6.4 – 13.0)	9.7*** (6.6 – 12.7)	7.9*** (3.5 – 12.3)	8.0*** (3.5 – 12.6)
Age, per 10 years		-3.5*** (-1.7 – -5.2)	-3.0** (-1.1 – -4.8)	-1.7 (-3.5 – 0.1)	-1.7 (-3.6 – 0.1)	-1.4 (-3.4 – 0.7)
Male sex		7.2** (2.9 – 11.4)	7.5*** (3.2 – 11.8)	2.3 (-2.3 – 6.9)	2.6 (-2.0 – 7.2)	3.0 (-1.8 – 7.8)
eGFR, per fall of 10mL/min/1.73m ²			-0.5 (-1.6 – 0.6)	-0.8* (-1.9 – 0.2)	-0.8 (-1.8 – 0.3)	-0.7 (-1.8 – 0.3)
AV_{max} , per 1m ²			3.9** (1.6 – 6.3)	3.8*** (1.6 – 6.0)	3.9*** (1.7 – 6.1)	3.9*** (1.7 – 6.2)
Body surface area, per m ²				26.4*** (15.3 – 37.5)	26.0*** (14.9 – 37.1)	26.7*** (15.2 – 38.2)
Log cTnI					1.4 (-1.2 – 4.0)	1.4 (-1.2 – 4.0)
Ischaemic heart disease						-1.6 (-5.7 – 2.6)
Diabetes mellitus						0.6 (-4.8 – 6.0)
Hypertension						-1.0 (-4.8 – 6.0)
Adjusted r^2	0.28	0.39	0.43	0.50	0.50	0.50

eGFR – estimated glomerular filtration rate. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

(B) Controls

Variable	Model 1 β	Model 2 β	Model 3 β	Model 4 β	Model 5 β	Model 6 β
Log cMyC	2.8 (-1.0 – 6.5)	1.7 (-1.9 – 5.4)	4.1* (0.7 – 7.5)	3.8* (0.7 – 6.8)	2.1 (-1.4 – 5.6)	1.8 (-1.6 – 5.2)
Age, per 10 years		-0.9 (-1.9 – 0.1)	0.1 (-0.9 – 1.0)	0.4 (-0.6 – 1.4)	0.2 (-0.8 – 1.2)	-0.2 (-1.3 – 0.8)
Male sex		7.4** (3.1 – 11.7)	4.7* (0.7 – 8.7)	1.2 (-3.0 – 5.3)	1.0 (-3.1 – 5.0)	0.5 (-3.4 – 4.5)
eGFR, per fall of 10mL/min/1.73m ²			-2.3*** (-1.1 – -3.6)	-2.1*** (-1.0 – -3.2)	-2.0*** (-0.9 – -3.1)	-1.7** (-0.6 – -2.8)
AV _{max} , per 1m ²			4.4 (-3.0 – 11.8)	2.0 (-4.7 – 8.7)	0.9 (-5.7 – 7.6)	-3.6 (-11.2 – 4.0)
Body surface area, per m ²				18.5** (7.3 – 29.7)	20.0*** (9.0 – 31.0)	20.6*** (9.9 – 31.3)
Log cTnI					1.7 (-0.1 – 3.6)	2.1* (0.2 – 3.9)
Ischaemic heart disease						6.5 (-2.0 – 15.0)
Hypertension						2.6 (-1.4 – 6.6)
Adjusted r ²	0.03	0.25	0.44	0.56	0.59	0.62

eGFR – estimated glomerular filtration rate. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$. Diabetes excluded from modelling as there were no positive cases within the control group.

Table 3.5 – Linear regression modelling for predictors of change in ECV % in (A) aortic stenosis patients and (B) controls. Model 1 unadjusted; Model 2 adjusted for age and sex; Model 3 additionally adjusted for glomerular filtration rate and AV_{max} ; Model 4 additionally adjusted for body surface area; Model 5 additionally adjusted for cardiac troponin; Model 6 additionally adjusted for comorbidity (ischaemic heart disease, diabetes mellitus and hypertension). β values represent change in extracellular volume in univariate and multivariate analyses. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

(A) Aortic Stenosis Patients

Variable	Model 1 β	Model 2 β	Model 3 β	Model 4 β	Model 5 β	Model 6 β
Log cMyC	1.2*** (0.7 – 1.7)	1.3*** (0.8 – 1.9)	1.5*** (0.8 – 2.1)	1.5*** (0.8 – 2.1)	1.3* (0.2 – 2.3)	1.3* (0.3 – 2.3)
Age, per 10 years		0.2 (-0.2 – 0.5)	0.2 (-0.2 – 0.6)	0.2 (-0.2 – 0.6)	0.2 (-0.2 – 0.6)	0.3 (-0.1 – 0.7)
Male sex		-1.4** (-0.6 – -2.3)	-1.4** (-0.5 – -2.3)	-1.3* (-0.3 – -2.4)	-1.3* (-0.3 – -2.4)	-1.3* (-0.2 – -2.4)
eGFR, per fall of 10mL/min/1.73m ²			-0.1 (-0.3 – 0.2)	-0.1 (-0.3 – 0.2)	-0.1 (-0.2 – 0.3)	-0.0 (-0.3 – 0.2)
AV_{max} , per 1m ²			0.0 (-0.5 – 0.5)	0.0 (-0.5 – 0.5)	0.0 (-0.5 – 0.5)	0.0 (-0.5 – 0.5)
Body surface area, per m ²				-0.3 (-2.8 – 2.2)	-0.4 (-2.9 – 2.1)	-0.4 (-3.0 – 2.2)
Log cTnI					0.2 (-0.4 – 0.7)	0.2 (-0.4 – 0.8)
Ischaemic heart disease						-0.2 (-1.2 – 0.7)
Diabetes mellitus						1.2* (0.0 – 2.4)
Hypertension						-0.7 (-1.7 – 0.3)
Adjusted r^2	0.10	0.16	0.16	0.15	0.15	0.17

eGFR – estimated glomerular filtration rate. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

(B) Controls

Variable	Model 1 β	Model 2 β	Model 3 β	Model 4 β	Model 5 β	Model 6 β
Log cMyC	-0.4 (-1.2 – 0.5)	-0.2 (-1.1 – 0.6)	0.1 (-0.8 – 1.0)	0.2 (-0.7 – 1.0)	0.5 (-0.5 – 1.5)	0.5 (-0.5 – 1.5)
Age, per 10 years		0.2 (0.0 – 0.5)	0.4** (0.1 – 0.7)	0.4** (0.1 – 0.6)	0.4** (0.1 – 0.7)	0.4* (0.1 – 0.7)
Male sex		-1.5** (-0.5 – -2.5)	-1.9*** (-0.9 – -2.9)	-1.3* (-0.2 – -2.5)	-1.3* (-0.2 – -2.4)	-1.4* (-0.2 – -2.5)
eGFR, per fall of 10mL/min/1.73m ²			-0.4* (-0.7 – 0.0)	-0.4* (-0.1 – -0.7)	-0.4** (-0.1 – -0.7)	-0.4* (-0.1 – -0.7)
AV _{max} , per 1m ²			0.1 (-1.8 – 2.0)	0.5 (-1.4 – 2.3)	0.7 (-1.2 – 2.6)	0.2 (-2.1 – 2.5)
Body surface area, per m ²				-2.9 (-6.0 – 0.2)	-3.2* (-6.3 – -0.2)	-3.1 (-6.3 – 0.1)
Log cTnI					-0.4 (-0.9 – 0.2)	-0.3 (-0.9 – 0.2)
Ischaemic heart disease						0.5 (-2.0 – 3.0)
Hypertension						0.4 (-0.8 – 1.6)
Adjusted r ²	0.00	0.21	0.27	0.32	0.34	0.31

*eGFR – estimated glomerular filtration rate. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$. Diabetes excluded from modelling as there were no positive cases within the control group.*

LGE was present in 57 (35.4%) of the aortic stenosis patients in the mechanism cohort. cMyC concentrations were almost double in those with evidence of LGE compared to those without (32.3 [21.3 – 56.3] ng/L vs 17.2 [11.5 – 24.2] ng/L, $p < 0.001$). By ROC analysis, cMyC improved discrimination for LGE beyond age and gender (area under curve [AUC] 0.77, 95% CI 0.70–0.85, Figure 3.6a). Similar results were obtained with AV_{\max} included in the model (AUC 0.71, 95% CI 0.63–0.79 for a model including AV_{\max} , age and sex, improving to 0.77, 95% CI 0.70–0.85 with addition of cMyC). A sensitivity analysis restricted to those with mid-wall fibrosis only (77% of those with any LGE) demonstrated similar discrimination (AUC 0.77, 95% CI 0.70–0.84). Using logistic regression modelling adjusted for age and sex, the predicted probability of LGE was seen to progressively increase with cMyC concentration (Figure 3.6b). Univariate correlations between cMyC and baseline variables used for covariate adjustment in the mechanism cohort are presented in Table 3.6.

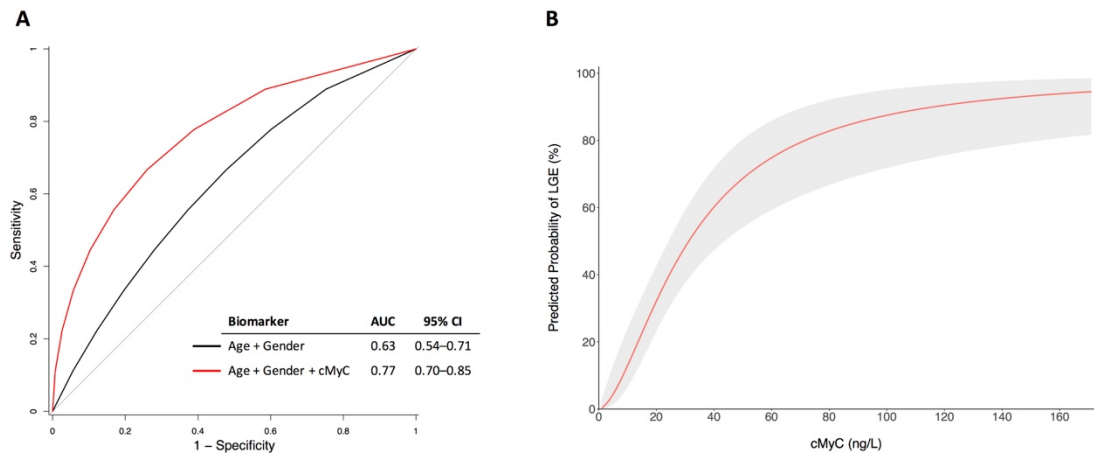


Figure 3.6 – cMyC and late gadolinium enhancement. (A) ROC curve analysis for outcome of LGE. AUC=area under the curve. Addition of cMyC improves the prediction of age and gender for LGE, $p=0.002$ (DeLong’s method with bootstrapping). (B) Logistic regression modelling for the predicted probability of LGE with serum cMyC concentration (adjusted for age and sex). Shaded area represents 95% confidence interval.

Table 3.6 – Univariate correlations between log cMyC and baseline variables used for covariate adjustment in aortic stenosis patients (mechanism cohort).

Variable	β coefficient for log cMyC (95% CI)	p-value
Age, per 10 years	0.21 (0.12 – 0.30)	<0.001
Male sex	0.40 (0.16 – 0.64)	0.001
eGFR, per fall of 10mL/min/1.73m ²	0.07 (0.02 – 0.13)	0.01
AV _{max} , per 1m ²	0.36 (0.25 – 0.48)	<0.001
Body surface area, per m ²	0.18 (-0.43 – 0.79)	0.57
cTnI, per log unit increase	0.36 (0.32 – 0.41)	<0.001
Ischaemic heart disease	0.37 (0.14 – 0.59)	0.002
Diabetes mellitus	0.18 (-0.14 – 0.49)	0.27
Hypertension	0.29 (0.05 – 0.52)	0.02

3.4.2 cMYC AND MYOCYTE DEATH

Clear differences were observed in staining patterns for oncosis and autophagy between subjects with low and high cMyC concentrations (Figure 3.7). Exploratory analysis suggested a relationship between cMyC and the rate of myocyte death (expressed as the sum of apoptosis, oncosis and autophagy counts) in 10 subjects with myocardial biopsy tissue taken at the time of aortic valve replacement ($r=0.67$, 95% CI 0.08–0.92, $p=0.03$).

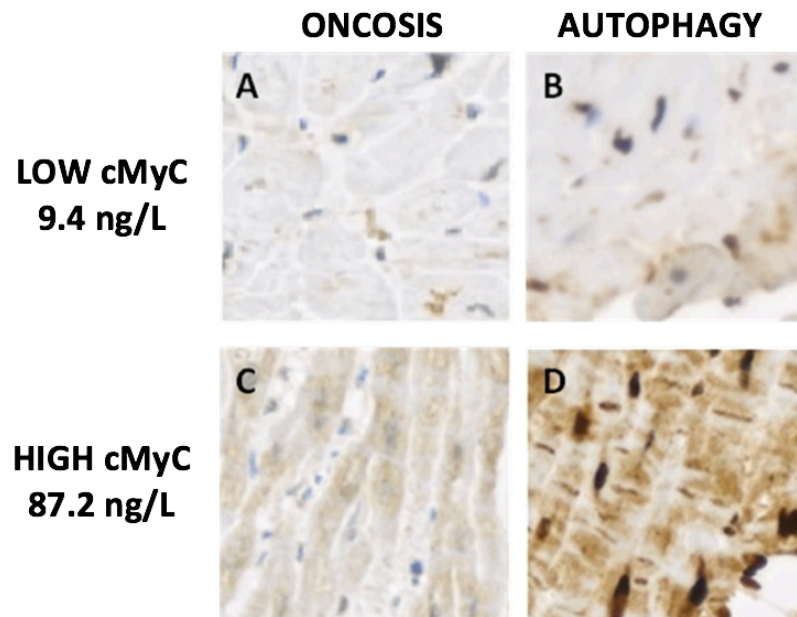
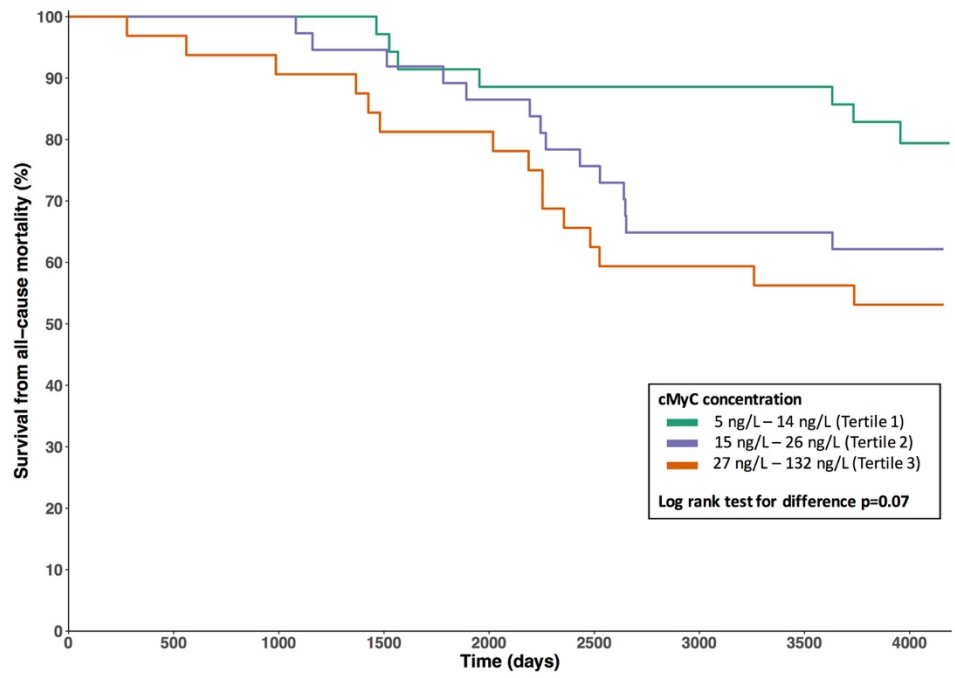


Figure 3.7 – Patterns of myocyte death in aortic stenosis patients. Images showing differing patterns of oncosis and autophagy visualized using a 3,3'-diaminobenzidine based detection kit in individuals with aortic stenosis. (A) oncosis and (B) autophagy in a patient with a low (9.4 ng/L) cMyC concentration. (C) oncosis and (D) autophagy in a patient with a high (87.2 ng/L) cMyC concentration. There is a marked difference in staining intensity by cMyC concentration for both oncosis and autophagy.

3.4.3 cMYC AND LONG-TERM OUTCOMES

Subjects in the outcomes cohort were stratified by tertile of cMyC (Table 3.2). As with CMR in the mechanism cohort, indexed LV mass by echocardiography increased across tertiles of cMyC (170.0 ± 56.8 g in lowest tertile vs 208.7 ± 52.0 g in highest, $p=0.004$). There were no consistent differences in cardiac risk factors across the tertiles. During the follow-up period, 36 (34.6%) subjects died, of which 16 (15.4%) were adjudicated as cardiac deaths. 48 patients (46.2%) within the cohort underwent surgical AVR, with no cases of transcatheter aortic valve implantation (TAVI) undertaken. There was a trend towards poorer survival over the period of follow-up with increasing tertile of cMyC (Figure 3.8, log-rank test for difference $p=0.07$).

In cox proportional hazards analysis, cMyC concentration was associated with an increased risk of all-cause mortality over the follow-up period after inclusion of AVR as a time-varying covariate (HR 1.49 per log unit increase of cMyC, 95% CI 1.11 – 2.01, $p=0.009$). However, following adjustment for age, sex, AV_{max} , CT coronary calcium scores or LV mass, statistical significance was lost. Age was significantly associated with cMyC in all models (Table 3.7).



Number at risk					
5 ng/L – 14 ng/L (Tertile 1)	35	35	31	31	21
15 ng/L – 26 ng/L (Tertile 2)	37	37	32	24	16
27 ng/L – 132 ng/L (Tertile 3)	32	29	26	19	15

Figure 3.8 – Survival by tertiles of cMyC in the outcome cohort.

Table 3.7 – Cox proportional hazard modelling (cMyC and all-cause mortality). For the relationship between cMyC and all-cause mortality at a median follow-up of 11.3 years. Model 1 – including only aortic valve replacement as a time-varying covariate; Model 2 – additionally adjusted for age and sex; Model 3 - as Model 2 plus adjustment for maximum velocity across the aortic valve (AV Max); Model 4 - as Model 2 plus adjustment for CT coronary calcium score; Model 5 – as Model 3 plus adjustment for LV mass. Values are hazard ratios (95% CI). ***p<0.001, **p<0.01, *p<0.05.

	Model 1	Model 2	Model 3	Model 4	Model 5
cMyC, per doubling	1.49** (1.11 – 2.01)	1.26 (0.88 – 1.80)	1.26 (0.88 – 1.80)	1.27 (0.88 – 1.82)	1.24 (0.85–1.80)
AVR (time dependent variable)	0.68 (0.31 – 1.49)	0.75 (0.34 – 1.65)	0.78 (0.33 – 1.85)	0.75 (0.34 – 1.66)	0.80 (0.33–1.90)
Age, per 10 years		2.14 ** (1.69 – 2.71)	2.14 ** (1.69 – 2.71)	2.12** (1.65 – 2.72)	2.06** (1.63–2.60)
Sex, male		1.64 (0.70 – 3.81)	1.64 (0.71 – 3.82)	1.61 (0.67 – 3.89)	1.72 (0.72–4.13)
AV _{max} , per 1m/s increase			0.94 (0.53 – 1.67)		0.96 (0.54–1.70)
CT Coronary Calcium Score, per 10 fold increase				1.02 (0.75 – 1.38)	
LV mass, per 10g					1.00 (0.99–1.02)

3.5 DISCUSSION

In a comprehensive series of clinical assessments, we report the relationship between serum cMyC concentration, a novel marker of myocardial injury, and cardiac structure and outcomes in patients with aortic stenosis. We have made several important observations. First, serum cMyC concentrations can be reliably quantified in the vast majority of patients with aortic stenosis. Second, cMyC strongly associates with markers of both diffuse interstitial and focal replacement fibrosis as well as indexed left ventricular mass, a marker of the hypertrophic response to aortic stenosis. These observations were independent of the severity of valve stenosis and cMyC concentrations were unrelated to coronary artery disease. Third, cMyC concentrations are strongly correlated with cardiac troponin, and in histological analyses, are associated with myocyte cell death, suggesting that sarcomeric protein release is a direct consequence of the maladaptive myocardial response to aortic stenosis. We believe these novel observations demonstrate the utility and validity of cMyC as a marker of sustained chronic myocardial injury.

3.5.1 ASSAY PERFORMANCE

The release profile of cMyC has been previously studied after acute injury in myocardial infarction, alcohol ablation for hypertrophic cardiomyopathy and coronary artery bypass grafting.^{93,144} These models have shown cMyC to be more abundant, to be released earlier following injury, and to decline more rapidly when compared to cardiac troponin. These observations suggest that cMyC has enhanced potential for dynamic monitoring of myocardial injury and disease. However, previous generations of the cMyC assay had limited sensitivity at the low levels that would be expected in the presence of chronic myocardial injury such as in aortic stenosis. For example, cMyC was only detectable in 2 out of 20 patients with hypertrophic cardiomyopathy.¹⁴⁹ Here we report cMyC measurements from a high-sensitivity assay using magnetic nanoparticle and single molecule counting technology, and demonstrate near universal quantification in patients with aortic stenosis and

healthy controls. The weak correlation with renal function observed for both cMyC and cardiac troponin is consistent with previous work, where chronic elevations reflect worsening cardiovascular disease and left ventricular hypertrophy observed in patients with chronic kidney disease.^{150,151} There appears to be limited convincing evidence of impaired clearance of these markers even in severe renal impairment.¹⁵²

3.5.2 cMYC AS A BIOMARKER OF DISEASE

Our observations suggest cMyC is a sensitive marker of the hypertrophic and subsequent fibrotic myocardial response to aortic stenosis. ECV fraction on CMR T1 mapping is a marker of diffuse interstitial fibrosis, which may precede the focal mid-wall replacement fibrosis detected by LGE.^{145,146} Histological studies have suggested that myocyte death drives this transition.²⁰ Our exploratory analysis in myocardial biopsy tissue relates combined measures of autophagy, oncosis and apoptosis to serum cMyC concentrations, providing histological correlation of biomarker release as a result of myocyte death. This combination of autophagy and oncosis in failing myocardial tissue has been independently associated with mortality in larger studies.¹⁵³

Our observations are consistent with our previous findings using a high-sensitivity cardiac troponin I assay. Using two independent sarcomeric proteins, we have now demonstrated that serum markers of myocardial injury mirror important pathological changes in the myocardium in response to aortic stenosis.^{88,154,155} Similar to cardiac troponin, although cMyC was related to AVmax by echocardiography in the mechanism cohort, its association with markers of hypertrophy, fibrosis and diastolic function were independent of the severity of aortic stenosis. This suggests that these novel markers of the myocardial response to stenosis will add to conventional clinical assessment in aortic stenosis. Whilst CMR imaging provides important detail on the extent of the progression to fibrosis and heart failure, it is a resource-intensive investigation.^{19,156} As such, there is great potential for simple blood measures of myocardial injury to improve decision making in aortic stenosis, to better target

detailed imaging or even help determine the timing of surgery. Current guidelines advocate aortic valve replacement in the presence of left ventricular impairment or symptoms, but these are challenging to define objectively and may signify that irreversible myocardial fibrosis has already developed. A precise and early marker of myocardial injury such as cMyC may have great clinical utility in patients with aortic stenosis.

We demonstrate an increased mortality risk with rising cMyC concentrations, but this association was not independent of other variables. Our outcomes cohort benefits from extensive follow-up over 11 years after sampling, but the cohort size was restricted by limited remaining stored samples. This analysis is likely to be underpowered and further work is required in larger studies to clarify whether cMyC will provide additional prognostic information in the assessment of patients with aortic stenosis.

3.5.3 LIMITATIONS

We acknowledge some limitations with the current study in addition to sample size. There is only limited overlap for comparison of imaging endpoints between our two cohorts, although the magnitude of associations between cMyC and LV mass are similar. The clinical utility of biomarkers such as cMyC is more likely to be of interest in severe cases of aortic stenosis approaching the need for valve replacement, where myocardial injury becomes more pronounced. Our mechanism and outcomes cohort contained only 84 and 52 patients with severe aortic stenosis respectively. Whilst this represents half of our study population, further evaluation in a larger population with severe disease would be informative. The histological analysis presented is exploratory and the tru cut technique used may provide limited volumes of myocardium compared to novel scalpel methods.¹⁵⁷

Our findings add to our previous observations with cardiac troponin.^{88,154,155} Compared to those assays, the latest generation cMyC assay is still in the early stages of development, with no definitive healthy population studies to derive a normal reference range. However,

our study suggests that the biomarker will be measurable in virtually all individuals and is likely to meet the criteria for a high-sensitivity assay.⁸⁶ Larger cohort studies are necessary to assess the relationship and potential differences in cMyC and cardiac troponin release, and to determine whether the association of cMyC and long-term outcomes is independent of other patient factors and cardiac biomarkers. Further validation with prospective serial sampling of cMyC in patients with aortic stenosis prior to clinical decompensation is now required.

3.6 CONCLUSIONS

Serum cMyC concentration is associated with myocardial hypertrophy, fibrosis and an unadjusted increased risk of mortality in aortic stenosis. The quantification of serum sarcomeric protein concentrations provide objective measures of disease severity and their clinical utility to monitor the progression of aortic stenosis merits further study.

CHAPTER 4

VARIABILITY OF CARDIAC TROPONIN I, B-TYPE NATRIURETIC PEPTIDE AND GALECTIN-3 IN OLDER ADULTS WITH AORTIC STENOSIS

CHAPTER 4: VARIABILITY OF CARDIAC TROPONIN I, B-TYPE NATRIURETIC PEPTIDE AND GALECTIN-3 IN OLDER ADULTS WITH AORTIC STENOSIS

4.1 OVERVIEW

Blood biomarkers of myocardial injury, ventricular wall stress and fibrosis are attractive targets for monitoring disease progression in aortic stenosis. However, the analytical and biological variability of these biomarkers in older multimorbid patients is uncertain. We assessed hourly and weekly variability of cardiac troponin I, B-type natriuretic peptide (BNP) and galectin-3 using fresh and frozen sampling regimes in older patients with aortic stenosis.

The study included fourteen subjects >65 years old with moderate to severe aortic stenosis in the absence of clinical or echocardiographic evidence of left ventricular decompensation. Participants underwent hourly blood draws for four hours with further testing 7 and 14 days later. Plasma samples were analysed for high-sensitivity cardiac troponin I, BNP and galectin-3 using a high-throughput commercial analyser within a controlled laboratory environment. Samples were tested in the fresh state directly after processing and repeated as a single frozen batch at the end of the study. Analytical (CV_a), biological (CV_i), and inter-individual variations were calculated alongside the index of individuality (II) for each assay. The reference change value (RCV) was determined for a significant change between serial samples.

All three biomarkers were detectable above the assay-specific limits of detection in every sample obtained. BNP demonstrated degradation in frozen compared to fresh samples, but troponin and galectin-3 remained highly stable. CV_a was $\leq 5\%$ for all three assays in both fresh and frozen sample processing. CV_i in fresh weekly samples was 15% for cardiac troponin, 20% for BNP and lowest for galectin-3 at just 5%. The II was < 0.6 for all assays, confirming that interpretation requires a change between serial samples. The RCV for

weekly fresh sampling was 42% for cardiac troponin, 55% for BNP and 14% for galectin. These values for cardiac troponin and BNP are lower than equivalent studies in healthy controls and in stable heart failure.

It is feasible to measure these putative biomarkers of disease progression in older aortic stenosis patients using a high-throughput platform with excellent analytical precision. These data demonstrate differences with prior studies of younger, healthier subjects, with significant change between serial samples indicated by smaller variations in biomarker concentrations amongst older patients. Further studies of analytical variability using multiple clinical laboratories would additionally inform real-world performance of these biomarker assays.

4.2 INTRODUCTION

Aortic stenosis is the most common form of valvular heart disease in the Western World, affecting up to 12% of those over 65 years old.¹³⁸ In the absence of definitive surgical treatment, progressive aortic stenosis results in decompensated heart failure and death. However, heterogeneity in the myocardial response creates discrepancy between the degree of stenosis and the symptoms that guide decisions for surgical intervention. It has previously been demonstrated by cardiac magnetic resonance (CMR) imaging that left ventricular hypertrophy and myocardial fibrosis are independent early markers of adverse prognosis in patients with aortic stenosis, with up to 8-fold increase in mortality.^{17-19,158} However, CMR is costly and may not be tolerated by an older group of patients. It is plausible that blood biomarkers of myocardial injury and ventricular wall stress may provide useful prognostic information and objectively track this disease progression in patients with aortic stenosis.

Before such markers could be considered for clinical use, there must be an understanding of what constitutes a significant change of each biomarker over time. Variability arises from two main components of measurement: test imprecision (analytical variability) and the natural variability of a marker in a subject over time (biological variability). Traditional approaches to the measurement of variability rely on repeated testing in healthy individuals generally of young to middle age. However, the underlying pathology of aortic stenosis may modify the homeostatic set-point of a given biomarker and alter the natural variation around this level.

Differences between the variability of biomarkers in diseased and healthy states have been noted for a number of organ specific biomarkers including creatinine, CA125 and alpha-fetoprotein.¹⁵⁹ In these cases, use of reference change values derived from healthy subjects may under or overestimate variability and thus reduce the value of serial testing in disease states by increasing false positive or false negative rates.

In this study, we tested the variability of three putative biomarkers of disease progression in aortic stenosis: cardiac troponin I, brain natriuretic peptide (BNP) and galectin-3. We used a population of older patients with asymptomatic moderate to severe aortic stenosis, the clinical group where a more objective assessment using simple blood testing would be most attractive. Cardiac troponin is a structural sarcomeric protein, with serum concentration acting as a highly specific marker for myocardial injury.⁸⁵ It has previously been demonstrated that higher cardiac troponin concentrations on a single measure in patients with aortic stenosis identifies those at highest risk of future decompensation and death.⁸⁸

BNP is released predominantly by ventricular cardiomyocytes in response to volume overload and stretch.⁹⁶ As a well-established marker of heart failure, it is likely to have a role in distinguishing symptomatic aortic stenosis from related comorbidity in elderly individuals, such as breathlessness due to chronic lung disease. Single measures of BNP have been suggested as a predictor of future symptom development or the requirement for valve replacement in patients with stable, asymptomatic aortic stenosis.⁹⁷ However, BNP measurement is not routinely undertaken in the evaluation of these patients and there is limited evidence for serial testing.

Galectin-3 is a beta-galactoside-binding lectin protein expressed by activated macrophages. Particularly high concentrations are observed in the lung, spleen, adrenal gland, gastrointestinal tract, ovary and uterus. Cardiac expression is relatively low, but importantly may be upregulated in disease states.¹⁰⁰ Higher circulating concentrations of galectin-3 are noted in heart failure, with a postulated role for the protein in the development of the disease through promotion of cardiac fibroblast proliferation.^{97,98}

We hypothesised that these three candidate biomarkers of disease progression, namely cardiac troponin, BNP and galectin-3, would demonstrate satisfactory variability in older adults with aortic stenosis to make clinical monitoring by serial sampling feasible. As many

biomarker studies with potential to influence patient care are performed retrospectively in large research cohorts using stored frozen sample, we additionally analysed the validity of fresh-frozen comparisons of these biomarkers to better understand the translation of research findings to the clinical setting.

4.3 METHODS

The methods and sample size are in keeping with other studies of variability of circulating blood biomarkers and best-practice guidance.^{91,160-163}

4.3.1 PARTICIPANT SELECTION

Fourteen subjects (8 males, 6 females) with moderate-severe aortic stenosis were recruited from outpatient cardiology clinics at the Royal Infirmary of Edinburgh. Participants >65 years old were included on the basis of a specialist cardiologist review determining stable moderate to severe aortic stenosis without current or prior clinical decompensation. Further, subjects required echocardiography within 6 months of enrolment demonstrating a peak velocity across the aortic valve (AV_{max}) greater than 3.5m/s, evidence of normal left ventricular function by visual assessment and without other significant valve dysfunction (i.e. not greater than mild severity). Potential participants were further excluded if there was any history of heart failure or current use of diuretic medication, current smoking habit, or if screening blood tests showed significant derangement of liver or renal function (calculated creatinine clearance <30mL/min). Finally, participants were required to have had no changes to any regular prescribed medication in the 4 weeks prior to the start of variability testing. During the testing period, subjects were excluded if they developed any new symptoms consistent with disease progression or intercurrent illness. Written informed consent was obtained from all participants. The study protocol was reviewed and approved by the local research ethics committee (SE/14/SS/1110).

4.3.2 BLOOD SAMPLING AND PROCESSING

Blood was drawn at six timepoints over 14 days (Figure 4.1). To assess short term variability (hour-to-hour), samples were taken every 60 minutes for four hours. To assess medium term variability (week-to-week), further samples were taken 7 and 14 days later at a similar time of day. All sampling occurred between 9am and 2pm. At each timepoint, blood was drawn

into 4.9mL EDTA plasma tubes (Sarstedt, Nümbrecht, Germany) and placed on ice. Within 30 minutes of blood draw, these samples were spun at 2000g in a refrigerated centrifuge at 4°C for 15 minutes to separate out the plasma layer. This was divided into four 500µl plasma aliquots, which were labelled as two pairs ('A' and 'B' aliquots). One pair was frozen at -80°C, while the other pair was directly analysed within 30 minutes to replicate testing on demand in a clinical laboratory. At the end of the study, frozen aliquots were allowed to thaw at room temperature before being analysed in a single batch.



Figure 4.1 – Blood sampling schematic for variability testing.

4.3.3 ASSAYS

High-sensitivity cardiac troponin I, BNP and galectin-3 were run concurrently using the high-throughput ARCHITECT STAT analyser (Abbott Diagnostics, Illinois, USA) held within a dedicated biomarker research laboratory. The same reagent lot was used for each assay throughout the study. As fresh and frozen testing occurred on different days, the machine was calibrated prior to any sample runs according to manufacturer guidance.

4.3.4 STATISTICAL ANALYSIS

Continuous data are summarised using the sample mean \pm standard deviation, while categorical data are presented as the total number within the sample (percentage). At completion of the study, results of each of the three assays were available in duplicate ('A' and 'B' aliquots) for each subject and at each timepoint in both fresh and frozen form. Variability was assessed using standard formulae described in the methods of Fraser and Harris.¹⁶⁴ Hourly variability was determined using only results from the first day of sampling, while weekly variability included all timepoints.

The variation in each biomarker within each subject was tested for normality using the Shapiro-Wilk test. The normality assumption for later calculations was considered true if >50% of subjects did not meet the criteria to reject the null hypothesis of normality at 5% significance. A key component of variability calculations is the coefficient of variation (CV) which is defined as the ratio of the standard deviation (σ) of the sample to its mean (μ):

$$CV = \left(\frac{\sigma}{\mu} \right) \times 100\%$$

4.3.4.1 ANALYTICAL VARIABILITY (CV_A)

The analytical coefficient of variation (CV_a) was determined for each pair of identical aliquots analysed (i.e. the 'A' and 'B' sample). This was summarised as a mean CV_a value

for each assay. Linear regression analysis was undertaken to investigate the effect of assay concentration on analytical variability, plotting the mean concentration of each pair of aliquots against its CV_a .

4.3.4.2 TOTAL SUBJECT VARIABILITY (CV_T)

The total variability in each individual was determined using the mean and standard deviation of all samples and the CV formula above. This represents the combination of analytical and biological variability, plus any unmeasured pre-analytical differences that persist despite controlled sampling methodology.

4.3.4.3 BETWEEN-SUBJECT VARIABILITY (CV_G)

The between-subject coefficient of variation (CV_g) was determined for each assay using the mean and standard deviation of biomarker concentrations across all fourteen participants. This represents the variability of each biomarker between individuals with aortic stenosis.

4.3.4.4 BIOLOGICAL VARIABILITY (CV_i)

The biological variability is the within-subject coefficient of variation (CV_i) after accounting for analytical variation. It was determined using the formula:

$$CV_i = \sqrt{(CV_t^2 - CV_a^2)}$$

4.3.4.5 INDEX OF INDIVIDUALITY (II)

The index of individuality represents the variation in the homeostatic setpoint for an assay and was calculated as the ratio of the within-subject biological variability to the between-subject variation:

$$II = \frac{\sqrt{(CV_a^2 + CV_i^2)}}{CV_g}$$

In keeping with the existing literature, an $II < 0.6$ was considered indicative of high variation in the homeostatic setpoint, such that serial testing would be justified in place of a universal reference range.

4.3.4.6 REFERENCE CHANGE VALUE (RCV)

The RCV describes the minimum percentage change between two serial measures that overcomes the calculated analytical and biological variability. It may therefore be considered as the minimum change between serial samples in an individual that represents a statistically significant change in a biomarker. The symmetrical limits of a normally distributed RCV were determined using the formula:

$$RCV = Z \times \sqrt{2} \times \sqrt{(CV_a^2 + CV_i^2)}$$

where $Z = 1.96$ corresponding to a 95% confidence level.

4.3.4.7 FRESH-FROZEN COMPARISONS

For each biomarker, correlation coefficients were determined for the comparison of fresh and frozen aliquot concentrations. Bland-Altman plots were used to visualise agreement and assess mean differences.

4.4 RESULTS

The baseline characteristics of the fourteen included subjects are summarised in Table 4.1. Following the initial day of sampling, one participant developed a chest infection and was therefore withdrawn from further weekly sampling. However, data for hourly variation was retained. A second participant developed a syncopal episode between Day 7 and 14 sampling and was subsequently referred for aortic valve replacement. Once more weekly sampling was withdrawn from the study but hourly data was retained. All samples for all three biomarkers were detectable above the assay-specific limits of detection.

Using all available results for each participant, the distribution of each biomarker concentration was assessed for normality by Shapiro-Wilk testing at the 5% significance level. For the fresh cardiac troponin assay, results from 12 (86%) participants did not reject the null hypothesis of normality. This was seen with 11 (79%) subjects on frozen sample testing. Similarly with BNP and galectin-3, 12 (86%) subjects in each of the fresh and frozen sample sets demonstrated insufficient evidence to reject the normal distribution. Given these findings, data were not log-transformed.

Table 4.1 – Baseline characteristics.

	n=14
Age, years	79.3 ± 8.3
Females	6 (43)
Body mass index, kg/m ²	27.9 ± 3.9
Creatinine clearance, mL/min	69.8 ± 24.0
AV _{max} , m/s	4.3 ± 0.5
<i>Comorbidity</i>	
Myocardial infarction	1 (7)
Stroke or TIA	3 (21)
Diabetes mellitus	1 (7)
Hypertension	9 (64)
Atrial fibrillation	2 (14)
Chronic kidney disease	1 (7)
Current smoker	0 (0)
Ex-smoker	7 (50)
<i>Biomarker concentrations</i>	
Cardiac troponin I, ng/L	10.8 ± 7.8
BNP, ng/L	145.2 ± 142.2
Galectin-3, µg/mL	18.1 ± 6.4

Values are mean ± SD or number (%)

Creatinine clearance was determined by the Cockcroft-Gault equation

AV_{max} = maximal velocity across the aortic valve determined by echocardiography

TIA = transient ischaemic attack

Chronic kidney disease defined by primary care or hospital coding

4.4.1 ANALYTICAL AND BIOLOGICAL VARIABILITY

Key components of variability for each biomarker are summarised for hourly (Table 4.2) and weekly (Table 4.3) sampling regimes. The spread of data across all subjects is shown in the representative plots for fresh and frozen cardiac troponin concentrations in Figure 4.2.

Overall analytical variability was $\leq 5\%$ for all three biomarkers, with no discernible improvement in performance with single-run batch testing of frozen samples when compared to immediate processing of fresh plasma. Between-subject variation (CV_g) was high for all three biomarkers which was reflected in universally low indices of individuality (all < 0.6).

Biological variability (CV_i) was similar in all three biomarkers on hourly sampling. However, galectin-3 remained stable on weekly testing, while the CV_i of both cardiac troponin and BNP increased. This is reflected in the RCVs, with galectin-3 results suggesting a $\sim 15\%$ change in concentration between serial tests at either hourly or weekly intervals would represent significant change in the biomarker. However, both cardiac troponin and BNP required larger changes of $\sim 40\%$ and $\sim 60\%$ respectively at weekly sampling.

Table 4.2 – Key variability data for hourly sampling with fresh and frozen protocols.

Variability component	Troponin I	BNP	Galectin-3
<i>Fresh</i>			
Mean concentration \pm SD	10.4 \pm 7.7ng/L	137.7 \pm 129.8ng/L	18.0 \pm 6.5 μ g/mL
Analytical (CV _a , %)	4.1	2.8	1.7
Between subject (CV _g , %)	75.4	96.7	36.3
Biological (CV _i , %)	5.9	4.8	3.6
Index of Individuality (II)	0.1	0.1	0.1
Reference change value (%)	19.9	15.4	11.0
<i>Frozen</i>			
Mean concentration \pm SD	10.9 \pm 9.8ng/L	82.8 \pm 80.2ng/L	18.5 \pm 6.8 μ g/mL
Analytical (CV _a , %)	5.0	3.4	1.6
Between subject (CV _g , %)	92.4	99.4	37.0
Biological (CV _i , %)	5.0	4.7	3.4
Index of Individuality (II)	0.1	0.1	0.1
Reference change value (%)	19.5	16.1	10.4

Table 4.3 – Key variability data for weekly sampling with fresh and frozen protocols.

Variability component	Troponin I	BNP	Galectin-3
<i>Fresh</i>			
Mean concentration ± SD	11.2 ± 8.1ng/L	153.3 ± 155.8ng/L	18.1 ± 6.3µg/mL
Analytical (CV _a , %)	3.4	1.9	1.4
Between subject (CV _g , %)	71.9	105.1	34.3
Biological (CV _i , %)	14.8	19.9	4.7
Index of Individuality (II)	0.2	0.2	0.1
Reference change value (%)	42.2	55.3	13.7
<i>Frozen</i>			
Mean concentration ± SD	12.4 ± 10.6ng/L	95.3 ± 99.9ng/L	18.8 ± 6.5µg/mL
Analytical (CV _a , %)	3.8	2.9	1.7
Between subject (CV _g , %)	90.3	106.9	34.4
Biological (CV _i , %)	14.2	21.4	5.0
Index of Individuality (II)	0.2	0.2	0.2
Reference change value (%)	40.8	59.8	14.8

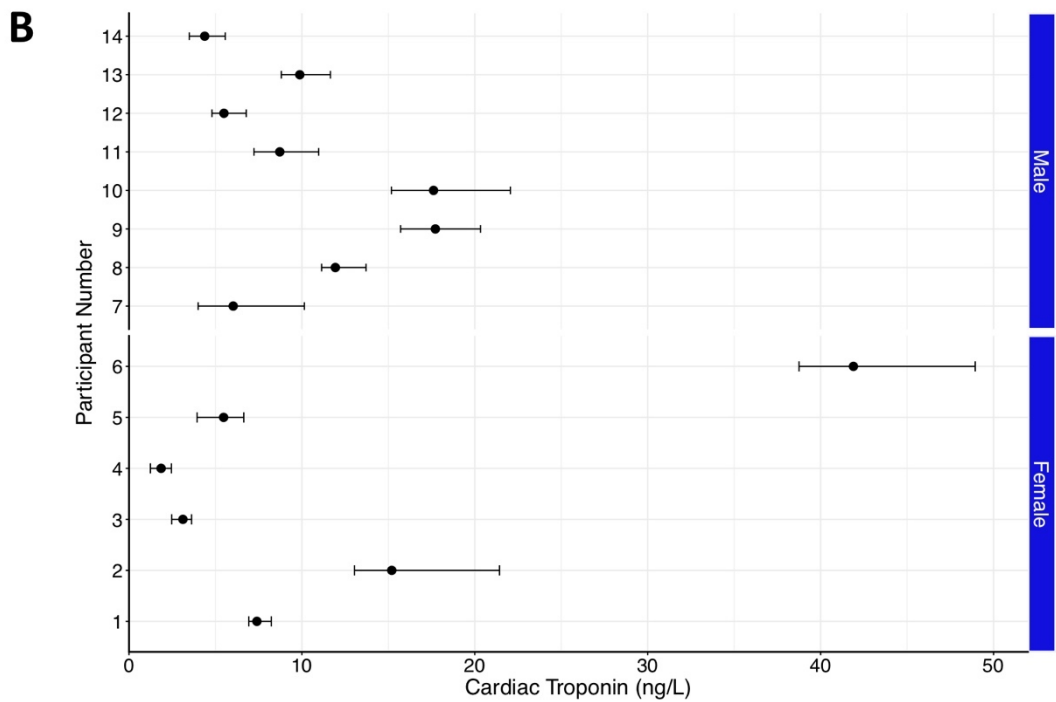
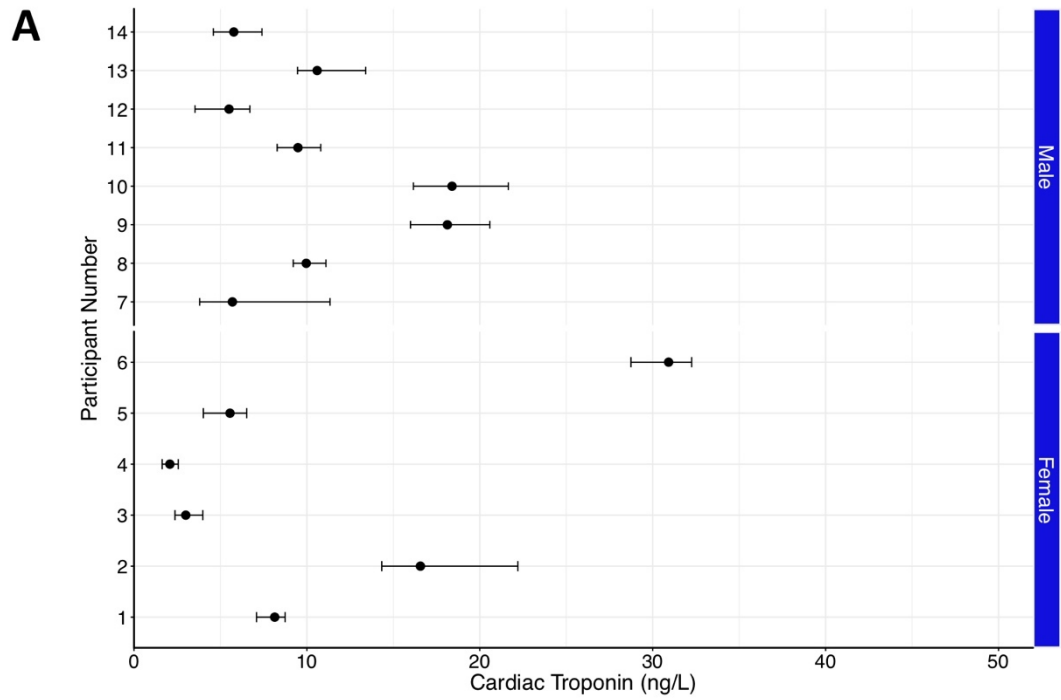


Figure 4.2 – Spread of troponin concentrations across all samples. Data is presented as mean and range for fresh (A) and frozen (B) sampling regimes.

4.4.2 VARIABILITY BY BIOMARKER CONCENTRATION

Linear regression was used to assess the relationship between CV_a in fresh samples and mean concentration at each timepoint (Figure 4.3). Concentrations from fresh samples were analysed as these demonstrated the lowest CV_a across all three biomarkers. The analytical variability was observed to decrease as each biomarker concentration increased. This was most notable for cardiac troponin (correlation coefficient, $r=-0.30$, 95% confidence intervals [CI] -0.08 to -0.49, $p=0.008$) and galectin-3 ($r=-0.29$, 95% CI -0.07 to -0.48, $p=0.01$). A similar pattern was observed for BNP, although this did not reach statistical significance ($r=-0.14$, 95% CI -0.35 to 0.09, $p=0.24$).

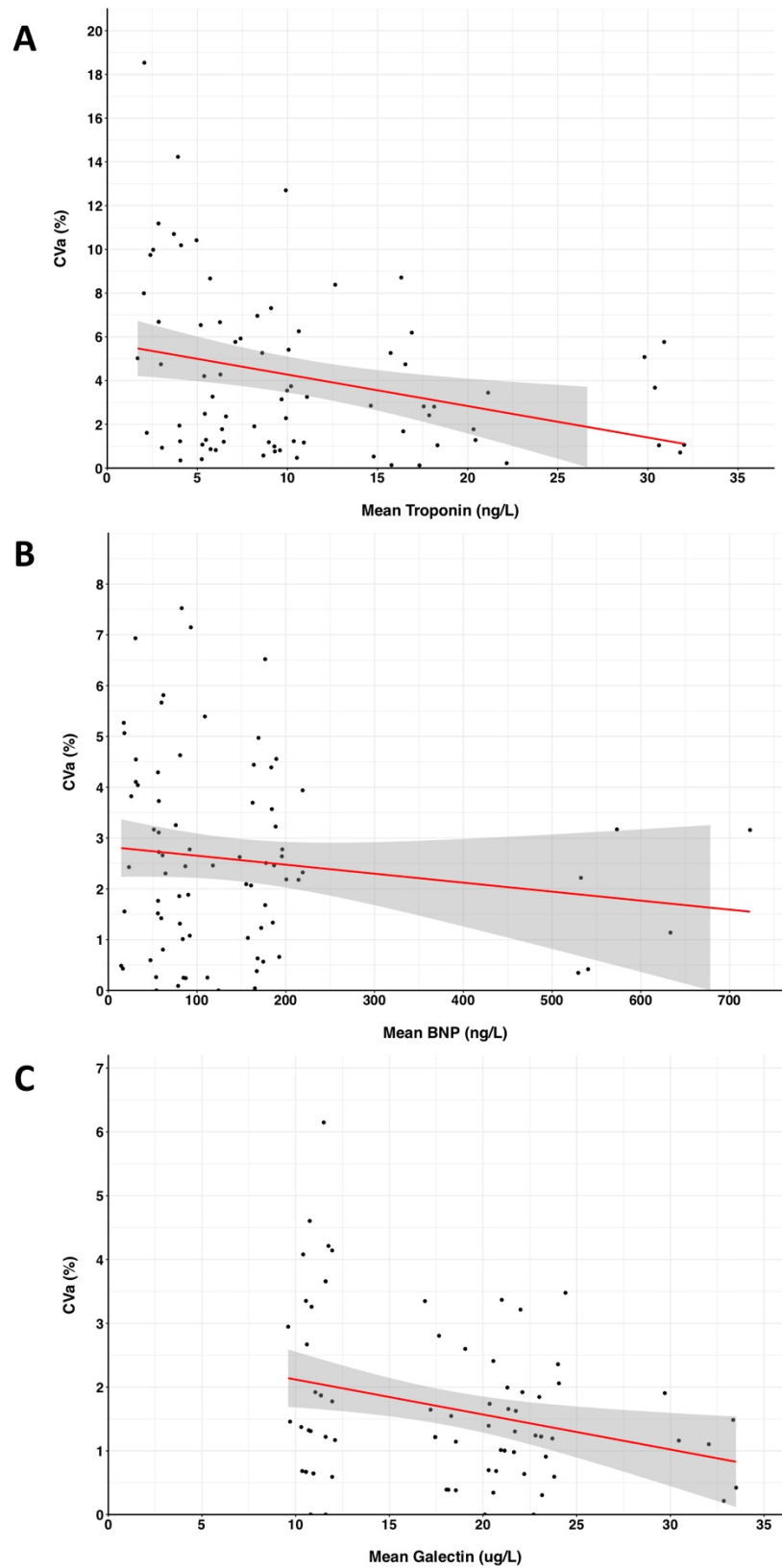


Figure 4.3 – Relationship between mean concentration and analytical variability. Linear regression line shown with shaded area representing 95% confidence intervals for cardiac troponin (A), BNP (B) and galectin-3 (C) fresh sampling.

4.4.3 FRESH-FROZEN COMPARISONS

Fresh and frozen sample runs were compared by correlation and Bland-Altman plots for mean difference. For cardiac troponin (Figure 4.4) correlation between fresh and frozen concentrations was strong ($r=0.97$, 95% CI 0.95 to 0.97, $p<0.001$), although results at the higher end of the spectrum in one subject appeared greater on frozen than fresh testing. The results for this individual subject lay outside of the limits of agreement (one standard deviation away from the mean difference) by Bland-Altman, although the overall mean difference across all samples was negligible.

When BNP was analysed in an identical manner, a clear trend for lower values in frozen samples was observed (Figure 4.5). Although simple correlation demonstrated excellent agreement ($r=0.99$, 95% CI 0.99 to 1.00, $p<0.001$), the mean difference between fresh and frozen samples was greater than 50ng/L. The Bland-Altman plot suggested a linear trend with the difference between fresh and frozen results rising with increasing BNP concentration. Owing to the large mean difference, only those samples tested at the highest concentrations lay outside of the limits of agreement.

Agreement between fresh and frozen galectin-3 samples was excellent ($r=0.99$, 95% CI 0.98 to 0.99, $p<0.001$), with a negligible mean difference (Figure 4.6). A small number of samples lay outside the limits of agreement by Bland-Altman, but these were symmetrical and appeared unrelated to galectin-3 concentration.

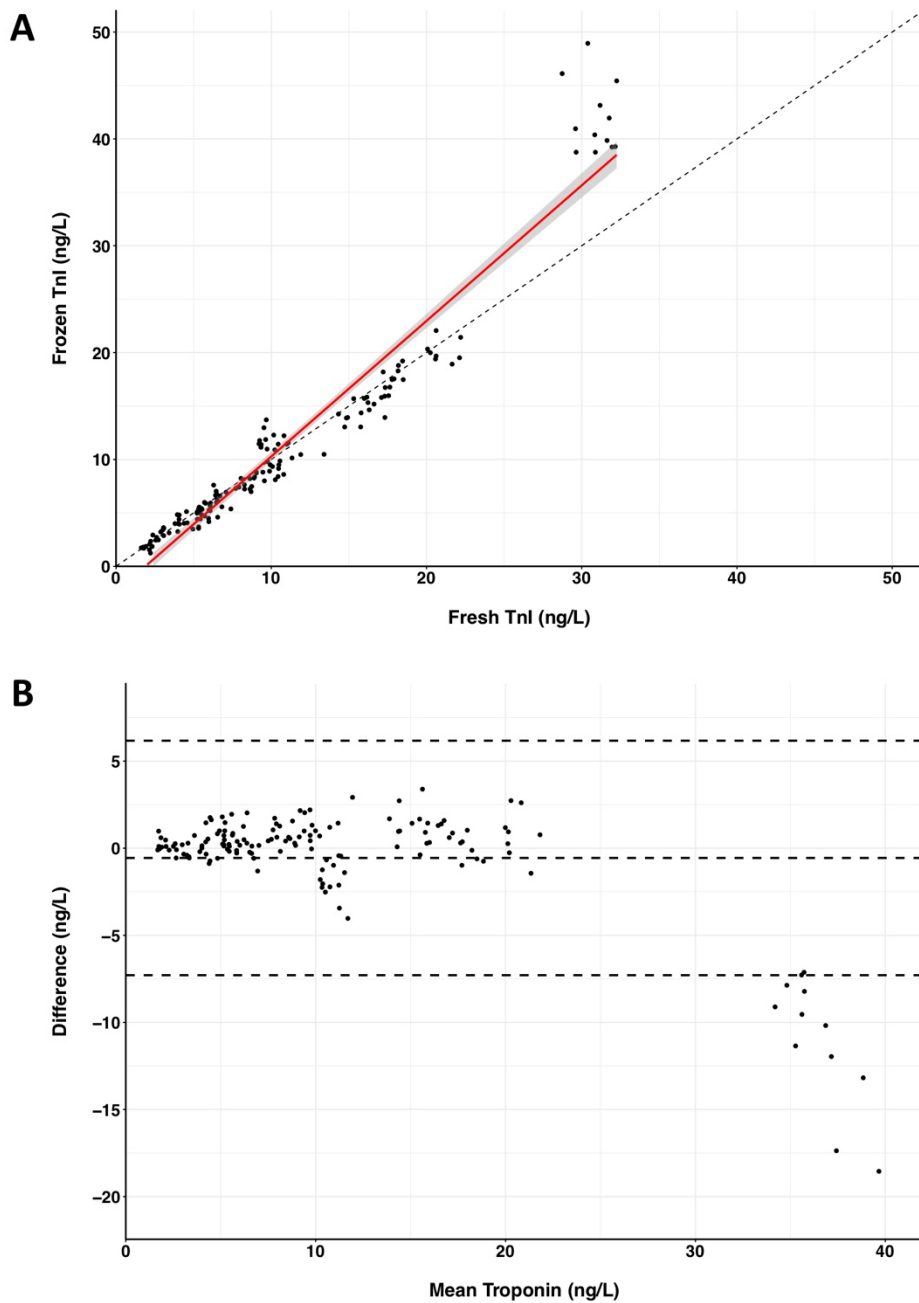


Figure 4.4 – Relationship between fresh and frozen cardiac troponin concentrations. In the correlation between fresh and frozen samples (A), the dashed line represents complete agreement and the red line is the best fit linear regression line through the results, with shaded 95% confidence intervals. A Bland-Alman plot (B) shows the mean difference (central line) with upper and lower lines of limits of agreement, defined as 1 standard deviation each side of the mean difference.

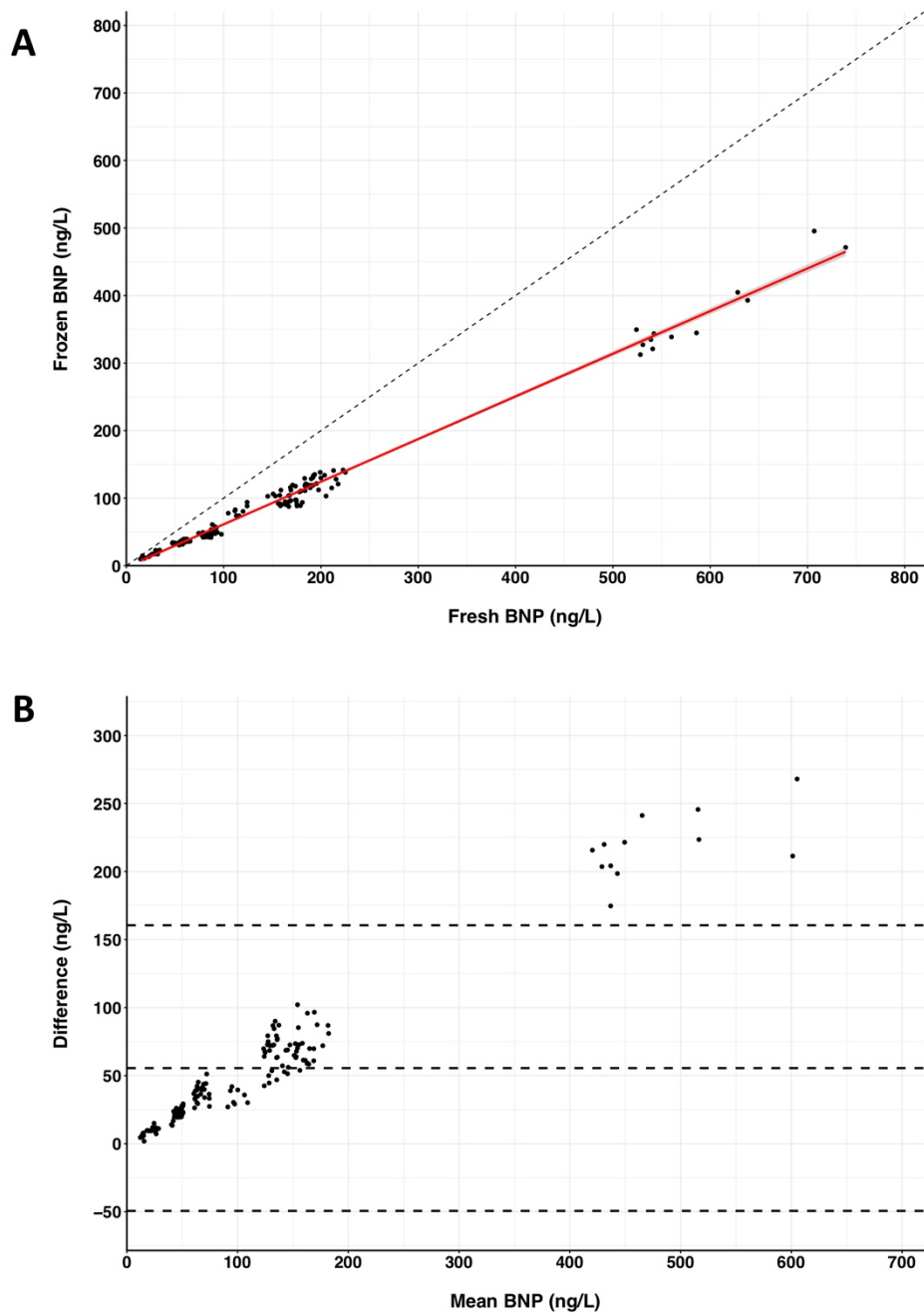


Figure 4.5 – Relationship between fresh and frozen BNP concentrations. In the correlation between fresh and frozen samples (A), the dashed line represents complete agreement and the red line is the best fit linear regression line through the results, with shaded 95% confidence intervals. A Bland-Altman plot (B) shows the mean difference (central line) with upper and lower lines of limits of agreement, defined as 1 standard deviation each side of the mean difference.

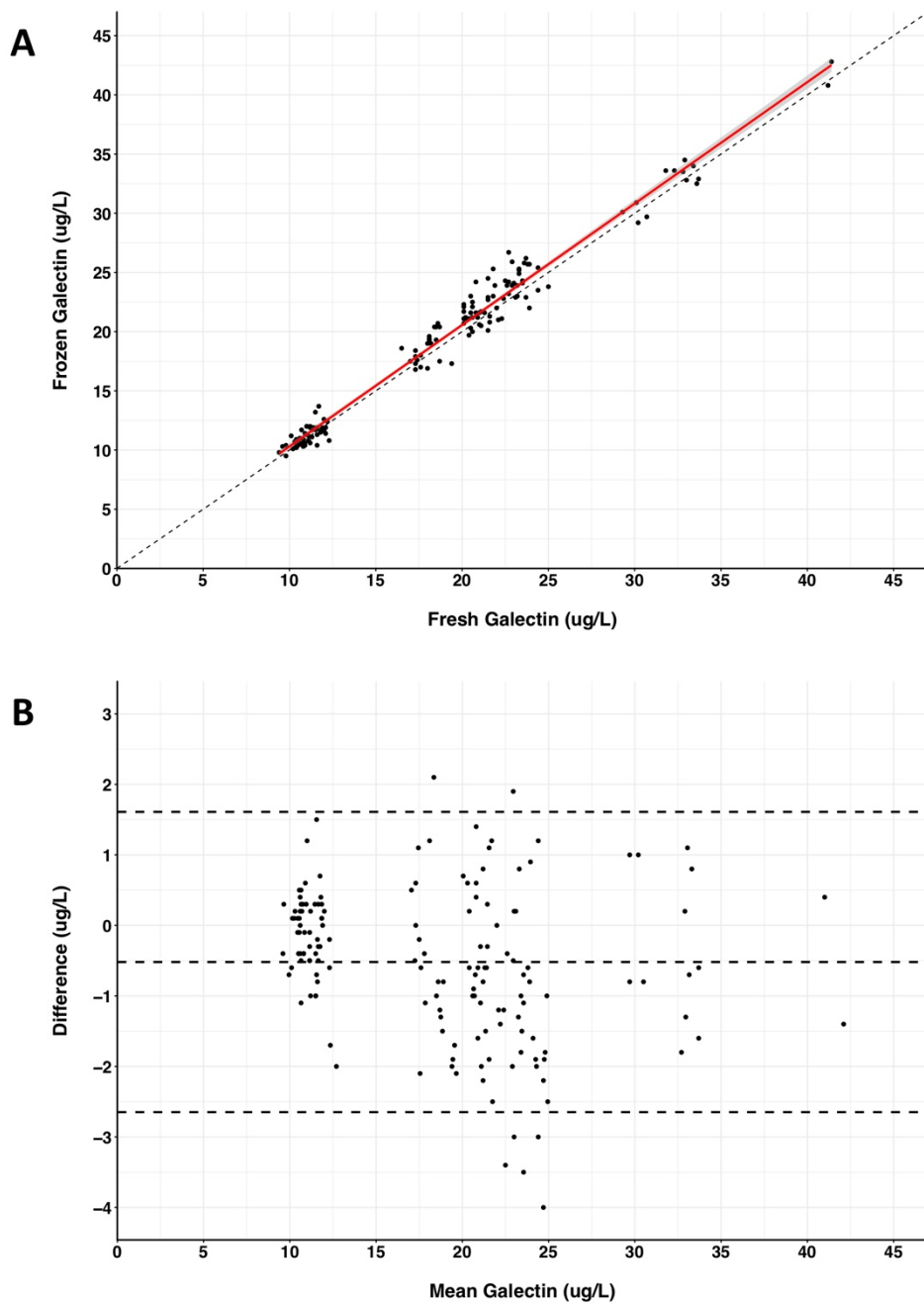


Figure 4.6 – Relationship between fresh and frozen galectin-3 concentrations. In the correlation between fresh and frozen samples (A), the dashed line represents complete agreement and the red line is the best fit linear regression line through the results, with shaded 95% confidence intervals. A Bland-Alman plot (B) shows the mean difference (central line) with upper and lower lines of limits of agreement, defined as 1 standard deviation each side of the mean difference.

4.5 DISCUSSION

In this analysis we have reported the performance of three putative blood biomarkers of disease progression in older subjects with aortic stenosis. We have made a number of important observations. Firstly, cardiac troponin I, BNP and galectin-3 demonstrated low analytical variability on a high-throughput platform, even using a fresh sampling protocol simulating clinical laboratory practices. Second, all three biomarkers were detectable in all subjects above assay limits of detection. Third, degradation of BNP on frozen sampling indicates that batch testing in this manner would not be valid for clinical use. Finally, the index of individuality across all three biomarkers was low, mandating serial testing to determine a clinically significant change in an individual subject. Reference change values to overcome inherent biological and analytical variability between samples taken weeks apart were 14% for galectin-3, 42% for cardiac troponin I and 55% for BNP. Taken together, these findings may inform the appropriate use of these blood biomarkers for monitoring disease progression in the clinical setting of older patients with moderate to severe aortic stenosis.

The use of blood biomarkers to monitor progression of aortic stenosis is currently not widespread, but the evidence base is growing.¹⁶⁵ Serial testing of stable, asymptomatic aortic stenosis patients could be an attractive enhancement to clinical assessment and routine echocardiography, and may help to stratify patients for more invasive or costly investigations. However, this approach could only be considered with sufficient understanding of the analytical and biological variability of these biomarkers. In contrast to other groups who have focused on young and healthy volunteers, we have assessed these biomarkers in the population with the most potential for clinical application, namely older, comorbid individuals with moderate to severe aortic stenosis.

Differences are observed with the variability of the same cardiac troponin I assay in a study of 12 healthy volunteers aged 19–58 years old by Wu *et al.*¹⁶² Analytical variability on weekly frozen sampling in this study was 15%, compared to just 3.8% in our study. This

may be explained by frequency of low concentrations of cardiac troponin near to the limit of detection of the assay amongst the healthy volunteers, with virtually no results above 5ng/L. In a different study of older patients with stable coronary disease (median age 68 years), the CV_a was also lower than healthy volunteers at 8% with a mean troponin concentration of 7.8ng/L.¹⁶⁰ In our older aortic stenosis population, the mean cardiac troponin was higher at 12.4ng/L, a level where analytical imprecision is even lower, as we have demonstrated by our comparison of CV_a across concentrations within our cohort. Interestingly, biological variability in the healthy volunteers cohort was 14%, which matches that seen in our subjects.¹⁶²

Therefore largely as a result of smaller analytical imprecision, our study generated a lower RCV for cardiac troponin between weekly serial samples. This is important if such testing were to enter clinical practice. Application of a higher RCV threshold derived from younger cohorts to define a clinically meaningful change in cardiac troponin may reduce test sensitivity in older patients, by increased false-negative rates in those with potentially progressive aortic stenosis.

Although our data suggest a decay of BNP on frozen sample analysis, this did not significantly alter analytical or biological variability resulting in a similar RCV to fresh sampling. However, this finding clearly adds uncertain pre-analytical variability to the interpretation of any BNP concentrations derived from frozen samples, limiting the translation of findings from such stored samples into clinical laboratory testing. The deterioration of endogenous BNP has been acknowledged before and may be overcome on frozen sampling by testing for the amino-terminal NT-proBNP, which appears more stable in this setting.¹⁶⁶ However, NT-proBNP and BNP concentrations are not directly comparable.⁹⁹ The RCV for BNP observed in our study at 55% was smaller than in cohorts of stable heart failure patients on frozen sampling, where threshold changes of 95–113% between weekly serial samples were indicated.^{99,167} This is likely to reflect the deliberate exclusion of

subjects with signs of heart failure within our sample, resulting in biological variability values that were approximately half that of the heart failure cohorts.

Galectin-3 proved the most stable of the three biomarkers, with little variation on hourly or weekly testing. The observed RCV of 14% is comparable to healthy cohorts, and slightly lower than the ~25% change observed in heart failure patients.^{167,168} It is interesting to note that the mean concentration of galectin-3 in aortic stenosis subjects included in our study was comparable to that in the CORONA trial of older patients with ischaemic heart failure, where the biomarker was predictive of cardiovascular and all-cause mortality.¹⁶⁹ However, in comparison to cardiac troponin and BNP, galectin-3 is the least widely adopted clinical biomarker.

4.5.1 LIMITATIONS

Unfortunately, two subjects had samples excluded from weekly sampling due to unforeseen intercurrent illness and the development of symptoms that could be consistent with disease progression. This limits the numbers included in this analysis, although at 12 subjects remains similar in size to other such studies.¹⁶² Although the findings for fresh sample analysis are reassuring for the translation of these findings to on-demand clinical laboratory settings, the pre-analytical phase was tightly controlled within this experiment. For true testing of analytical variability in 'real-world' laboratory settings, test samples of known concentration could be randomly included within routine clinical samples. It is likely that different operators obtaining and processing blood samples, as well as multiple analysers, reagent lots and calibration regimes would increase analytical variability and thereby the true RCV threshold. Application of the tighter RCVs obtained from controlled experiments may therefore reduce test specificity by increasing false positive triggering, whereby individuals may be incorrectly identified with an apparent significant change in their biomarker concentration, which is driven by higher analytical variation. It is important to acknowledge

that RCV calculations determine the threshold for significant statistical change in a biomarker between serial measures, but further clinical correlation studies are required to interpret the implications for disease progression. Testing for variability is performed in stable patients and so interpretation of these thresholds in an individual must occur in the context of stability of non-cardiac disease, particularly with biomarkers that are not organ-specific. Clinical instability is more likely in older patients due to greater comorbidity, which may make interpretation of many biomarkers challenging.

As a small variability study of 14 subjects, the spread of possible biomarker concentrations was limited, which may impair interpretation of these findings at the extremes of concentration. It is also clear that one subject had BNP levels that would be expected with significant wall stress (repeated testing >500ng/L) and heart failure. However, this did not correlate with their clinical presentation, raising the possibility of an idiosyncratic heterophilic antibody reaction falsely elevating the plasma concentration.¹⁷⁰ These data were retained in the analysis as the results were internally consistent for the subject and therefore did not meet criteria for outlier exclusion. Furthermore, the high BNP concentration was not matched by extreme cardiac troponin or galectin-3 results. However, this inclusion of an atypical subject may have influenced the biological variability results for BNP.

4.6 CONCLUSIONS

In a population of older patients with asymptomatic moderate to severe aortic stenosis, cardiac troponin I, BNP and galectin-3 were all detectable on a high-throughput platform above the limit of detection on multiple repeated hourly and weekly blood draws. Galectin-3 proved the most stable biomarker tested, with variability similar to other reported cohorts. However, reference change values for cardiac troponin I and BNP were lower than that reported in healthy volunteer and stable heart failure cohorts. This suggests added value from studying variability in subjects drawn from the clinical group to which testing is likely to be applied. These findings may inform the appropriate use of these blood biomarkers with serial testing for differences greater than the newly defined reference change values for older patients with moderate to severe aortic stenosis.

CHAPTER 5

THE RELATIONSHIP BETWEEN PREOPERATIVE FRAILITY AND OUTCOMES FOLLOWING TRANSCATHETER AORTIC VALVE IMPLANTATION: A SYSTEMATIC REVIEW AND META-ANALYSIS

Contents based on the following published material with minor changes:
Anand A, Harley C, Visvanathan A, Shah ASV, Cowell J, MacLulich A, Shenkin SD, Mills NL. The relationship between preoperative frailty and outcomes following transcatheter aortic valve implantation: a systematic review and meta-analysis. *European Heart Journal - Quality of Care and Clinical Outcomes*. 2017;3:123–132.

CHAPTER 5: THE RELATIONSHIP BETWEEN PREOPERATIVE FRAILITY AND OUTCOMES FOLLOWING TRANSCATHETER AORTIC VALVE IMPLANTATION: A SYSTEMATIC REVIEW AND META-ANALYSIS

5.1 OVERVIEW

Transcatheter aortic valve implantation (TAVI) is an increasingly common intervention for patients with aortic stenosis deemed high-risk for major cardiac surgery, but identifying those who will benefit can be challenging. Frailty reflects physiological reserve and may be a useful prognostic marker in this population. We performed a systematic review and meta-analysis of the association between frailty and outcomes after TAVI.

Five databases were searched between January 2000 and May 2015. From 2,623 articles screened, 54 were assessed for eligibility. Ten cohort studies (n=4,592) met the inclusion criteria of reporting a measure of frailty with early (≤ 30 days) or late (> 30 days) mortality and procedural complications following TAVI as defined by the Vascular Academic Research Consortium (VARC).

Frailty was associated with increased early mortality in four studies (n=1,900) (HR 2.35, 95% CI 1.78-3.09, $p < 0.001$), and increased late mortality in seven studies (n=3159) (HR 1.63, 95% CI 1.34-1.97, $p < 0.001$). Objective frailty tools identified an even higher risk group for late mortality (HR 2.63, 95% CI 1.87-3.70, $p < 0.001$). Frail individuals undergoing TAVI have a mortality rate of 34 deaths per 100 patient years, compared to 19 deaths per 100 patient years in non-frail patients. There was limited reporting of VARC procedural outcomes in relation to frailty, preventing meta-analysis.

Frailty assessment in an already vulnerable TAVI population identifies individuals at even greater risk of poor outcomes. Use of objective frailty tools may inform patient selection, but this requires further assessment in large prospective registries.

5.2 INTRODUCTION

Aortic stenosis is the most common valvular disease in the Western World, affecting 1 in 8 individuals over the age of 75 years. The incidence of functionally important disease is rising in line with the ageing population, providing challenges for conventional valve replacement surgery.¹³⁸ Patients over 80 years old undergoing elective cardiac surgery have more operative complications and a 10 percent mortality rate at 30 days; therefore decisions around intervention in older patients are complex.²⁶ Transcatheter Aortic Valve Implantation (TAVI) has become a widespread and viable alternative for patients considered high-risk for conventional surgery. Population modelling suggests in excess of 91,000 people fall into this category across North America each year.¹³⁸ The Society of Thoracic Surgeons (STS)⁷³ and EuroSCORE¹⁷¹ tools are often used to guide treatment based on the predicted risk of poor outcomes, but these scoring systems have not been designed or formally tested in TAVI populations. The application of such scores in elderly patients suitable for conventional surgery has also been questioned.^{80,172} Many believe that a holistic approach through frailty assessment may improve the decision making process.

Frailty is a multimodal concept describing loss of strength, endurance and physiological reserve across multiple systems that increases vulnerability for developing dependency or death.¹⁰⁵ It becomes more common with age, but is a very distinct concept of biological rather than chronological years; indeed the majority of individuals over 85 years old are not frail. Common models focus on the development of a phenotype or the gradual accumulation of deficits over time, but there is no clear consensus on the best form of measurement.^{105,107,108} Within non-cardiac surgical cohorts, frailty is predictive of mortality, post-operative complications and institutionalisation.¹⁷³⁻¹⁷⁶ It is plausible that such measures applied to high-risk patients undergoing TAVI may improve the discrimination of current risk assessment tools for important patient outcomes. In this systematic review, we evaluate the effect of pre-operative frailty on important patient outcomes after TAVI.

5.3 METHODS

5.3.1 SEARCH STRATEGY

We conducted a systematic literature review of Medline, EMBASE and CINAHL databases between 1st January 2000 and 1st June 2015 using the key search terms of frailty (and its synonyms) and TAVI (and its synonyms) (Table 5.1). Earlier dates were not searched as TAVI procedures were not performed routinely until 2001. Reference and forward citation searching via the Web of Science (Thomson Reuters) was performed on papers meeting the criteria for inclusion. Hand-searching using the primary search terms was performed within the three most commonly identified journals from the initial search. This was repeated using the Google Scholar search engine. This review was prospectively registered on the PROSPERO database:

https://www.crd.york.ac.uk/prospero/display_record.php?RecordID=17791.

5.3.2 ELIGIBILITY CRITERIA

We included any primary peer-reviewed paper where a measure of frailty was defined by the authors prior to TAVI, and where this was related to at least one of the predefined post-TAVI outcomes. No other assessments were adjudicated to represent frailty unless stipulated as a determinant of frailty by the authors of a study. No restrictions were placed on the age of study participants, specific vascular route or operator technique by which TAVI was performed. Results in all languages were considered, using translation services where required to adjudicate eligibility.

Table 5.1 – Example search string (shown for Medline).

Search number	Search term
1.	TAVI.ti,ab.
2.	"transcatheter aortic valve implant*".ti,ab.
3.	"transcatheter aortic valve replac*".ti,ab.
4.	Aortic Valve Stenosis/
5.	Heart Valve Prosthesis/
6.	exp Cardiac Surgical Procedures/
7.	Heart Valve Prosthesis Implantation/
8.	TAVR.ti,ab.
9.	Cardiac Catheterization/
10.	or/1-9
11.	Frail Elderly/
12.	frail*.ti,ab.
13.	exp Geriatric Assessment/
14.	(frail adj2 elderly).ti,ab.
15.	(frail* adj3 (index or phenotype or assess*)).ti,ab.
16.	Rockwood.ti,ab.
17.	(Fried adj5 (frail* or index* or phenotype)).ti,ab.
18.	(frail* adj2 scor*).ti,ab.
19.	"Activities of Daily Living"/
20.	Geriatrics/rh, su [Rehabilitation, Surgery]
21.	11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20
22.	10 and 21

The primary outcome was all-cause mortality after TAVI, either reported in the short (≤ 30 days) or long term (>30 days). Secondary outcomes comprised procedural complications as defined by the Valve Academic Research Consortium (VARC) standardized endpoint definitions. These include cardiovascular mortality, myocardial infarction, major stroke, bleeding, acute kidney injury requiring dialysis and numerous other vascular complications.¹⁷⁷ Any measures of functional capacity or patient independence after TAVI were sought as secondary outcomes where the relationship to a pre-TAVI frailty measure was presented. Review articles and non-peer reviewed material (such as conference proceedings and poster abstracts) were excluded.

5.3.3 DATA EXTRACTION

All extracted abstracts and full-text articles meeting the inclusion criteria were assessed between three researchers (AA, AV and CH), such that two people independently reviewed each submission. Disagreements were resolved by consensus including the third reviewer. For each study meeting the inclusion criteria, a standardised data extraction form was developed to record study design, TAVI population demographics, assessed risk of the population (STS and EuroSCORE), specific frailty measure, length to follow-up and any data related to the primary and/or secondary outcomes. Where the relationship between frailty and outcome was qualitatively but not quantitatively expressed, primary authors were contacted in an attempt to gain additional primary data. Where the same study appeared to be reported across more than one article, only the most complete submission was included, with the aim of maximising the volume of frailty data included.

5.3.4 QUALITY AND BIAS ASSESSMENT

No validated quality assessment tool has been widely established to assess observational studies that are not designed to directly compare two groups. The Newcastle-Ottawa Scale was used to provide a structured assessment of sample selection (4 points), comparability (2

points) and outcomes (3 points).¹⁷⁸ This tool was chosen as an objective measure with clear descriptors to derive a maximum score of 9 points. Studies were independently assessed by two reviewers and disagreement resolved by consensus: ≥ 7 points considered high quality for frailty reporting, < 7 moderate or low quality. Publication bias was assessed in the primary endpoint with the greatest number of studies by creating a funnel plot and using Egger's regression test.¹⁷⁹ We then corrected for asymmetry using the trim and fill method to determine an adjusted effect size.¹⁸⁰

5.3.5 DATA SYNTHESIS AND ANALYSIS

All included studies were observational cohorts with respect to frailty. Meta-analysis was performed when at least three studies reported a comparable endpoint to generate a meta-estimate. Given the wide number of frailty tools available, significant heterogeneity was expected across the studies and therefore a random-effects model (maximum likelihood approach) was chosen to calculate summary effect estimates.¹⁸¹ Statistical analysis was performed using the *metafor* statistical package within R version 3.1.3 (<http://www.r-project.org>) and GraphPad Prism version 6.0 (GraphPad Software, San Diego, CA, USA). A value of $p < 0.05$ was considered statistically significant.

5.4 RESULTS

5.4.1 SEARCH RESULTS AND PATIENT CHARACTERISTICS

We identified 2,623 abstracts from our initial search, resulting in 54 articles for full-text review to assess eligibility. Ten studies from Europe and North America met the full inclusion criteria (Figure 5.1). These comprised 4,592 patients undergoing TAVI in whom a frailty measure was made prior to surgery. The mean age was 80 to 86 years, 34% to 53% of participants were men, and the STS-predicted 30-day mortality rates where available were between 6.3% and 16.6%. In those studies detailing the access route chosen for TAVI, the femoral approach was the most common, although this ranged from 47% to 100% of cases. The proportion of TAVI patients identified as frail varied greatly across the included studies, from 5% to 83% (Table 5.2).

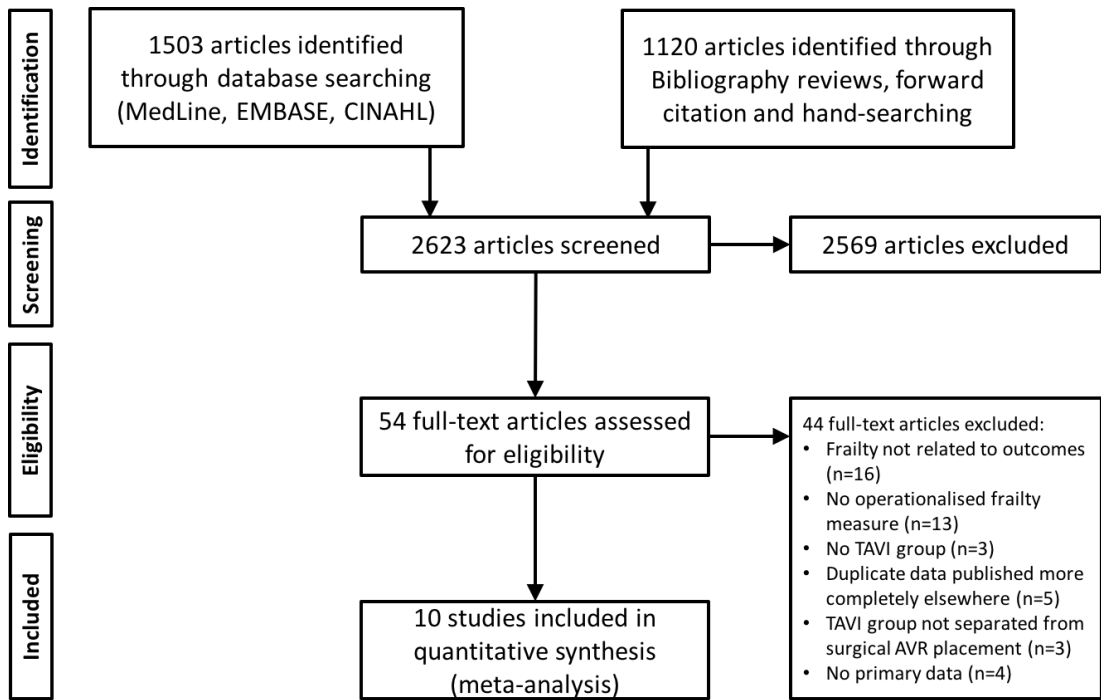


Figure 5.1 – Flow diagram of reviewed studies.

Table 5.2 – Contextual detail of included studies.

Author, year	Country	Definition of frailty	n	Mean age (years)	Male gender (%)	Proportion frail (%)	TAVI access route	30-day mortality (%)	1-year mortality (%)
Ewe, 2010 ¹⁸²	Netherlands/ Italy	Fried criteria based on gait speed, grip strength, weight loss, physical activity and exhaustion	147	80	43	32.7	Femoral 51%, apical 49%	6.8	15.0
Stortecky, 2012 ¹²⁰	Switzerland	Frailty Index based upon geriatric assessment of cognition, nutrition, timed get-up-and-go, ADLs and disability. Scored 0-7 with ≥ 3 considered frail	100	84	40	49	Femoral 85%, apical 14%, subclavian 1%	8.0	19.0
Rodes-Cabau, 2012 ¹⁸³	Canada	Subjective assessment of multidisciplinary team	339	81	45	25.1	Femoral 48%, apical 52%	10.6	-
Kamga, 2013 ¹⁸⁴	Belgium	ISAR score (self-reported functional dependence, recent hospitalisation, impaired memory, difficulties with vision and polypharmacy) SHERPA score (age, ADLs, cognitive decline, falls and self-perceived health)	30	86	53	ISAR: 83.3% moderate or high risk SHERPA: 73.3% moderate or high risk	Femoral 100%	-	26.7

Zahn, 2013 ¹⁸⁵	Germany	Presumed subjective assessment (limited detail)	1318	82	42	17.7	Femoral 88%, apical 9%, subclavian 2%, aortic 1%	-	19.9
Puls, 2014 ¹⁸⁶	Germany	Katz Index of ADLs (score <6 frail)	300	82	34	48	Femoral 47%, apical 53%	11.3	28
Seiffert, 2014 ¹⁸⁷	Germany	Subjective assessment guided by CHSA Clinical Frailty Scale ¹¹⁰ score ≥ 6	347*	81	52	4.6	-	-	24.2
Capodanno, 2014 ¹⁸⁸	Italy	Geriatric Status Scale based upon ADLs, cognition, continence and mobility. Scored 0-3 with ≥ 2 frail	1256 [†]	82	42	24.4	-	6.1	-
Debonnaire, 2015 ¹⁸⁹	Netherlands/ Italy	Presumed subjective assessment	511	82	38	19.2	Femoral 52%, apical 48%	5.7	15.7
Green, 2015 ¹⁹⁰	USA	Frailty score composed of serum albumin, grip strength, gait speed and ADLs. Scored between 0-12 with ≥ 6 considered frail	244	86	52	45.1	Femoral 49%, others presumed apical	8.6	23.5

Abbreviations: ADLs = activities of daily living. Observed mortality data refer to the whole study population including frail and non-frail individuals.

*Only the Bonn subgroup that received frailty assessment considered from this multicentre study.

[†]Only the development cohort of this study included. The validation data set does not contain frailty related outcome data.

5.4.2 DEFINITIONS OF FRAILITY

Frailty was identified by authors as either *subjective* (four studies) or *objective* (six studies). Subjective frailty was based on the judgement of a clinical team without reporting use of a specific tool. Objective frailty was determined by use of a tool specifically with the purpose of defining frailty, such as activity of daily living assessments, comprehensive geriatric assessment and frailty indices. With the exception of one small study of 30 patients by Kamga *et al.*¹⁸⁴, frailty data was available as a dichotomised variable when related to outcomes, even where it had been measured on a continuous scale.

5.4.3 FRAILITY AND MORTALITY

Four studies (n=1,900) reported frailty (using objective measures) and early (≤ 30 days) mortality after TAVI (Table 5.3 and Figure 5.2), identifying greater than doubling of the risk of early death amongst patients identified as frail (HR 2.35, 95% CI 1.78-3.09, $p < 0.001$). All papers reported unadjusted univariate analyses for the association between frailty and mortality. There was no significant heterogeneity between studies ($I^2 = 0\%$, $p = 0.33$).

Seven studies (n=3,159) quantified the relationship between frailty and late mortality > 30 days after TAVI, with every study completing at least one year of follow-up (Table 5.4 and Figure 5.2). All reported an increased risk of death amongst frail patients, with an overall effect size of HR 1.63 (95% CI 1.34-1.97, $p < 0.001$). This was only marginally increased by restricting analysis to studies undertaking adjustment for potential confounders (5 studies, HR 1.85, 95% CI 1.34-2.55, $p < 0.001$) or including only studies of higher quality for frailty reporting (4 studies, HR 1.79, 95% CI 1.28-2.50, $p < 0.001$). There was moderate heterogeneity ($I^2 = 66\%$, $p = 0.01$), which was reduced by performing a sensitivity analysis by the type of frailty measure used (Figures 5.3 and 5.4). The mortality risk for frail patients was greater amongst those studies using an objective measure (HR 2.63, 95% CI 1.87-3.70, $p < 0.001$) rather than subjective assessment (HR 1.42, 95% CI 1.28-1.59, $p < 0.001$).

Table 5.3 – Early (≤ 30 days) outcomes related to frailty in included studies.

Author, year	Outcome(s) related to frailty	Adjustment	Effect estimate*	Lower 95% CI	Upper 95% CI	p-value
Stortrecky, 2012 ¹²⁰	30 day MACCE	Nil	4.78	0.96	23.77	0.05
	30 day MACCE (per unit increase in frailty index)	Nil	1.66	1.14	2.44	0.01
	30 day all-cause mortality	Nil	8.33	0.99	70.48	0.03
Puls, 2014 ¹⁸⁶	30 day all-cause mortality (per unit increase in frailty index)	Nil	2.18	1.32	3.61	0.002
	All-cause mortality	Nil	3.05	1.4	5.7	0.003
	Procedural myocardial infarction	Nil	1.08	0.15	7.59	0.94
	Procedural major stroke	Nil	0.98	0.41	2.33	0.95
	Procedural TIA	Nil	1.08	0.07	17.16	0.95
	Life-threatening or disabling bleeding	Nil	0.86	0.45	1.62	0.63
	Major bleeding	Nil	2.17	0.84	5.62	0.11
	Minor bleeding	Nil	1.50	1.05	2.16	0.03
	Renal failure requiring dialysis	Nil	2.01	1.09	3.70	0.02

Capodanno, 2014 ¹⁸⁸	All-cause mortality	Nil	2.09	1.30	3.37	0.003
Green, 2015 ¹⁹⁰	All-cause mortality	Nil	1.34	0.59	3.04	0.48
	Cardiovascular mortality	Nil	1.22	0.47	3.14	0.68
	Major stroke	Nil	0.61	0.06	6.63	0.68
	Major bleeding	Nil	1.74	0.69	4.42	0.24
	Major vascular complications	Nil	1.42	0.49	4.11	0.52
	Permanent pacemaker insertion	Nil	1.02	0.46	2.26	0.97
	Renal failure requiring dialysis	Nil	1.57	0.60	4.07	0.36

*Where not presented directly by authors, relative risk ratios calculated from 2 by 2 tables for those with and without frailty.
Abbreviations: MACCE = major adverse cardiovascular and cerebral events.

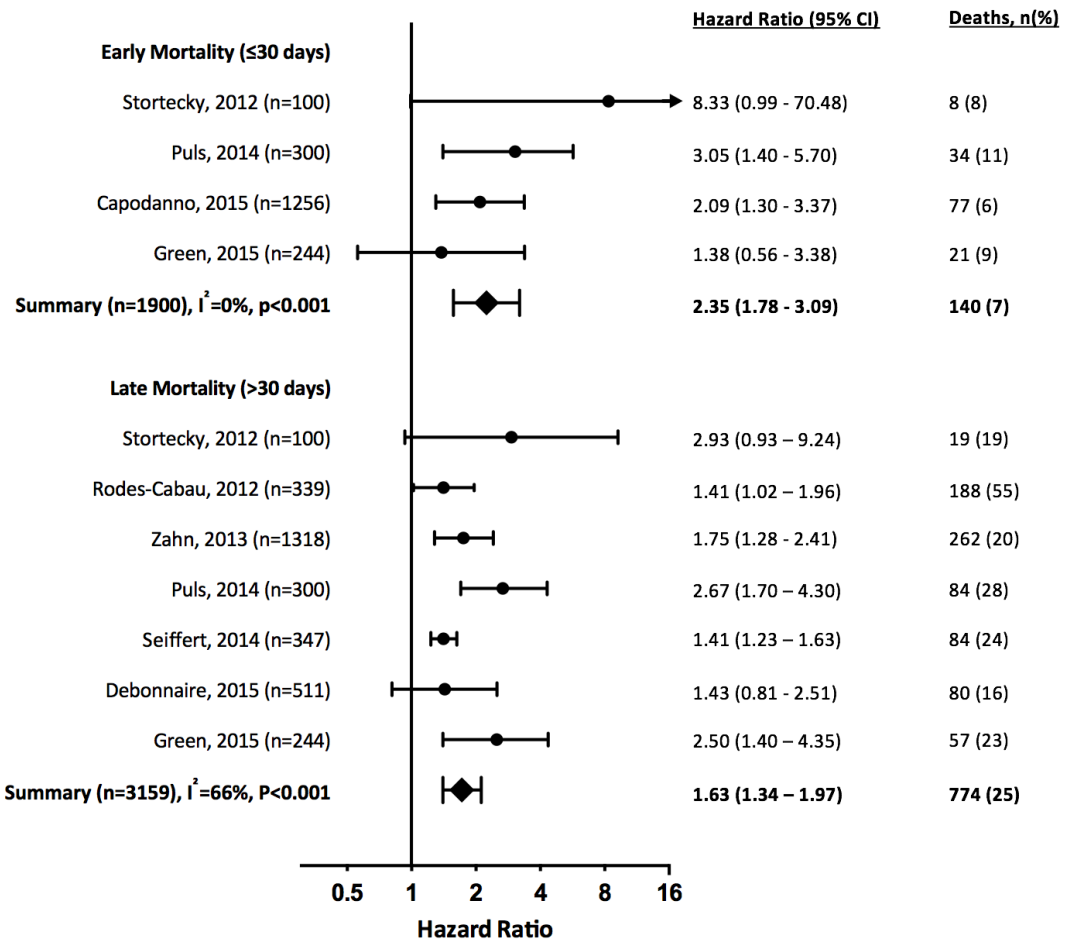


Figure 5.2 – Risk of early (≤30 days after TAVI) and late (>30 days) mortality in studies suitable for meta-analysis ordered by date of publication. Summary meta-estimate calculations based on random-effects model analysis

Table 5.4 – Late (>30 days) outcomes related to frailty in included studies.

Author, year	Outcome(s) related to frailty	Adjustment	Effect estimate*	Lower 95% CI	Upper 95% CI	p-value
Ewe, 2010 ¹⁸²	MACCE defined as composite of death, nonfatal stroke, heart failure or nonfatal myocardial infarction (mean follow-up 9.1 months)	Logistic EuroSCORE, peripheral vascular disease, previous CABG, baseline LVEF	4.20	2.00	8.84	<0.001
Stortrecky, 2012 ¹²⁰	1 year MACCE	Nil	4.89	1.64	14.6	0.003
	1 year MACCE	STS score	4.17	1.37	12.72	0.01
	1 year MACCE	Logistic EuroSCORE	4.48	1.48	13.53	0.01
	1 year MACCE (per unit increase in frailty index)	Nil	1.80	1.33	2.45	<0.001
	1 year all-cause mortality	Nil	3.68	1.21	11.19	0.02
	1 year all-cause mortality	STS score	2.93	0.93	9.24	0.07
	1 year all-cause mortality	Logistic EuroSCORE	3.29	1.06	10.15	0.04
	1 year all-cause mortality (per unit increase in frailty index)	Nil	1.80	1.31	2.47	<0.001
Rodes-Cabau, 2012 ¹⁸³	All-cause mortality (mean follow-up 42 ± 15 months)	Atrial fibrillation, cerebrovascular disease, COPD, eGFR, pulmonary hypertension	1.41	1.02	1.96	0.034

	Late all-cause mortality (excluding mortality within 30 days of TAVI)	Age, atrial fibrillation, COPD, eGFR	1.52	1.07	2.17	0.021
Kamga, 2013 ¹⁸⁴	1 year all-cause mortality (per 1 unit increase in SHERPA score)	Unclear but likely gender, BMI, pulmonary hypertension, diabetes	2.74	1.39	5.39	0.004
Zahn, 2013 ¹⁸⁵	1 year mortality	Nil	1.50	1.19	1.89	<0.001
Puls, 2014 ¹⁸⁶	All-cause mortality (median follow up 537 days)	Age and sex	2.67	1.7	4.3	<0.0001
Seiffert, 2014 ¹⁸⁷	1 year mortality	Age and sex	1.41	1.23	1.63	<0.001
Debonnaire, 2015 ¹⁸⁹	1 year mortality	Nil	1.29	0.80	2.06	0.29
Green, 2015 ¹⁹⁰	1 year all-cause mortality (frailty dichotomised)	Nil	2.18	1.27	3.75	0.005
	1 year all-cause mortality (frailty dichotomised)	Stepwise inclusion of variablest with entry/stay criteria of 0.1/0.1 and a maximum of one covariate for every 10 events.	2.5	1.40	4.35	0.002
	1 year all-cause mortality (per unit increase in frailty score)	Nil	1.12	1.02	1.22	0.01
	Poor outcome (death or poor quality of life‡) at 6 months	Stepwise inclusion of variablest as above	2.21	1.09	4.46	0.03
	Poor outcome (death or poor quality of life‡) at 1 year	Stepwise inclusion of variablest as above	2.40	1.14	5.05	0.02

Abbreviations: MACE = major adverse cardiovascular and cerebral events; CABG = coronary artery bypass grafting; LVEF = left ventricular ejection fraction; COPD = chronic obstructive pulmonary disease; eGFR = estimated glomerular filtration rate; BMI = body mass index; TIA = transient ischaemic attack; STS = Society of Thoracic Surgeons.

*Where not presented directly by authors, relative risk ratios calculated from 2 by 2 tables for those with and without frailty.

†Candidate variables: age, sex, body mass index, access route, STS score, diabetes, hypertension, angina, heart failure, New York Heart Association class IV, coronary artery disease, previous coronary angioplasty, previous CABG, cerebrovascular disease, peripheral vascular disease, previous balloon aortic valvuloplasty, permanent pacemaker, renal disease, liver disease, chronic pulmonary disease, aortic valve mean gradient, ejection fraction, moderate or severe mitral regurgitation.

‡ Poor quality of life defined as Kansas City Cardiomyopathy Questionnaire Overall Summary score < 45, or a decrease of ≥ 10 points on serial testing before and after TAVI.

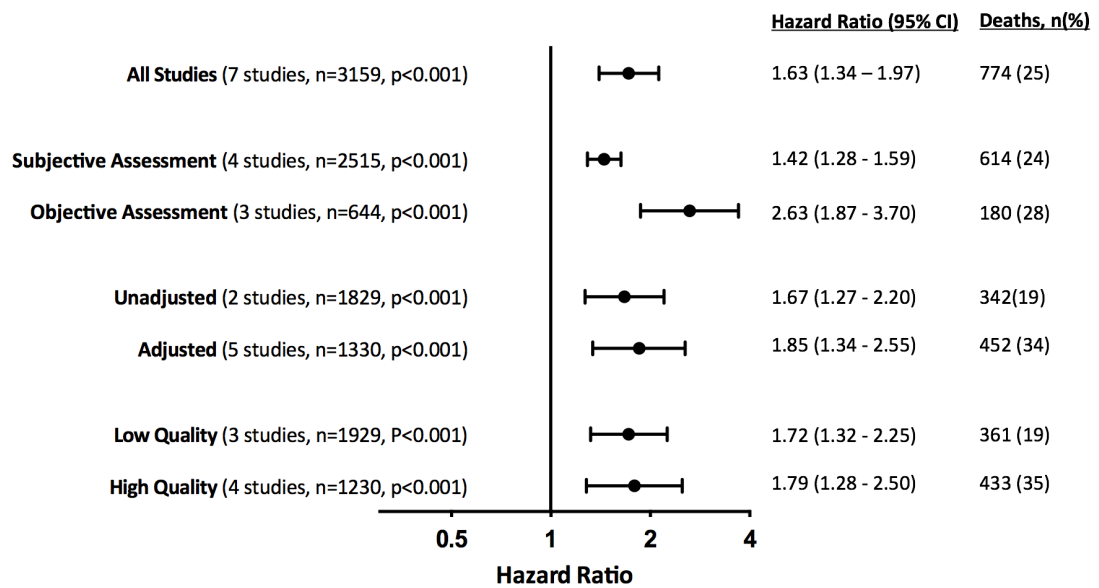


Figure 5.3 – Risk of late (>30 days after TAVI) mortality amongst frail patients. Summary meta-estimates presented grouped by type of frailty assessment used (subjective vs. objective), adjustment for confounders (unadjusted vs. adjusted) and study quality with regard to frailty reporting (low vs. high). All summary meta-estimate calculations based on random-effects model analysis. Individual study level data are shown in Figure 5.4.

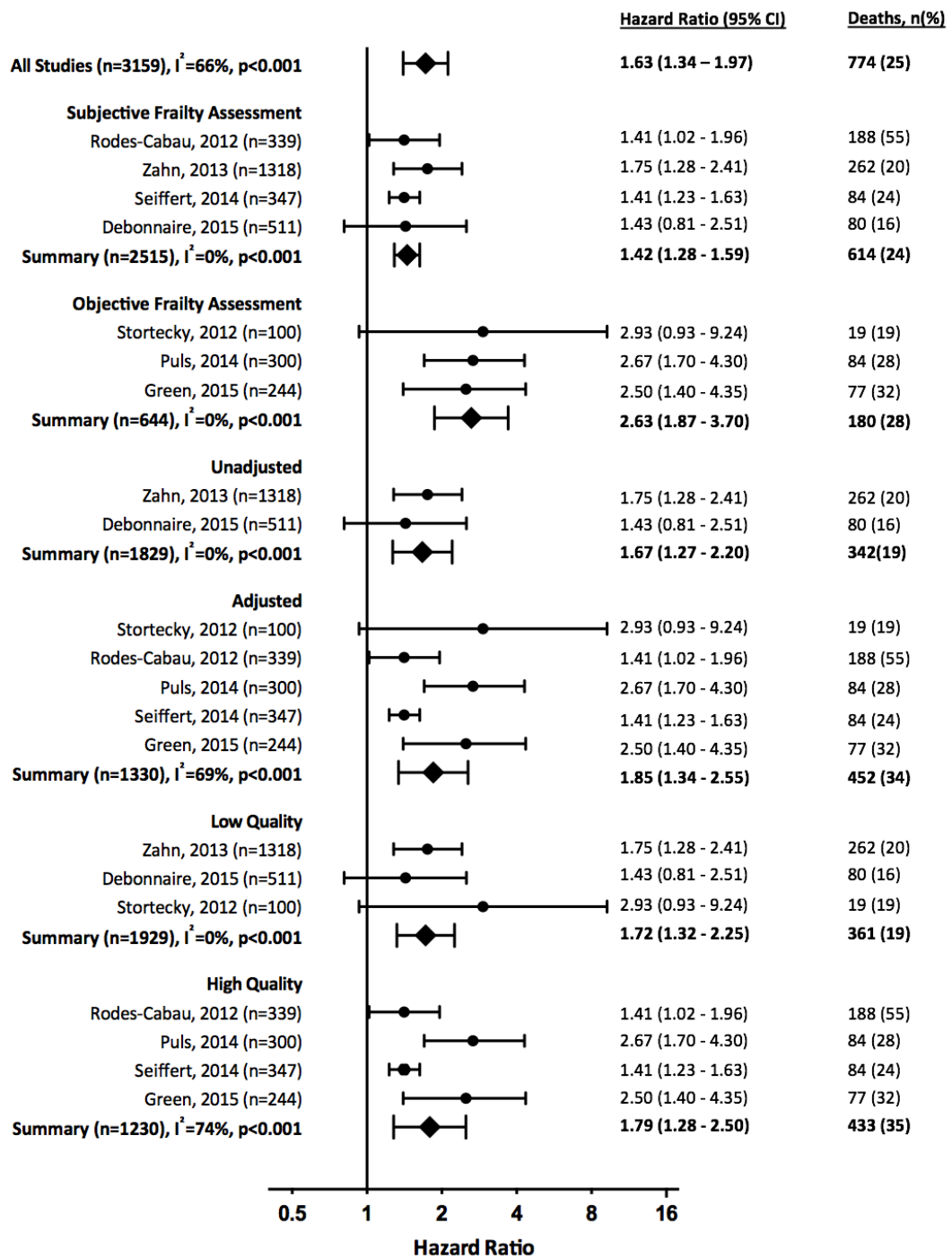


Figure 5.4 – Sensitivity analysis for late mortality (>30 days) after TAVI. Individual and summary meta-estimates presented for subjective vs. objective frailty assessment, unadjusted vs. adjusted analyses, and high vs. low quality for frailty reporting. All summary meta-estimate calculations based on random effects model analysis.

Five studies provided the absolute number of deaths by frailty status allowing combined incidence estimations. This calculation totalled 3629 TAVI patients (24.6% frail) followed for the equivalent of 2717 patient years. Amongst those with frailty, 34 deaths/100 patient years were observed, against 19 deaths/100 patient years in non-frail individuals (Table 5.5). Two studies could not be included in the meta-analysis due to frailty being reported as a continuous variable (Kamga *et al.*¹⁸⁴), or because only a composite end point of MACCE (major adverse cardiovascular or cerebrovascular event) rather than all-cause mortality was reported (Ewe *et al.*¹⁸²). However, both studies did report significant associations of frailty with poorer outcomes including late mortality.

Table 5.5 – Comparison of mortality in frail and non-frail patients after TAVI.

Author, year	Zahn 2013 ¹⁸⁸	Puls 2014 ¹⁸⁶	Capodanno 2014 ¹⁸⁸	Debonnaire 2015 ¹⁸⁹	Green 2015 ¹⁹⁰	Overall
Frail (n)	233	144	306	98	110	891
Frail deaths (n)	70	80	30	20	36	236
Non-frail (n)	1085	156	950	413	134	2738
Non-frail deaths (n)	217	37	47	60	21	382
Follow-up period	Mean 12.9 months	Median 537 days	30 days	1 year (censored)	1 year (censored)	-
Frail years of follow-up	250	212	25	98	110	695
Non-frail years of follow-up	1166	230	78	413	134	2021
Death rate/100 frail patient years	28	38	120	20	33	34
Death rate/100 non-frail patient years	19	16	60	15	16	19

5.4.4 FRAILTY AND VARC OUTCOMES

There was wide variation in the reporting of secondary outcomes across the included studies, with only three studies reporting comparable outcomes in relation to frailty. Meta-analysis of these endpoints was therefore not possible. VARC outcome measures ≤ 30 days after TAVI were reported in relation to frailty status in only two of the included studies, totalling 544 patients (Table 5.3). Both used objective tools, and reported increased effect estimates for the risk of major bleeding and renal failure requiring dialysis in frail patients, but only the latter complication reached significance in the paper by Puls *et al.* (OR 2.23, 95% CI 1.12-4.47, $p=0.02$). Both studies reported no increase in the risk of stroke amongst frail individuals after TAVI.

5.4.5 QUALITY AND RISK OF BIAS

Six studies met our frailty-defined criteria for high quality (Newcastle-Ottawa scale score ≥ 7) and four were considered moderate or low in quality (Table 5.6). No study scored maximum points. All those considered of lower quality did not include adjustment for potential confounders of the relationship between frailty and outcomes. Publication bias was suggested amongst the seven studies reporting late mortality (Egger's test for asymmetry $p=0.02$). Adjustment by the trim and fill method (Figure 5.5) had no effect on the size estimate, which remained statistically significant (HR 1.59, 95% CI 1.33-1.90, $p<0.001$ vs HR 1.63, 95% CI 1.34-1.97, $p<0.001$ before adjustment).

Table 5.6 – Newcastle-Ottawa Scale quality assessment of included studies.

Author, year	Selection (max 4)	Comparability (max 2)	Outcome (max 3)	Total (max 9)	Quality group
Ewe, 2010	****	**	*	7	High
Stortecky, 2012	****	*	*	6	Low
Rodes-Cabau, 2012	***	**	**	7	High
Kamga, 2013	****	**	*	7	High
Zahn, 2013	***		*	4	Low
Puls, 2014	****	**	**	8	High
Seiffert, 2014	***	**	**	7	High
Capodanno, 2014	****		**	6	Low
Debonnaire, 2015	***		**	5	Low
Green, 2015	****	**	*	7	High

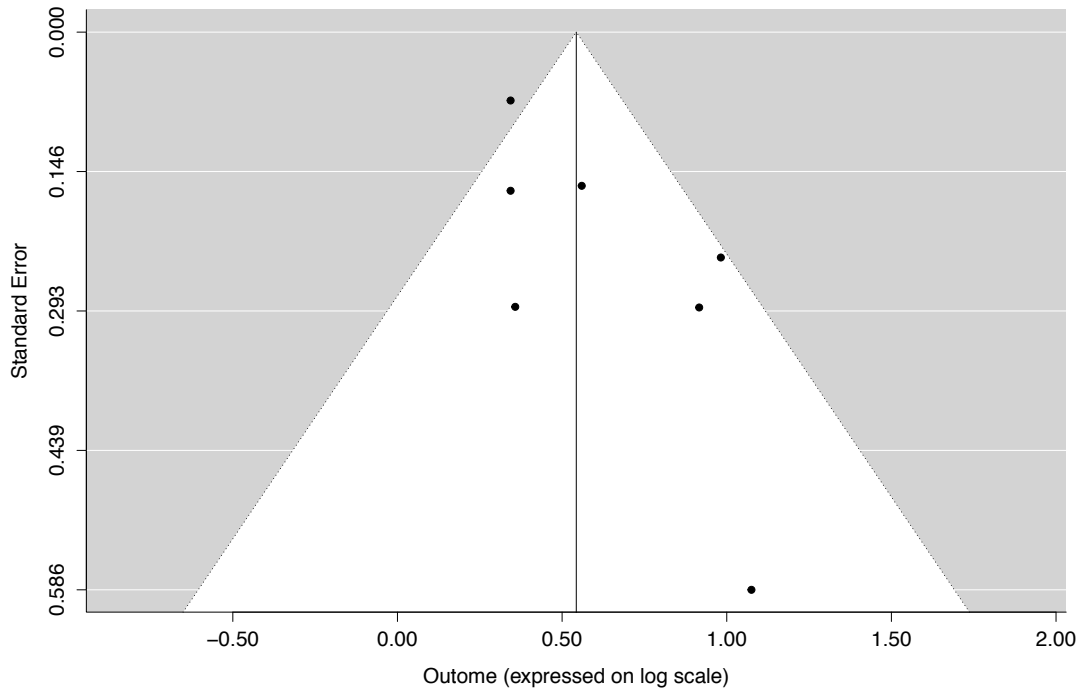


Figure 5.5 – Funnel plot for publication bias assessment in seven studies reporting late mortality. Each point represents one study, with the model estimating two missing studies from the left side of plot.

5.5 DISCUSSION

In this systematic review and meta-analysis we explored the relationship between pre-procedure frailty and outcomes after TAVI in 10 studies from Europe and North America comprising 4,592 patients. We have made several important observations. First, the measurement of frailty detects a population at double the risk of both early and late mortality after TAVI. Second, using objective measures of frailty appears to identify an even more vulnerable group than 'end-of-the-bed' subjective assessment. However, it is worth acknowledging that such subjective frailty assessment still provides important discrimination of risk within a population already considered at 'high-risk' for conventional surgery. Third, VARC complication rates in relation to frailty status are not well reported, with only very limited data to suggest increased risk of dialysis requirement and bleeding risk in frail patients. However, these observations were not suitable for meta-analysis and are subject to competing risk bias from the increased early mortality observed amongst those with frailty.

A recent review by Puri *et al* has emphasised the potential value of frailty assessment in TAVI candidates.¹⁹¹ Through the process of systematic review and meta-analysis, we have further clarified the growing body of research in this area and have numerically quantified the mortality risk of frailty identified by both objective and subjective measures. Established methods for determining those most likely to benefit from TAVI over medical management or conventional surgical aortic valve replacement are lacking. The PARTNER randomised controlled trial of high-risk severe aortic stenosis patients, demonstrated improved survival with TAVI, but 43% of patients had still died within 2 years of intervention compared to 68% with standard medical care. The stroke rate of 13.8% in the TAVI cohort was also more than double that of medically managed patients,^{27,192-194} although rates are falling as procedural techniques improve.¹⁹⁵ TAVI as an intervention may therefore have population level survival benefits over medical management, but the severe aortic stenosis population is heterogeneous and individual risk is likely to vary greatly.

Mortality prediction using traditional risk assessment tools such as the STS mortality score and logistic EuroSCORE was commonly reported amongst the reviewed papers. It is possible to directly compare these figures to observed early (≤ 30 days) mortality in six of the included studies (Table 5.7). This comparison highlights the poor correlation of predictive scores with actual outcomes in this population, which is perhaps unsurprising given these tools were developed in younger cohorts excluding TAVI. Others have also identified the weakness of existing risk scores.^{80,172} It is noteworthy that these predictive algorithms only provide prognostic estimates for early surgical outcomes, which may not be the most important endpoint after TAVI. In such complex older patients approaching the end of life, quality of life after intervention may be more important than survival or avoidance of procedural complications. A systematic review by Kim *et al* of function and quality of life after TAVI reported mixed patient outcomes, with improvements in physical function amongst survivors not matched by changes in psychological and general health measures.⁶⁸

Table 5.7 – Surgical risk prediction and observed mortality in included studies.

Author, year	Predicted STS Operative Mortality* (%)	Predicted Logistic EuroSCORE 30-day mortality (%)	Observed 30-day mortality (%)
Ewe, 2010 ¹⁸²	NR	21.8	6.8
Stortecky, 2012 ¹²⁰	6.3	25.8	8.0
Rodes-Cabau, 2012 ¹⁸³	9.8	NR	10.6
Puls, 2014 ¹⁸⁶	7.3	26.0	11.3
Debonnaire, 2015 ¹⁸⁹	16.6	18.3	5.7
Green, 2015 ^{†190}	11.3	NR	10.0

*STS operative mortality refers to the predicted risk of death within 30 days of a procedure or index hospitalisation episode (where death may occur after 30 days).

†Matched predicted and observed data only available for frail individuals within this study.

NR= not reported

Frailty has gained traction within surgical and cardiovascular literature as a potential metric for the currently unmeasured risk of older patients undergoing complex interventions.¹⁷³⁻¹⁷⁶ Whilst this may be seen as positive for the holistic care of older patients, there is wide variation in definitions and measurement. In this review, the six studies that sought to objectively measure frailty each used different tools, varying from functional scales to composite scores including nutrition, cognition and mobility. Many of these assessments are described as measures of frailty, but are distinct from the consensus definition by Morley *et al.*¹⁰⁵ However, several of these concepts are correlated with the frailty construct and clearly have some predictive value. In the absence of trial data with randomisation based upon frailty, it is not possible to infer which elements of these measures will carry the most prognostic weight. However, it is notable that all the tools used included some estimation of participation in activities of daily living. It is possible that such measures are particularly sensitive to procedural risk in severe aortic stenosis populations, as impairments may reflect established heart failure at the time of consideration for TAVI.

There remains no consensus on the optimum approach to frailty assessment. The majority of studies included in this review considered frailty as a dichotomised variable for the purpose of outcome analysis. This reflects the phenotypic model of frailty and is perhaps attractive as a simple clinical concept.¹⁰⁷ However, forcing a continuous variable into a binary form limits the consideration of a 'pre-frail' status, and may be open to criticism for the potentially arbitrary nature of the threshold used to define frailty. Dichotomous phenotypic frailty assessment may also suffer from saturation amongst the highest risk populations and therefore provide limited discrimination compared to an index of deficits.¹⁹⁶ A formal Frailty Index, such as that first described by Rockwood *et al*¹¹⁰ is an alternative frailty construct, and may better reflect the accumulation of markers of frailty over time, although deficits are rarely amenable to dynamic change or reversal. Therefore, whilst a potentially useful marker of risk at a single timepoint, such indices are not capable of evaluating interventions to improve frailty which may be of interest in TAVI populations. Three of the included studies

do present some outcome data per unit change in the chosen frailty index, but given the differences in the structure of these scales meta-estimation of a combined effect size was not possible or logical.

Although the included studies comprise 4,592 patients undergoing TAVI, there are even larger published population registries in America, the United Kingdom, France, Germany, Italy and Belgium. Unfortunately, there is currently no systematic measurement of frailty within any of these cohorts of consecutive patients.^{42,197-200} It is likely that these registries will be used to produce future TAVI-specific surgical risk assessment tools similar to STS and EuroSCORE, and therefore inclusion of frailty measurement would provide a valuable opportunity to test effectiveness in large populations. It is important that future work looks beyond the prediction of mortality after TAVI to assess the impact of the procedure on symptoms and quality of life amongst frail individuals.

5.5.1 LIMITATIONS

Several limitations of our review should be considered. First, there are no studies randomised by frailty status, and so it is likely that patient selection in the observational cohort studies included in our meta-analysis was already influenced by underlying and unmeasured frailty. This is inevitable given the nature of TAVI as a treatment reserved for high-risk aortic stenosis patients requiring valve replacement. Whilst this selection bias may limit interpretation of frailty measurement in a broader aortic stenosis population, the results are representative of real-world TAVI cohorts. Studies evaluating frailty and outcomes in patients referred for TAVI, but in whom the procedure was felt too high risk by their multidisciplinary team, would be informative but to our knowledge, no such studies have been reported.

Second, we have only included studies where frailty was defined by the researchers. It is possible that other data exist including similar measurements without specific use of the term frailty. However, such studies would be less likely to report outcomes directly related to these measures without acknowledging the concept of frailty. Combining these disparate tools into meta-estimates may be challenged as the constructs described as frailty are heterogeneous. However, subgroup analyses of similar tools demonstrated low heterogeneity and support a predictive effect of frailty. Third, the meta-estimate for early mortality is based on a small number of studies, without adjustment for potential confounders. We were limited by the infrequent reporting of standardised VARC complications in relation to frailty status and these interpretations are open to competing risk bias. Therefore, whilst the observations of the effect of frailty on early outcomes are important, further work is required in this area. It is in this light that the addition of objective frailty measures to ongoing large TAVI registries would be helpful.

5.6 CONCLUSIONS

We demonstrate that frailty is associated with poorer early and late outcomes in TAVI patients. Objective frailty tools identify an even more vulnerable population at greater than double the late mortality risk of non-frail patients. There is currently a lack of consistency in frailty measures and clarity in reporting against standardised early VARC outcomes. Given the ongoing uncertainty in appropriate patient selection for TAVI, randomised controlled trials should consider including patients based on an objective assessment of frailty status.

CHAPTER 6

FRAILTY MEASURES AND SURGICAL RISK SCORES IN OLDER PATIENTS WITH AORTIC STENOSIS

CHAPTER 6: FRAILITY MEASURES AND SURGICAL RISK SCORES IN OLDER PATIENTS WITH AORTIC STENOSIS

6.1 OVERVIEW

Patient selection for aortic valve replacement is challenging in an ageing population.

Conventional surgical risk estimates are based on important cardiac status, comorbidity and age, but do not include frailty. This chapter examines the relationship between measures of frailty, quality of life and surgical risk in older patients with moderate-severe aortic stenosis.

Across two cohorts, 185 patients (mean age 80 ± 8 years, 51% female) with aortic stenosis were included (mean AVmax 4.2 ± 0.5 m/s). Frailty was measured using the Fried phenotype, the Short Physical Performance Battery (SPPB), Edmonton Frail Scale (EFS) and Clinical Frailty Scale (CFS). Surgical risk estimates were calculated from the online Society of Thoracic Surgeons (STS) operative risk and EuroSCORE II algorithms using clinical, echocardiographic, angiographic and laboratory data. Quality of life measures were taken from the Short Form (SF-12) questionnaire composite physical and mental component scores.

Frailty assessment using the four tools was feasible and rapidly performed in all patients. Frailty was identified in 27-53% of the study population, varying with the measure used: 70 (38%) by Fried assessment, 98 (53%) by SPPB, 50 (27%) by EFS and 52 (28%) using the CFS. Agreement between these measures was moderate by Cohen's kappa (range 0.33–0.53 with strongest agreement between Fried and SPPB –kappa 0.53, 95% confidence interval [CI] 0.41–0.65). Mean STS and EuroSCORE risk estimates increased with frailty between not frail, pre-frail and frail subgroups ($p < 0.05$ using all frailty tools). However, principal component analysis without defined frailty thresholds demonstrated a divergence between frailty tools and surgical risk scores. Quality of life indicators declined in patients with frailty and higher surgical risk.

In a population of older patients with moderate to severe aortic stenosis, frailty assessments are feasible and show moderate agreement. While frail patients have higher surgical risk scores, deeper analysis suggests differences in the information captured by frailty measures and both the STS and EuroSCORE. Evaluation including outcomes after surgical intervention is now needed to understand if these frailty measures hold value beyond conventional risk calculators in the assessment of patients with aortic stenosis for intervention.

6.2 INTRODUCTION

Aortic stenosis is a progressive degenerative condition that affects up to 12% of older adults.¹ In the absence of disease modifying therapy, the mainstay of treatment in those who develop symptomatic, severe aortic stenosis remains valve replacement, either through conventional cardiac surgery or transcatheter approaches. These procedures carry significant surgical risk. In an increasingly aged and frailer population, patient selection is complex. Surgical risk tools may assist in this process, using large database records to derive risk models that predict poorer outcomes in the perioperative period. Such outputs may guide clinician or patient preference for intervention and support in the delivery of individualised management plans for patients with severe aortic stenosis.

The Society of Thoracic Surgeons (STS) risk calculator is based on 67,292 procedures performed between 2002 and 2006.⁷³ The covariates include age, gender, ethnicity, body mass index, comorbidities and preoperative cardiac status. Similarly, the EuroSCORE II tool is based on 6,753 consecutive procedures across 154 surgical units in 2010.⁷⁴ However, these risk calculators do not include measures of frailty or disability, which may plausibly affect the risk of complications and recovery from major cardiac interventions. Furthermore, these models were developed using outcomes from conventional cardiac surgery, but are frequently applied to judge the risk of harm from Transcatheter Aortic Valve Implantation (TAVI).²⁰¹ The population of patients undergoing TAVI is generally older and with greater comorbidity²⁰², resulting in a need to update risk tools specifically for this population. In an older population approaching the end of life, survival metrics may be less valuable than quality of life, although this is challenging to objectively measure.

Frailty is increasingly recognised as a marker of surgical risk^{203,204} and functions as a measure of biological rather than chronological age. In patients undergoing TAVI, frailty is associated with a greater than doubling of early mortality after the procedure²⁰⁵, but the interaction with conventional surgical risk estimates is not clear. Numerous frailty tools have

been developed²⁰⁶ since Fried *et al.* first described the physical frailty phenotype in 2001.¹⁰⁷

The Fried criteria describes frailty in the presence of any three of five possible traits: weakness, slowness, reduced physical activity, exhaustion and unintentional weight loss. However, other tools such as the Short Physical Performance Battery (SPPB) is a more specific measure of physical performance, while the Edmonton Frail Scale (EFS) and Clinical Frailty Scale (CFS) include disability and a recognition of cognitive impairment.

The aim of this study is to evaluate the feasibility and agreement of frailty tools in older patients with severe aortic stenosis, and how these measures compare with quality of life estimates and surgical risk scores.

6.3 METHODS

We evaluated frailty tools and surgical risk scores in two cohorts: a longitudinal observational cohort study of older patients with asymptomatic moderate-severe aortic stenosis and a quality improvement study register of patients referred to the Scottish TAVI assessment clinic (Royal Infirmary of Edinburgh, Edinburgh, Scotland). Four tools were chosen based on likely acceptability to patients, speed of measurement to be feasible within a busy clinic environment, and the use of minimal specialist equipment. Written informed consent was obtained from all participants in the observational cohort and the study protocol was reviewed and approved by the local research ethics committee (SE/14/SS/1110). The quality improvement study protocol was also reviewed by the Scientific Officer for the local research ethics committee who provided approval for collection and use of data. The study was registered on the local quality improvement register and performed with the full cooperation and support of clinical staff in the TAVI assessment clinic.

6.3.1 PARTICIPANT SELECTION

Participants were included if they had evidence of moderate-severe aortic stenosis, defined by echocardiographic evidence of a maximum velocity across the aortic valve (AVmax) of greater than 3.5m/s or an aortic valve area less than 1.0cm². There were no exclusion criteria to ensure assessment of a broad and generalisable cohort.

6.3.2 FRAILITY ASSESSMENT

Four frailty measures were undertaken in the same manner using identical equipment in all study participants: the Fried frailty phenotype, SPPB, EFS and CFS. Detailed methodology for each measure is provided in Chapter 2. All measures were performed by AA, with the exception of the CFS which was rated by an independent nurse who assessed each participant. Fried frailty was considered present if 3 or more of the five possible phenotype traits was detected.¹⁰⁷ For selected analyses a pre-frailty label was applied where 1 or 2 traits

were present. For the SPPB, a lower score out of a maximum of 12 indicates greater frailty; a frailty cutoff of ≤ 5 was applied in keeping with previously described use amongst cardiac populations.¹²³ An intermediate pre-frail group was assigned for scores of 6-9 with scores ≥ 10 indicating no frailty. The EFS is scored out of 17 points and frailty was assigned at ≥ 8 points. Pre-frailty was determined as 6-7 points and lower scores indicated no frailty.¹³³ The CFS uses a 9-point scale where scores ≥ 5 indicate frailty. Pre-frailty or vulnerability was indicated at 4 points with lower scores denoting no frailty.¹¹⁰

6.3.3 QUALITY OF LIFE ASSESSMENT

Quality of life was assessed using the Short Form (SF-12) questionnaire as detailed in Chapter 2.¹³⁶ Summary estimates for the Physical Component Score (PCS) and Mental Component Score (MCS) were used in all cases, with a lower value indicating greater impairment.

6.3.4 SURGICAL RISK CALCULATORS

The STS operative mortality risk for isolated Aortic Valve Replacement (AVR) surgery was calculated in all patients using its online calculator (<http://riskcalc.sts.org/stswebriskcalc/#/>). This estimates the percentage risk of death within the episode of continuous hospital care including AVR surgery, or within 30 days for patients discharged from hospital earlier. The EuroSCORE II risk was also calculated using its online calculator (<http://www.euroscore.org/calc.html>) and similarly estimates the risk of death within the index hospitalisation. Data required for completion of these calculations was taken from electronic patient records, echocardiography, angiography and laboratory databases.

6.3.5 STATISTICAL ANALYSIS

Statistical analysis was performed using the statistical software R version 3.3.3 (<http://www.r-project.org>). Continuous variables are presented as mean (standard deviation)

and categorical variables as absolute numbers (percentage). Agreement between frailty measures was assessed by Cohen's Kappa, using the dichotomised frailty thresholds described above for each test. STS and EuroSCORE were compared by Bland-Altman plot. Baseline characteristics, mean EuroSCORE and STS risk scores were compared across frailty groups (not frail, pre-frail and frail) by ANOVA. In the absence of a true gold standard frailty measure, principal component analysis was used to reduce the dimensionality of frailty measures and explore the relationship with surgical risk score values. Principal component analysis uses orthogonal transformation to produce variables that account for as much of the variance in the dataset as possible; in the analysis performed sufficient variance was explained by two principal components, so allowing the original individual patient data to be plotted as points on a biplot of these transformed components. To allow comparison of frailty and surgical risk scores, arrows that best summarise the direction of these scores on the transformed scale were plotted using the *ggbiplot* R package.

In a further analysis, a principal component biplot for the four frailty measures alone was created with the study sample divided at an intermediate surgical risk threshold of STS and EuroSCORE estimated operative mortality $\geq 4\%$. In these analyses, SPPB scores were reversed to ensure directionality of the frailty measures was consistent (i.e. higher scores indicating greater frailty). Quality of life composite measures were assessed in those with and without frailty by each measure, and in those above and below the median STS and EuroSCORE risk. Comparisons were made by unpaired two-sided t-test. Absolute p-values are reported at 0.001 and greater, and $p < 0.05$ was considered statistically significant.

6.4 RESULTS

A total of 185 patients with aortic stenosis were included in the study. This comprised 80 subjects from the longitudinal cohort study and 105 from the TAVI assessment clinic.

Baseline characteristics are summarised in Table 6.1. The study sample was representative of an older population (mean age 80 ± 8 years, 51% female) with moderate-severe aortic stenosis (mean AVmax 4.2 ± 0.5 m/s). Frailty was present in 27-53% of the cohort according to the measure used: Fried frailty was determined in 70 (38%), SBBP in 98 (53%), EFS in 50 (27%) and CFS in 52 (28%) participants. Pre-frailty was present in 23-47%, varying from 87 (47%) participants by Fried, 65 (35%) by SPPB, 43 (23%) by EFS and 52 (28%) by CFS. Using the Fried phenotype (Table 6.1), pre-frail and frail patients were more likely to be female, suffer heart failure and chronic kidney disease than those without any frailty markers. However, no relationship was observed between frailty status and age, severity of aortic valve disease or other comorbidity.

Table 6.1 – Baseline characteristics across all patients and stratified by Fried frailty status

	All (n=185)	Fried Frailty Status			p-value
		Frail (n=70)	Pre-frail (n=87)	Not Frail (n=28)	
Age, years	79.8 ± 7.5	80.5 ± 7.9	80.1 ± 7.1	76.8 ± 7.2	0.07
Females	95 (51)	45 (64)	44 (51)	6 (21)	<0.001
AV _{max} , m/s	4.2 ± 0.5	4.4 ± 0.6	4.2 ± 0.4	4.3 ± 0.7	0.26
<i>Comorbidity</i>					
Myocardial infarction	22 (12)	9 (13)	10 (11)	3 (11)	0.90
Heart failure	21 (11)	14 (20)	7 (8)	0 (0)	0.04
Stroke	22 (12)	8 (11)	14 (16)	0 (0)	0.13
Diabetes mellitus	35 (19)	16 (23)	18 (21)	1 (4)	0.19
Hypertension	115 (62)	39 (56)	57 (66)	19 (68)	0.55
Atrial fibrillation	46 (25)	19 (27)	22 (25)	5 (18)	0.80
CKD	37 (20)	23 (33)	14 (16)	0 (0)	0.005
COPD	25 (14)	16 (23)	8 (9)	1 (4)	0.08
Osteoarthritis	81 (44)	44 (47)	28 (32)	9 (32)	0.57
Depression	15 (8)	8 (11)	6 (7)	1 (4)	0.59
<i>Frailty Scores</i>					
Fried	2.1 ± 1.4	3.6 ± 0.7	1.5 ± 0.5	0	–
SPPB	5.6 ± 3.2	3.1 ± 2.0	6.5 ± 2.8	9.2 ± 1.7	<0.001
EFS	5.7 ± 3.0	7.9 ± 2.6	4.8 ± 2.3	2.9 ± 2.2	<0.001
CFS	3.6 ± 1.4	4.6 ± 0.9	3.2 ± 1.3	2.0 ± 1.1	<0.001
<i>SF-12 Quality of Life</i>					
Physical Component	34.2 ± 10.2	28.3 ± 8.4	36.0 ± 9.2	43.1 ± 9.2	<0.001
Mental Component	50.1 ± 9.9	46.7 ± 10.8	50.9 ± 9.0	56.3 ± 6.2	<0.001
<i>Surgical Risk Scores</i>					
STS Operative Risk	2.9 ± 1.8	3.5 ± 2.0	2.8 ± 1.7	1.8 ± 1.3	<0.001
EuroSCORE II (%)	4.0 ± 3.7	5.0 ± 4.3	3.5 ± 2.9	2.7 ± 3.5	0.006

Values are mean ± SD or number (%).

P-values presented for differences between the frail, pre-frail and not frail groups by

ANOVA for normally distributed continuous data and chi-squared test for categorical data.

AV_{max} = maximal velocity across the aortic valve determined by echocardiography; COPD =

chronic obstructive pulmonary disease; CKD = chronic kidney disease; SPPB = Short

Physical Performance Battery; EFS = Edmonton Frail Scale; CFS = Clinical Frailty Scale;

STS = Society of Thoracic Surgeons.

6.4.1 AGREEMENT BETWEEN FRAILTY ASSESSMENTS

Agreement between measures for the allocation of frailty reached moderate agreement between each tested pair, except for the relationship between the SPPB and EFS where only fair agreement was observed (Table 6.2). The strongest association was noted between the Fried and SPPB tools (Cohen's kappa 0.53, 95% confidence interval [CI] 0.41–0.65).

Table 6.2 – Agreement between frailty scores by Cohen’s Kappa

	Fried	SPPB	EFS	CFS
Fried		0.53 (0.41–0.65)	0.46 (0.33–0.60)	0.49 (0.36–0.63)
SPPB			0.33 (0.19–0.46)	0.47 (0.35–0.60)
EFS				0.43 (0.28–0.58)
CFS				

Values are Cohen’s Kappa statistics (95% CI) for the agreement between frailty measures using dichotomised frailty thresholds (frail vs not frail or pre-frail).

SPPB = Short Physical Performance Battery; EFS = Edmonton Frail Scale; CFS = Clinical Frailty Scale

6.4.2 AGREEMENT BETWEEN SURGICAL RISK SCORES

Agreement between the estimated operative risk by STS and EuroSCORE was strong ($r=0.66$, 95% CI 0.57–0.73, $p<0.001$). However, at higher predicted mortality, EuroSCORE estimates appeared greater than for the equivalent STS calculation (Figure 6.1).

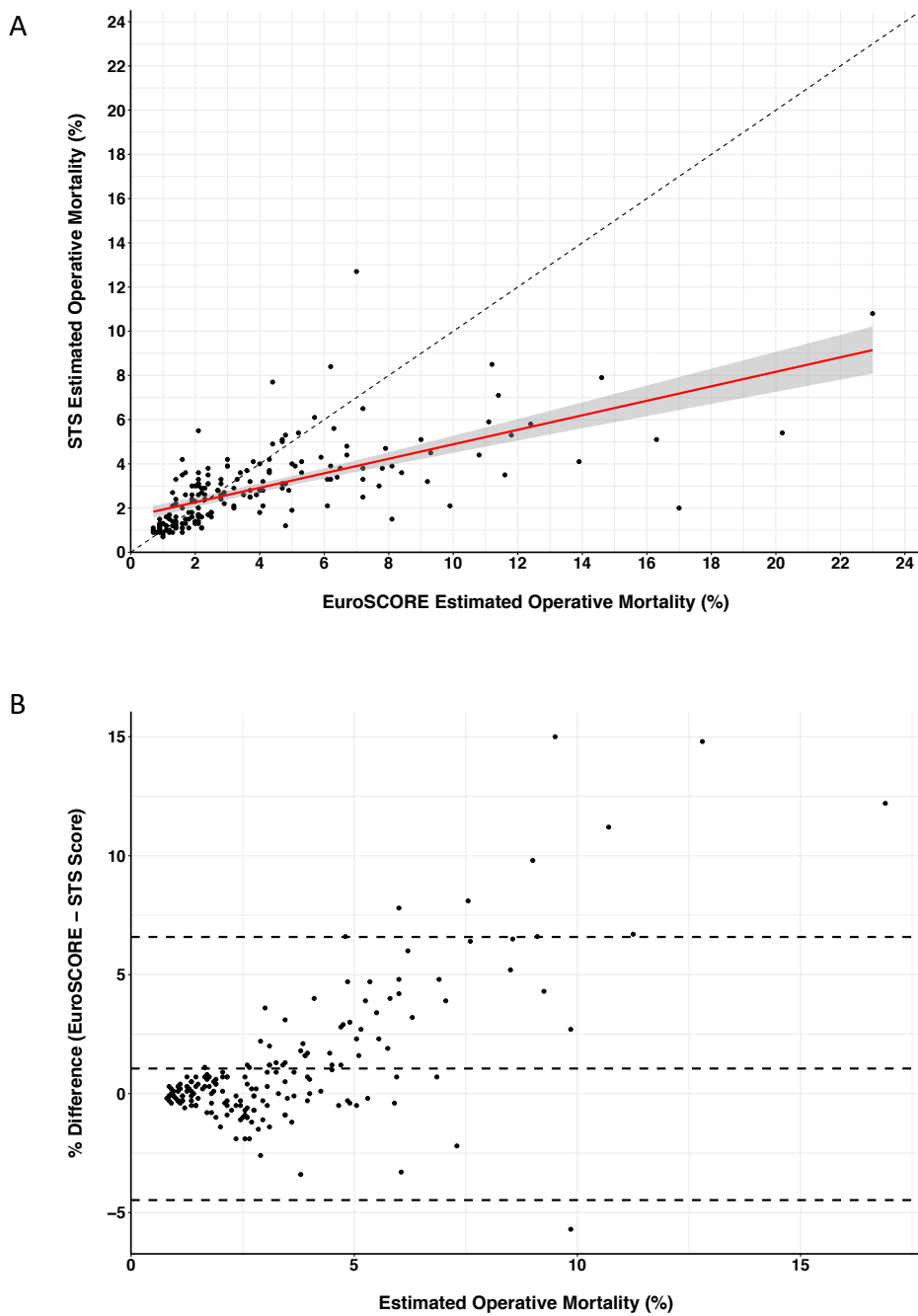


Figure 6.1 – Relationship between EuroSCORE and STS estimated operative risk. (A) Linear regression line shown with the shaded area representing 95% confidence intervals. The dotted black line demonstrates where complete agreement between the measures would lie. (B) Bland-Altman plot for the difference between these surgical risk scores.

6.4.3 AGREEMENT BETWEEN FRAILTY AND SURGICAL RISK SCORES

STS and EuroSCORE estimated operative mortality risk increased significantly with increasing frailty status using all four tools (Table 6.3). The most marked separation of risk estimates was observed using the Fried phenotype (Figure 6.2). Principal component analysis showed the four frailty measures and two surgical risk scores could be successfully reduced to a two component plot which explained 78% of the variance in measurement. In this reduced dimensional state, all four frailty measures acted in a similar direction, but the two surgical risk estimates were divergent with near perpendicular separation (Figure 6.3). A separate biplot using only the four frailty tools explained 83% of the variance in measurement (Figure 6.4). Within this plot, the closest directional agreement was observed between the three frailty tools using physical measures (Fried, SPPB and EFS) with some separation from the CFS. Further division by operative risk for those with high ($\geq 4\%$) and low ($< 4\%$) STS score (Figure 6.4a) and EuroSCORE (Figure 6.4b) demonstrated extensively overlapping summary estimates on the frailty biplot, and therefore no clear relationship between frailty and these surgical risk scores.

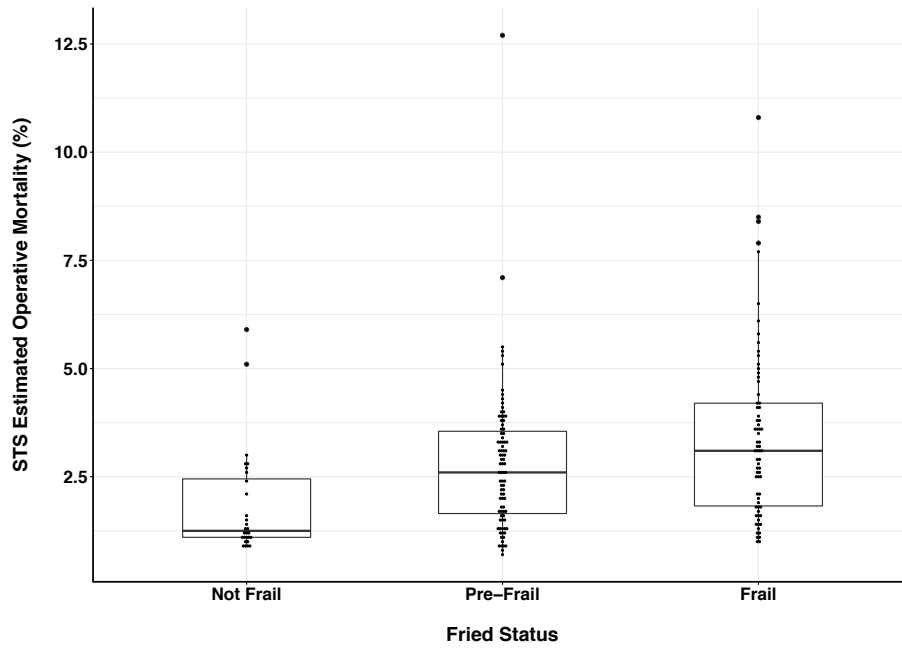
Table 6.3 – Mean STS and EuroSCORE estimated operative risks by frailty assessment and status

	Not Frail	Pre-frail	Frail	p-value
<i>STS Operative Risk</i>				
Fried	1.8 ± 1.2	2.8 ± 1.7	3.5 ± 2.0	<0.001
SPPB	2.3 ± 1.6	2.3 ± 1.3	3.5 ± 2.0	<0.001
EFS	2.5 ± 1.7	3.2 ± 1.6	3.4 ± 2.0	0.007
CFS	2.3 ± 1.3	2.6 ± 1.4	4.1 ± 2.3	<0.001
<i>EuroSCORE II</i>				
Fried	2.7 ± 3.5	3.5 ± 2.9	5.0 ± 4.3	0.006
SPPB	3.2 ± 3.1	3.2 ± 3.4	4.6 ± 3.9	0.04
EFS	3.2 ± 3.3	4.3 ± 3.1	5.1 ± 4.5	0.009
CFS	2.9 ± 2.7	3.8 ± 3.4	5.7 ± 4.5	<0.001

Values are mean ± standard deviation of STS and EuroSCORE II operative risk (%). P-value represents the ANOVA for difference in means across frailty groups.

SPPB = Short Physical Performance Battery; EFS = Edmonton Frail Scale; CFS = Clinical Frailty Scale.

A



B

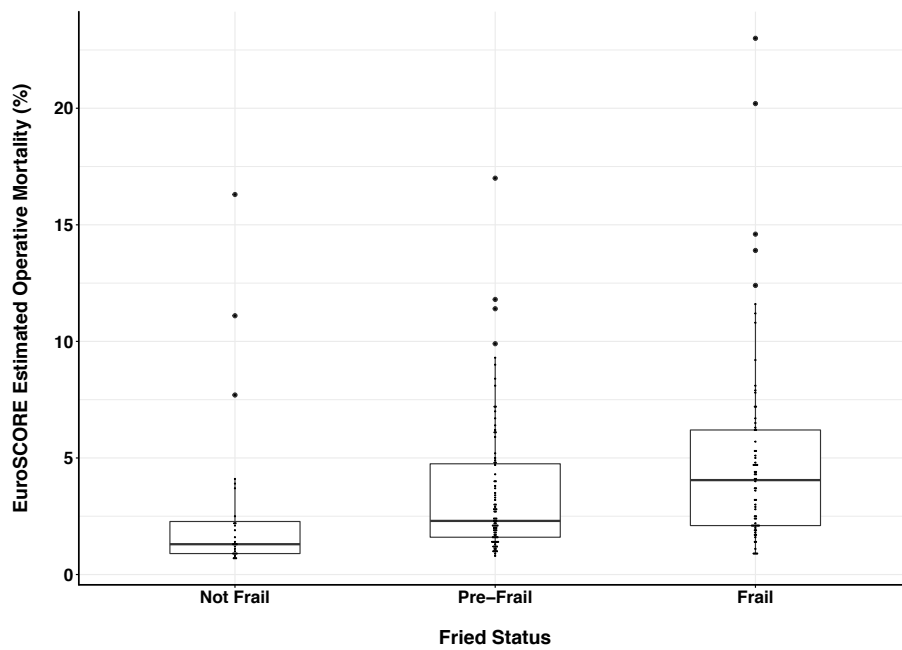


Figure 6.2 – Box and whisker plots for the relationship between Fried frailty status and surgical risk scores. Box represents median and interquartile range for risk estimates using the STS score (A) and EuroSCORE (B). By non-parametric ANOVA testing, median STS ($p < 0.001$) and EuroSCORE ($p < 0.004$) values are significantly greater as frailty increases.

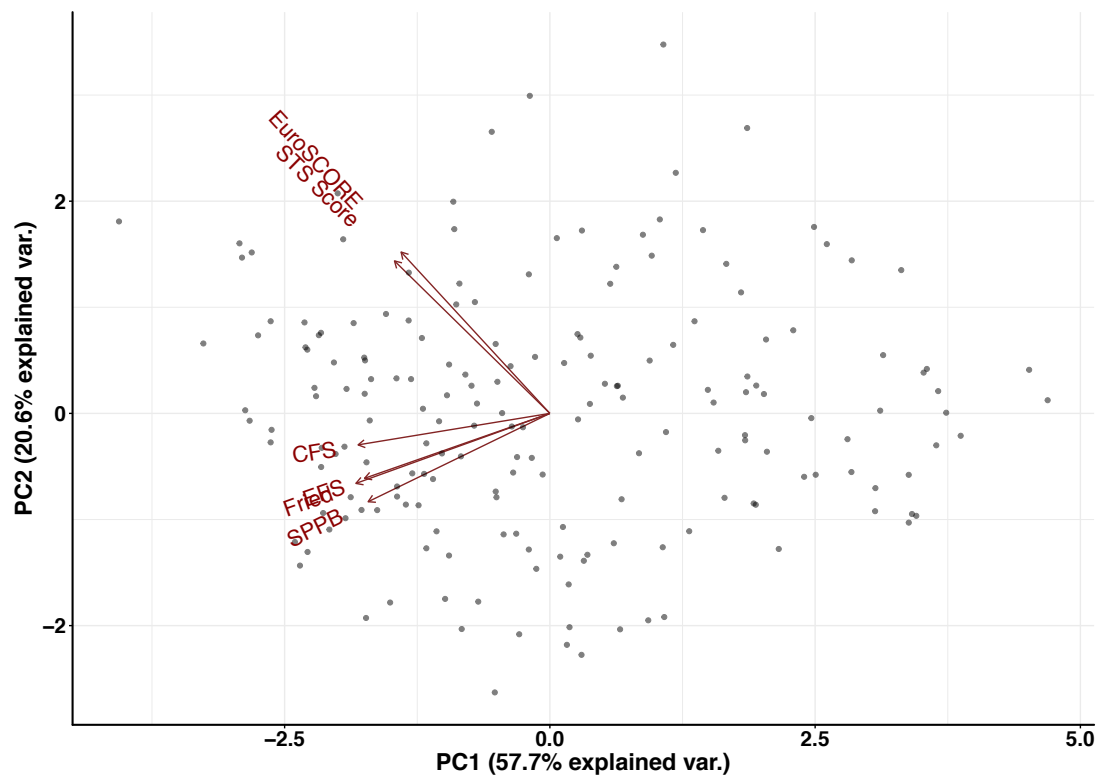


Figure 6.3 – Principal component analysis biplot for frailty and surgical risk scores. The plots demonstrate similarity in frailty measures (arrows showing the direction of variables are similar), but divergence with the STS and EuroSCORE.

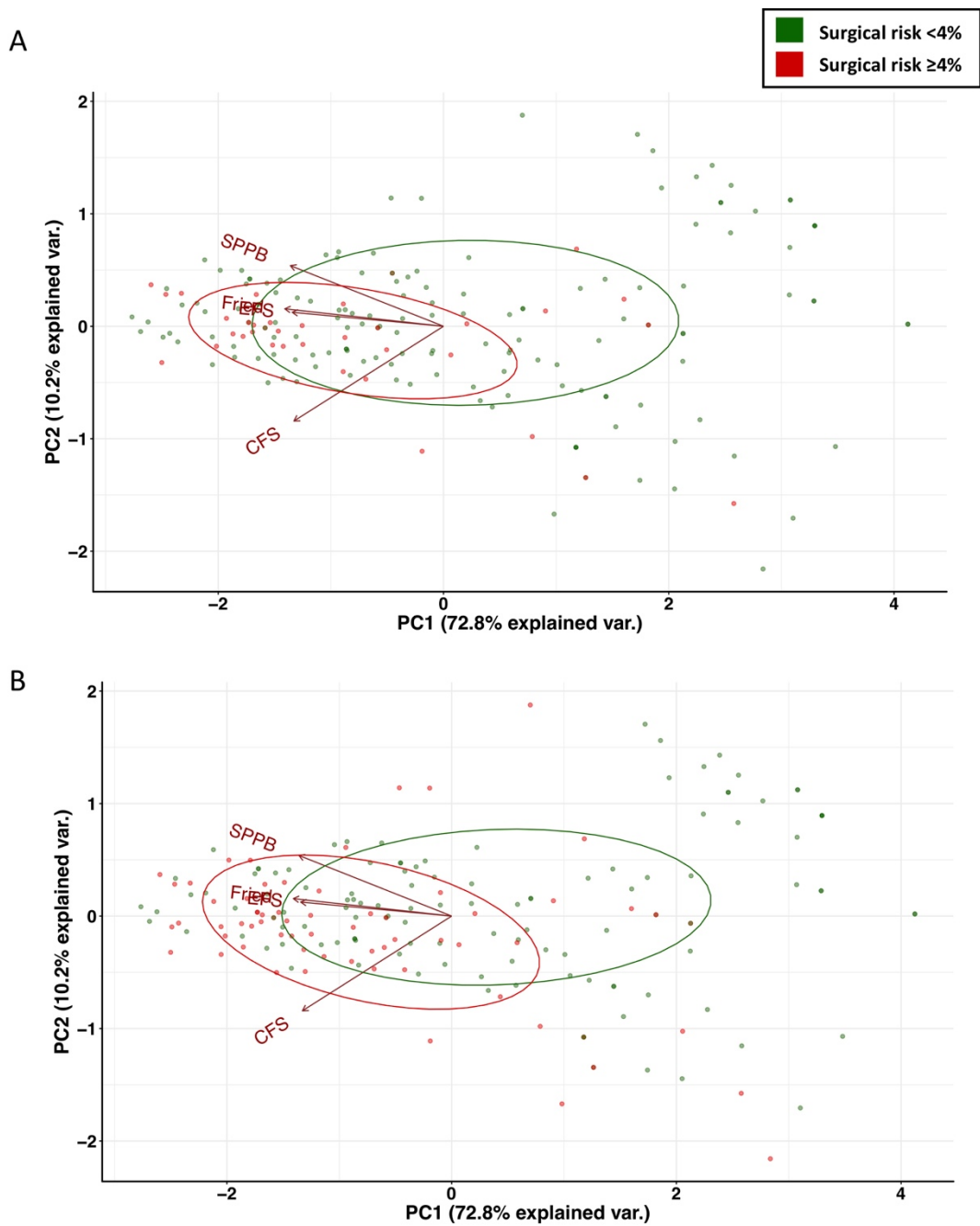


Figure 6.4 – Principal component analysis plots for frailty separated by surgical risk score. The plots demonstrate similarity in frailty measures (arrows showing the direction of variables are similar), but failure of separation of those with high ($\geq 4\%$) and low ($< 4\%$) estimated surgical risk by STS score (A) and EuroSCORE (B).

6.4.4 RELATIONSHIP WITH QUALITY OF LIFE

Both frailty and surgical risk tools demonstrated lower physical component quality of life scores in those with greater frailty or estimated surgical risk (Table 6.4). Frailty determined by Fried, SPPB and EFS tools identified patients with lower mental component scores, where no relationship was observed using the surgical risk calculators.

Table 6.4 – SF-12 physical and mental component scores

		PCS	p-value	MCS	p-value
Fried	Frail	28.3 ± 8.4	<0.001	46.7 ± 10.8	<0.001
	Not frail	37.7 ± 9.6		52.2 ± 8.7	
SPPB	Frail	29.8 ± 8.6	<0.001	48.2 ± 10.2	0.004
	Not frail	39.1 ± 9.8		52.3 ± 9.1	
EFS	Frail	26.6 ± 7.1	<0.001	43.0 ± 10.4	<0.001
	Not frail	37.0 ± 9.8		52.7 ± 8.3	
CFS	Frail	28.5 ± 8.6	<0.001	48.0 ± 11.5	0.09
	Not frail	36.4 ± 10.0		51.0 ± 9.1	
STS Score	Above median	32.5 ± 9.6	0.02	50.6 ± 9.6	0.50
	Below median	35.9 ± 10.6		49.6 ± 10.2	
EuroSCORE	Above median	31.4 ± 9.3	<0.001	50.7 ± 9.8	0.42
	Below median	37.0 ± 10.4		49.5 ± 10.0	

Values are mean ± standard deviation of Physical Component Score (PCS) and Mental Component Score (MCS) from the SF-12 questionnaire. P-value determined by unpaired two-sided T-test. The not frail group includes pre-frail patients.

SPPB = Short Physical Performance Battery; EFS = Edmonton Frail Scale; CFS = Clinical Frailty Scale.

6.5 DISCUSSION

This study has assessed frailty, surgical risk scores and quality of life in older patients with moderate-severe aortic stenosis and produced several relevant observations. First, four different frailty tools demonstrated moderate agreement in the determination of frailty, although prevalence varied from a quarter to half of the study population depending on the tool used. Second, frailty was unrelated to the age of patients or severity of aortic valve disease. Third, frailty assessment identified patients with poorer physical and mental wellbeing. Fourth, the group of patients identified as frail by fixed thresholds were observed to have higher surgical risk by STS and EuroSCORE estimates. However, considering frailty as a continuous variable showed separation from surgical risk estimates by principal component analysis. Future studies including outcomes after aortic valve replacement are required to establish if this observed divergence between frailty and existing surgical risk estimates is meaningful for the improved prediction of outcomes after surgery.

The disparate number of frailty tools makes research in this area challenging. We focussed on four measures that could be rapidly performed with potential for inclusion in busy clinical environments. Indeed, evaluation of all four frailty scores was achievable within 10 minutes per patient. The physical frailty phenotype described by Fried *et al.* was included given the extensive validity for risk estimation in a diverse range of surgical settings.^{119,207,208} However, this measurement includes use of a hand-grip dynamometer which may limit adoption into widespread practice. Further, the validity of the self-reported exhaustion component of the Fried phenotype has been questioned.²⁰⁹

There was variation in the numbers identified as frail using each tool. The SPPB, as the most physically demanding test identified over half of patients as frail, which may reflect the degree of physical limitation incurred in patients with advanced aortic valve disease. Indeed, physical component scores from the SF-12 questionnaire were low across the study

population, with 96% of aortic stenosis patients demonstrating composite physical scores lower than the median value for a UK population.²¹⁰ In contrast, the impairment in mental component scores was less marked, but still meaningfully lower in those with physical frailty and aortic stenosis.

The variation in numbers identified as frail is likely the result of forced dichotomisation using thresholds that have not been validated *between* tools. The agreement between frailty measures and common variable direction observed by principal component analysis supports a class effect and shows the value of considering frailty as a continuous marker. This shared effect appears distinct from the STS and EuroSCORE prediction, even though at a superficial level, increasing surgical risk is observed in those with frailty across all measures. Dichotomisation into a frail state using potentially arbitrary thresholds is frequently desirable by clinicians, but may limit the value of the holistic frailty assessment undertaken. Systematic reviews of outcome prediction in this area have demonstrated limited standardisation of frailty measures, failure to test frailty inclusion within the STS or EuroSCORE models and a lack of calibration testing.²¹¹⁻²¹³ All patients exist on a spectrum of frailty and meaningful outcome prediction should be undertaken through continuous modelling including STS and EuroSCORE estimates to test for the additive effect of frailty.

The data collected also provided an opportunity to assess the agreement of STS and EuroSCORE risk estimates in an older population with aortic stenosis. Whilst there was good correlation between these scores, EuroSCORE appeared to outweigh the STS estimate at higher risks. This is similar to findings from Kirmani *et al.* who described a divergence in the scores at >15% EuroSCORE operative mortality risk.²¹⁴ Others have noted the procedure-specific risk modelling on the STS platform results in a more refined prediction than the broader EuroSCORE model that groups all non-cardiac bypass grafting procedures together.²¹⁵

The strong relationship between frailty measures and quality of life indicators highlights an important consequence of living with frailty for older adults. This must not be overlooked in the assessment of a frailer patient for whom quality of life may take precedence over quantity. The challenge for a multidisciplinary heart team is to determine whether any impairment in physical and mental wellbeing is driven by symptomatic aortic stenosis with potential for reversibility following valve replacement. In such patients, accepting a ‘higher risk, higher reward’ strategy may be appropriate, but patients should be included in shared decision making. The SF-12 questionnaire provides some objectivity to the challenging assessment of general wellbeing in the research setting. However, results must be interpreted with some caution; the tool assumes physical and mental health scores are independent and uncorrelated when in reality an impairment in one domain is likely to affect the other.²¹⁶

6.5.1 LIMITATIONS

There are several limitations to this study. Without surgical outcomes, it is currently unclear if the observed differences between frailty measures and surgical risk estimates are meaningful. The cohort is also small in comparison to those that were used to derive surgical risk tools, but the patients included are thoroughly characterised and with multiple measures of frailty not usually collected in routine clinical care.

Despite the high prevalence of frailty, few patients were at very high surgical risk. In the original PARTNER trial comparing conservative medical management to TAVI, patients were only considered suitable for randomisation with estimated STS operative mortality $>10\%$.²⁷ Despite including 105 patients under direct assessment for TAVI, only 2 individuals in our cohort met this criterion. This is likely to reflect the changing risk profile of TAVI patients and increasing acceptance of the procedure for patients previously considered at intermediate risk. Indeed in the latest ESC guidelines, a decision for TAVI over conventional surgery is supported in those with STS or EuroSCORE II predicted operative mortality $\geq 4\%$.²¹⁷ This reflects randomised trial evidence suggesting non-inferiority for TAVI when

compared to conventional surgical AVR in lower risk groups.²¹⁸ The STS calculator estimate is frequently updated to include the latest surgical outcomes data, which continue to improve with time; one study demonstrated that 58% of patients determined as high-risk in 2008 would be reclassified as intermediate risk on the latest version of the calculator used in this study.²¹⁹ It is therefore likely that patients included in this study and assessed against the latest STS model would have higher predicted operative mortality in the era of the PARTNER trial.

6.6 CONCLUSIONS

In a population of older patients with moderate to severe aortic stenosis, frailty assessments are feasible and show moderate agreement. While frail patients have higher surgical risk scores, deeper analysis suggests differences in the information captured by frailty measures and both the STS and EuroSCORE. Increasing frailty is also associated with poorer physical and mental wellbeing in this patient group. Evaluation including outcomes after surgical intervention is now needed to understand if these frailty measures hold value beyond conventional risk calculators to improve prediction of outcomes for patients with aortic stenosis.

CHAPTER 7

CONCLUSIONS AND FUTURE DIRECTIONS

CHAPTER 7: CONCLUSIONS AND FUTURE DIRECTIONS

7.1 SUMMARY OF THESIS FINDINGS

Aortic stenosis is a condition that commonly affects older adults. In the absence of lifestyle or pharmacological methods of ameliorating unpredictable disease progression, complex cardiac intervention has remained the mainstay of treatment for over 50 years. Even in the age of less invasive transcatheter valve implantation, the risks of complications have steered guidelines against valve replacement unless necessitated by the development of symptoms of syncope or heart failure. This makes the accurate diagnosis of symptomatic aortic stenosis crucial to the management of these patients. However, in an increasingly aged population, the establishment of symptoms independent of the expression of multimorbidity is challenging. Furthermore, these comorbidities result in frailer candidates for surgery, where the balance between operative risks and functional benefit is less clearly discernible.

The core aims of this thesis were to first investigate the validity of novel blood biomarkers as candidate objective markers of disease progression in older patients with aortic stenosis. Second, in those considered for aortic valve replacement, this thesis aimed to assess frailty as a predictor of outcomes after surgery, and to compare this with existing risk assessment tools.

7.1.1 cMYC AS A NOVEL MARKER OF MYOCARDIAL INJURY AND FIBROSIS

It was hypothesised that cMyC as a novel biomarker of myocardial injury would predict progression of aortic stenosis and outcomes. To test this, cMyC was measured in stored serum from 265 patients with aortic stenosis and 46 healthy controls, across two cohorts designed to test mechanistic disease progression and outcomes.²²⁰ Rising cMyC concentrations were independently associated with increasing left ventricular mass and markers of myocardial fibrosis on MRI imaging after adjustment for age, sex, renal function, severity of aortic stenosis, cardiac troponin and comorbidity. There was also an unadjusted

association with all-cause mortality over 11 years of follow-up. These results add to previously conducted experiments with cardiac troponin and together suggest a role for the measurement of cardiac sarcomeric proteins in the objective assessment of the progression of disease in aortic stenosis.

However, it must be noted that measurement of cMyC only occurred at a single timepoint and therefore this study does not yet truly validate this test as a tracking biomarker of disease. Furthermore, the outcomes analysis was limited by sample size and was unpowered to demonstrate any independent effect of cMyC. These findings would be enhanced by more observations in patients with severe disease, in whom sufficient endpoint events would accumulate closer to the time of blood sampling.

7.1.2 VARIABILITY OF BLOOD BIOMARKERS IN OLDER PATIENTS WITH AORTIC STENOSIS

To use a blood biomarker monitoring strategy, clinicians would require confidence that changes in observed blood concentrations overcome natural biological variation in an individual and the limits of analytical variation of sample processing. It was hypothesised that three candidate biomarkers, namely cardiac troponin, BNP and galectin-3, would demonstrate satisfactory variability in older adults with aortic stenosis to make clinical monitoring by serial sampling feasible. To test this, 14 older subjects with stable moderate-severe aortic stenosis underwent repeated blood sampling at hourly and weekly intervals, with assay testing occurring in both fresh and frozen samples.

All biomarkers could be detected at all timepoints in every subject with a low index of individuality confirming the requirement for serial sampling. Fresh and frozen sampling was comparable for galectin-3 and cardiac troponin, providing some reassurance as to the validity of previous analyses of these biomarkers in stored samples from clinical trials. However, BNP proved unstable with significant degradation after a single freeze-thaw cycle; this biomarker only appears suitable for analysis in fresh blood.

Compared to other studies in healthy controls, analytical variation was lower for all three biomarkers, reflecting higher median concentrations in older patients with aortic stenosis. However, biological variation did not appear significantly different from findings in other cohorts, resulting in reference change values between serial samples for cardiac troponin and BNP (42% and 55% respectively) that were lower than in equivalent studies of healthy controls and patients with heart failure. Using these lower RCV thresholds in older patients with severe aortic stenosis could appropriately improve the sensitivity of testing for disease progression, by reducing the false negative rate for significant change between serial samples. However, it is important to acknowledge that these biomarkers still require validation as clinical tools and that the variability study only provides information on the statistical likelihood of serial measures being significantly different.

7.1.3 FRAILITY MEASUREMENT IDENTIFIES PATIENTS AT HIGH RISK AFTER TAVI

The hypothesis here was that pre-operative frailty would predict important patient outcomes including mortality after TAVI. This was investigated by systematic review and meta-analysis, which included ten relevant cohort studies of 4,592 patients who underwent TAVI with a prior formal assessment of frailty. Frailty was associated with increased early and late mortality after the procedure, particularly when identified using an objective measure such as determination of the frailty phenotype or deficit accumulation via a frailty index approach.²⁰⁵ Subjective or ‘end-of-the-bed’ assessment did identify mortality risk, but to a lesser extent. The importance of this finding is emphasised by guidelines suggesting that candidate patients for TAVI should have an expected life expectancy beyond one year after the procedure³; the meta-analysis suggests that 1 in 4 patients included in these studies died within that period. The latest European guidelines acknowledge the lack of evidence in this area, stating that:

“criteria for when TAVI should no longer be performed since it

would be futile need to be further defined.”²¹⁷

A further finding of this systematic review was a lack of reporting of non-mortality outcomes in relation to frailty. This could be addressed through the inclusion of frailty assessment within existing TAVI registries.

7.1.4 FRAILITY TOOLS AND DIVERGENCE WITH SURGICAL RISK SCORES

It was hypothesised that frailty measures would identify markers not captured by conventional surgical risk scores in an older population with aortic stenosis. This was tested in two cohorts totalling 185 patients with moderate-severe aortic stenosis, who underwent frailty testing to calculate individual Fried, SPPB, EFS and CFS scores. These tools proved feasible and quick to perform in this population. Using accepted dichotomised thresholds for frailty, agreement between these scores was moderate with frailty varying between 27–53% of the study population according to the tool used. Frail patients also had higher STS and EuroSCORE estimated operative risk from conventional aortic valve replacement, and lower physical and mental components scores on the SF-12 quality of life questionnaire. However, analysis of frailty as a continuous measure suggested divergence with surgical risk scores. An analysis including outcomes after valve replacement is required to fully determine any additional value of these frailty scores beyond STS and EuroSCORE estimates in the prediction of harmful outcomes after surgery.

Taken together these four chapters have assessed the emerging role for blood biomarkers and frailty assessments in older patients with aortic stenosis. Serum sarcomeric proteins offer potential to non-invasively track important markers of disease progression, by indirect measurement of myocyte necrosis and replacement fibrosis. Cardiac troponin, BNP and galectin-3 are quantifiable in an older aortic stenosis population and demonstrate a clear requirement for serial sampling to detect significant change above defined reference change values. Frailty is associated with early and late mortality after TAVI, particularly in those

who are identified using an objective tool. Some of this risk is already explained within STS and EuroSCORE risk estimates, but specific frailty measures may identify additional patient factors that merit further evaluation.

7.2 FUTURE DIRECTIONS

The findings of this thesis raise important potential uses of clinical biomarkers in older patients with aortic stenosis that now warrant further investigation. The following sections describe ongoing, planned or suggested future studies to better understand the role of non-invasive testing to improve decision-making in this patient group.

7.2.1 SERIAL BIOMARKER MEASURES IN AORTIC STENOSIS

Having observed the potential importance of serum sarcomeric proteins as markers of disease progression and demonstrated the need for serial testing, it is now crucial for prospective studies to assess this. I am leading a prospective observational cohort study entitled *Cardiac biOMarkers in older Patients with Aortic Stenosis (COMPASS)*, collecting serial blood samples from patients with asymptomatic moderate-severe aortic stenosis before any clinical decompensation (Figure 7.1).

A total of 80 patients are due to be recruited to this study, with the expectation that half will decompensate to the point of symptoms within 18 months. This is based on prior observational studies in this area.² The outcome measure will be a composite of hospitalisation with heart failure, elective or urgent aortic valve replacement for new symptoms of heart failure, cardiovascular death, or echocardiographic evidence of new left ventricular systolic impairment. Early symptomatic decline will also be assessed in 6 monthly measures of function using a 6 minute walk test, which is well validated as a measure in this patient population^{221,222}, and the Kansas City Cardiomyopathy Questionnaire.²²³

It is anticipated that increases in markers of myocardial necrosis, such as cardiac troponin or cMyC, will occur before left ventricular decompensation is evident clinically or detectable through changes in BNP. Significant change in serial testing will be determined by the

thresholds defined in Chapter 4 of this thesis. This study will prospectively determine whether biomarker assessment of the myocardial response to aortic stenosis could identify patients with functionally significant valve disease prior to the development of symptoms. The growing body of evidence relating to irreversibility of myocardial fibrosis has led to another ongoing trial randomising patients with asymptomatic aortic stenosis and myocardial fibrosis to early or late valve replacement ([clinicaltrials.gov NCT03094143](https://clinicaltrials.gov/ct2/show/study/NCT03094143)). Should this strategy prove effective in preserving post-surgery ventricular function, early blood biomarkers of the destructive path to fibrosis would be attractive screening tests for detailed cardiac MR imaging and early surgical review.

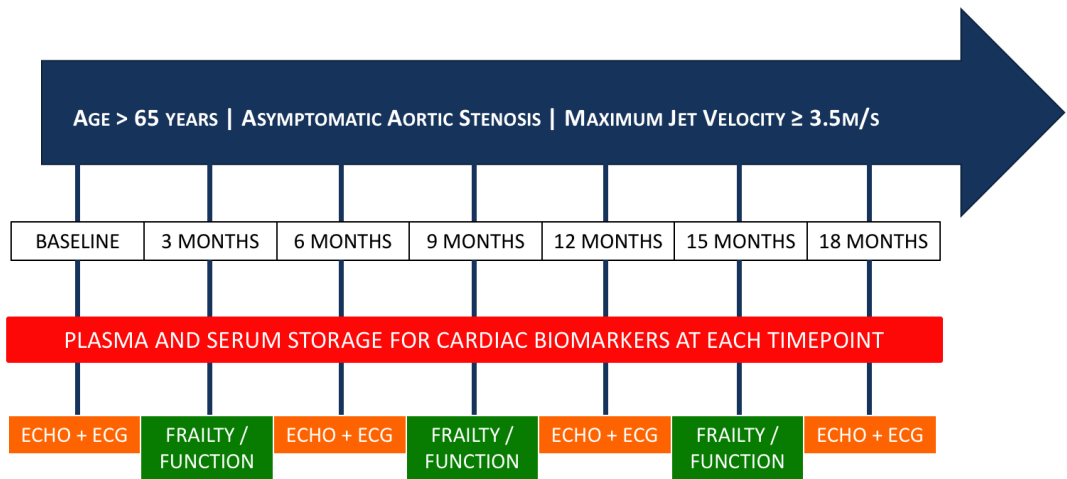


Figure 7.1 – Schematic for the serial sampling protocol of the Cardiac biOMarkers in older Patients with Aortic Stenosis (COMPASS) study

7.2.2 ADDITIONAL BIOMARKER STUDIES

This thesis has focussed on cMyC and cardiac troponin as markers of myocardial injury, BNP as a natriuretic peptide measure of ventricular wall stress and galectin-3 as a marker of fibrosis. However, there are further blood biomarkers that may be of interest in aortic stenosis. ST-2 is a member of the interleukin-1 receptor family and is secreted by mechanically overloaded cardiomyocytes into the circulation. It is therefore a marker of early ventricular failure but appears to provide additional risk prediction information beyond BNP in the setting of myocardial infarction.^{224,225} In a small study in aortic stenosis, soluble serum ST-2 concentrations were independently predictive of future cardiovascular events and only modestly correlated with BNP levels.²²⁶

Growth differentiation factor 15 (GDF15) is involved in inflammatory and apoptotic pathways as part of the transforming growth factor superfamily. Circulating concentrations of this biomarker are increased when cardiomyocytes undergo biomechanical stress such as the pressure overload of decompensating aortic stenosis.²²⁷ GDF15 levels also appear predictive of mortality in aortic stenosis and outcomes after TAVI.^{228,229}

It would appear that both ST-2 and GDF15 are biomarkers of potential interest in aortic stenosis and complementary to those examined within this thesis. A logical future extension of the proposed COMPASS programme would include measurement of these promising novel biomarkers within stored sample to assess any value beyond existing clinical assessment and surgical risk scores.

7.2.3 BIOMARKER CHANGES AFTER AORTIC VALVE REPLACEMENT

Biomarkers of myocardial injury may also hold predictive value after aortic valve replacement. In studies of mixed cardiac surgery including valve replacement, post-operative cardiac troponin is an independent predictor of mortality.^{230,231} I am a local co-investigator for the ongoing VISION Cardiac Surgery study, which will further inform the significance of this relationship in 15,000 patients undergoing cardiac surgery across the world (clinicaltrials.gov NCT01842568). It is plausible that the kinetics of myocardial injury biomarker release and recovery following valve replacement may inform the likelihood of symptomatic improvement.

As shown in Chapter 3, serum cMyC concentrations in aortic stenosis are related to left ventricular mass, a finding that has also been demonstrated using cardiac troponin.⁸⁸ Successful replacement of a stenotic aortic valve relieves afterload and should facilitate remodelling of the left ventricle with regression of hypertrophy. This would be expected to be accompanied by reductions in markers of wall stress (such as BNP) and myocardial injury (such as cardiac troponin and cMyC). However, adverse remodelling has been recognised in a subgroup of patients with poorer recovery after aortic valve replacement.²³² This may be related to a variety of factors including post-operative blood pressure control and ethnicity.^{233,234}

An extension of the COMPASS study outlined above may provide further evidence in this area. In an exploratory pilot study in those who undergo aortic valve replacement, early perioperative measures of cardiac blood biomarkers including cMyC, cardiac troponin and BNP may help understand the significance of perioperative injury and stress on cardiac recovery. A further measure at 6 months combined with repeated functional testing and echocardiography would inform the relationship between circulating cardiac biomarkers and ventricular remodeling. A proposed outline of such a study is shown in Figure 7.2.

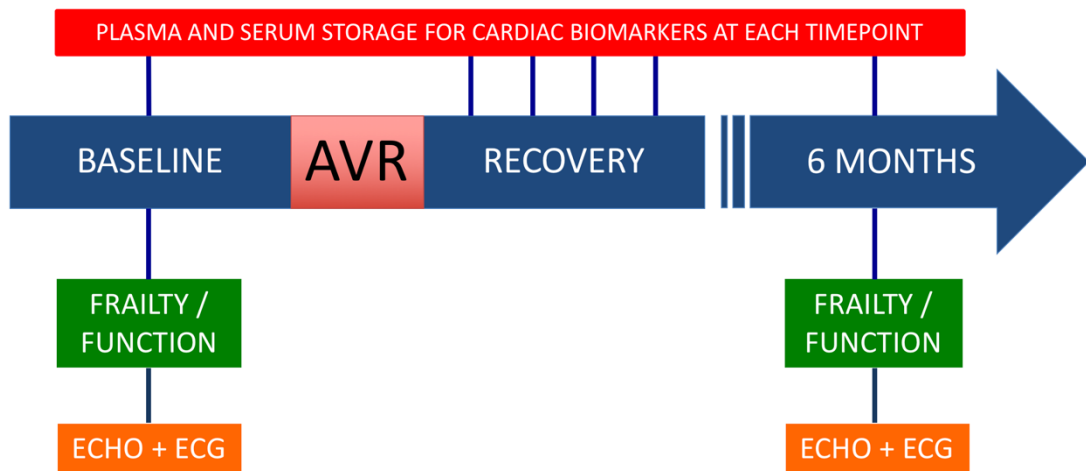


Figure 7.2 – Schematic for a study of serial cardiac biomarker changes before and after aortic valve replacement (AVR)

7.2.4 VARIABILITY OF NOVEL BLOOD BIOMARKERS

Chapter 4 of this thesis reported the analytical and biological variability of cardiac troponin, BNP and galectin-3 in older patients with aortic stenosis. Given the findings of Chapter 3 and the potential utility of cMyC in these patients, an extension of this research would include assessment of the variability of cMyC. This could be completed in currently stored sample to define the parameters reported for the other biomarkers. Given the ten-fold greater abundance of cMyC compared to cardiac troponin⁹², it would be expected that concentrations in the same patient group may be further from the assay LoD and therefore subject to lower analytical variation. If the release and turnover of both cardiac troponin and cMyC are similar in aortic stenosis given their shared sarcomeric lineage, it would be expected that both assays would have similar biological variability. It is therefore plausible that cMyC may have a smaller RCV than cardiac troponin, given the expected lower analytical variability, and be more sensitive to meaningful change in this patient group.

7.2.5 FRAILTY IN RELATION TO OUTCOMES AFTER AORTIC VALVE REPLACEMENT

In older patients who are candidates for aortic valve replacement, frailty assessment captures patient factors that appear distinctive from components of existing surgical risk scores (Chapter 6). As identified by multiple systematic reviews²¹¹⁻²¹³, there is currently a lack of high quality statistical modelling using the STS or EuroSCORE with addition of robust frailty measurement. This would truly test the potential of frailty measures and answer whether the additional time and effort required to collect these data add predictive value. Such an approach acknowledges that frailty exists on a spectrum and allows analysis free from forced dichotomisation into a frail state using arbitrary thresholds. The cohort presented in Chapter 6 have been robustly characterised for frailty and will be followed to collect important outcomes data for such an analysis.

The study presented focussed on physical frailty and determination of the phenotype through measures not normally collected even within geriatric clinical environments. However,

similar assessment measures have been successfully included in Proactive care of Older People undergoing Surgery (POPS) clinics.^{173,235} Such an approach acknowledges that frailty measurement offers risk stratification as discussed within this thesis, but also an opportunity to proactively address modifiable risks identified by comprehensive geriatric assessment. This approach has been shown to reduce operative complications, delirium and length of stay in a randomised controlled trial in vascular surgery.²³⁶

An alternative approach to frailty assessment reflects the accumulation of health deficits to generate a frailty index. The recent work of Clegg *et al.* has utilised routine healthcare data to generate such an electronic frailty index (eFI) from primary care read codes.¹¹² This score is highly predictive of hospitalisation, loss of independence and mortality in a community population. Such a ‘big data’ approach allows frailty to be measured in all patients, with minimal additional costs and no requirement for face-to-face assessment. This may be an attractive approach for stretched surgical clinics, although the validity of this risk score in relation to postoperative outcomes has not yet been demonstrated. For example, primary care data may be relatively insensitive to change in the presence of rapidly progressing symptomatic aortic stenosis. However, the ability to generate an eFI score at scale should allow large cohort testing of this measure within existing registries where preoperative surgical risk scores and outcomes have already been collected.

7.2.6 UNDERSTANDING TRAJECTORIES OF FRAILTY

While observational studies including baseline frailty measures in surgical cohorts are numerous, longitudinal studies to understand the trajectory of frailty after intervention are uncommon. This is critical to understanding predictors of meaningful gain after surgery. It is plausible that in a frail patient with multisystem decline, valve surgery to reverse the deleterious effects of aortic stenosis will not improve underlying frailty. This may at least partly explain the observation of 1 in 4 patients dying within 12 months of TAVI (Chapter 5) and a failure to meet patient expectations for improvement amongst some survivors. To

understand these trajectories of frailty, detailed studies are required before and after surgery (Figure 7.2).

As suggested in Figure 7.1, it would also be important to quantify the change in frailty markers over time in patients with severe aortic stenosis in the absence of surgery. If patient frailty is being driven by the consequences of severe aortic stenosis, for example through the effects of early left ventricular decompensation or development of syncopal symptoms, it is plausible that aspects of frailty may be reversed by aortic valve replacement. This phenomenon is anecdotally observed following successful cardiac surgery but has not been objectively demonstrated. A recent systematic review by Kim *et al.* identified no studies in cardiac surgery that measured frailty pre-operatively and again at least 6 months after surgery.²¹³ There is a clear need to address this gap in knowledge to improve understanding and informed decision making in this potentially vulnerable patient group.

Measuring frailty may therefore help guide aortic stenosis patients towards three broad treatment strategies. First, a group without frailty in whom surgical risk is considered low and the decision to intervene with surgery or TAVI is clear. Second, a group with presumed severe irreversible frailty and high perceived surgical risk in whom the benefits of intervention are likely to be outweighed by the surgical risks. Finally, a third group with intermediate surgical risk and/or some frailty markers who require further detailed assessment prior to surgery.

It is possible that blood biomarkers of aortic stenosis progression hold the most value in this latter group. In the absence of progressive elevation of biomarkers of myocardial injury, ventricular wall stress or fibrosis, it could be hypothesised that any frailty and symptoms observed are independent of aortic stenosis and would be unlikely to improve with valve replacement. It is plausible that a proportion of patients will have such irreversible frailty

and will be liable to the complications of cardiac intervention without scope to improve their wellbeing due to overwhelming intrinsic non-cardiac disease driving the frail state.

In contrast, in a frail patient with progressive blood biomarkers of disease, the risks of intervention may be worth considering to improve symptoms; this may be described as the ‘high risk, high reward’ strategy. As such, measurement of frailty and blood biomarkers of disease progression hold potential for more individualised assessments of risk, providing objective evidence to help patients, families and clinicians make balanced judgements. In a ‘high risk, low reward’ patient group, such measures could also strongly challenge the rationale for invasive interventions. The recently launched NHS Scotland ‘Realistic Medicine’ initiative has brought such concepts to the heart of clinical practice, with acceptance of the need to assess the “point of optimality” between benefits and harms of any healthcare intervention.²³⁷

7.2.7 CLINICAL PERSPECTIVE OF FRAILITY

For many the term ‘frailty’ carries stigma with negative connotations. In a survey of older adults performed by Age UK and the British Geriatric Society, the term was not well received with few participants self-identifying as frail.²³⁸ This was related to a perception that the term implied an “irreversible state” with loss of independence, dignity and control over body functions. However, the challenges that frail people experience in everyday life were well recognised by respondents. Ultimately, direct use of the term frailty may not be helpful in a patient interaction, but discussion around the risks related to the expression of the frail state are likely to be highly relevant.

It is important that any research involving frailty includes patients’ views as the aims must be to improve individualised or person-centred care. Qualitative research such as the survey described above must feed into quantitative research. For example, within this thesis, outcome measures for aortic valve disease have been discussed, but defining a suitable

endpoint for trials involving frail older adults is challenging. Does quality of life outweigh longevity? Does the burden of tablets and time spent in hospital outweigh small but statistically significant improvements in functional measures? Meaningful benefit from any intervention is a challenging entity and may lack the rigor of a so-called 'hard' endpoint such as all-cause mortality. Perception of benefit may vary between individuals for reasons as diverse as mood, cognitive function, values and prior expectations. Minimal Clinically Important Differences (MCID) provide some rigor to assessment of change in markers but are usually based on meaningful statistical change or the consensus view of an expert panel.²³⁹ These attributes have some value in improving endpoints for trials including older people, but further involvement of patients in study design is a clear future direction in this area.

In 1999, Bowling commented on ageism in cardiac care.²⁴⁰ While these attitudes may have declined in the last two decades, some have argued that frailty is a modern equivalent to ration treatments. However, this characterisation neglects the potential benefits of holistic assessment for an older adult. Further, including frailty assessment in routine care identifies patients who are surprisingly robust, who may otherwise have been considered at higher risk of poor outcomes due to the bias of subjective assessment. For healthcare professionals, frailty offers a language to communicate risk that is independent of age. It is therefore critical that this professional assessment is based on objectivity and careful measurement.

7.2.8 SARCOPENIA

Whilst this thesis has focussed on frailty, sarcopenia is a related state that refers to the loss of skeletal muscle mass and function.²⁴¹ The European Working Group on Sarcopenia in Older People (EWGSOP) definition requires objective evidence of loss of muscle mass, together with either reduced muscle strength or performance. Whilst physical frailty measures such as the Fried phenotype include grip strength and gait speed for strength and performance, these

do not include measures of muscle mass.¹⁰⁷ This requires more specialist equipment such as dual energy X-ray absorptiometry (DEXA), computerised tomography, magnetic resonance imaging or bioimpedance analysis.²⁴² This requirement has limited the adoption of sarcopenia measurements in clinical practice; even the EWGSOP guideline suggest case-finding through strength and performance measures to limit diagnostic muscle mass measurement to a smaller numbers of older adults. Muscle mass may also not add significantly to prognostication beyond measures of muscle strength and performance, which may question the value of the additional resources required to define sarcopenia.

However, while many individuals with frailty will have evidence of sarcopenia, due to broader and varied definitions, frailty may occur in the absence of physical deterioration through health deficits and cognitive decline.²⁴³ Sarcopenia is therefore a more precise term focusing solely on physical muscle health. For example, in a community cohort study of over 80 year old adults in Belgium (BELFRAIL), sarcopenia was only observed in 12.5% by EWGSOP criteria. However, 56% of the cohort demonstrated muscle weakness by grip strength and 61% impaired muscle performance by low SPPB score.²⁴⁴ The specificity of sarcopenia is attractive for targeted interventions and there is some evidence of the ability to improve and reverse the process with resistance muscle training.^{245,246} There has been further interest in the role of nutritional supplementation and pharmacological therapy including angiotensin-converting enzyme (ACE) inhibitors, which are subject to ongoing randomised controlled trials.^{247,248}

Resistance exercise training is challenging in a patient developing heart failure secondary to decompensated aortic stenosis. However, should non-exercise based interventions prove successful in improving sarcopenia, this could open new avenues for the optimisation of the perioperative patient. Extending the work of the thesis to include measures of muscle mass before and after aortic valve replacement would inform the burden of disease-related sarcopenia and its response to alleviation of aortic stenosis.

7.3 CONCLUSIONS

This thesis has explored the components of a future holistic approach to older patients with aortic stenosis– where blood biomarkers reveal functionally significant aortic valve disease, and frailty biomarkers refine surgical risk assessment and inform shared decision making. As currently described, frailty is a nebulous term with numerous measures that may lack the objectivity of blood biomarkers, but the variables that are included in frailty assessment are likely to be highly relevant to the likelihood of patient benefit from aortic valve replacement. Combining blood biomarkers of disease related risk, frailty biomarkers of surgical risk and patient preference holds potential as an integrated approach to complex older patients with aortic stenosis.

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*denotes equal contribution of authorship

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APPENDIX I: FRAILTY QUESTIONNAIRE

Combined questionnaire for completion of patient-reported components of the Fried, EFS and SF-12 assessments

Please answer the questions by ticking the relevant boxes.

1. In the past year, how many times have you been admitted to hospital?

NO ADMISSIONS 1-2 TIMES 3 OR MORE TIMES

2. In general, how would you describe your health?

EXCELLENT VERY GOOD GOOD

FAIR POOR

3. With which of the following activities do you require help (tick all that apply)?

MEAL PREPARATION SHOPPING TRANSPORTATION

USING THE TELEPHONE HOUSEKEEPING DOING LAUNDRY

MANAGING MONEY TAKING MEDICATIONS Total _____

4. When you need help, can you count on someone who is willing and able to support your needs?

ALWAYS SOMETIMES NEVER

5. Do you use 5 or more different prescription medications on a regular basis?

YES NO

6. At times, do you forget to take your prescription medications?

YES NO

7. Have you recently lost weight such that your clothing has become looser?

YES NO

8. Have you unintentionally lost more than 10lbs (4.5kg) in weight in the last year?

YES NO

9. Do you often feel sad or depressed?

YES NO

10. Do you have a problem with losing control of urine when you don't want to go?

YES NO

11. a) I felt that everything I did was an effort in the last week:

RARELY or NONE OF THE TIME (<1 day)

SOME or A LITTLE OF THE TIME (1-2 days)

MODERATE AMOUNT OF THE TIME (3-4 days)

MOST OF THE TIME (>4 days)

b) I could not get going in the last week:

RARELY or NONE OF THE TIME (<1 day)

SOME or A LITTLE OF THE TIME (1-2 days)

MODERATE AMOUNT OF THE TIME (3-4 days)

MOST OF THE TIME (>4 days)

These next questions ask for views about your health and activities. **Please answer each question by choosing just one answer.** If you are unsure how to answer a question, please just give the best answer you can.

1. The following questions are about activities you might do in a typical day. Does your health now limit you in these activities. If so, how much?

Moderate activities (e.g. moving a table, pushing a vacuum cleaner, playing bowls or golf)?

YES, limited a lot YES, limited a little NO, not limited at all

Climbing **several** flights of stairs?

YES, limited a lot YES, limited a little NO, not limited at all

2. During the **past 4 weeks**, have you had any of the following problems with your regular daily activities as a result of your **physical health**?

Accomplished less than you would like? YES NO

Were limited in the kind of activities you could do? YES NO

3. During the **past 4 weeks**, have you had any of the following problems with your regular daily activities as a result of any **emotional problems** (such as feeling anxious or depressed)?

Accomplished less than you would like? YES NO

Did work less carefully than usual? YES NO

4. During the **past 4 weeks**, how much did pain interfere with your normal activities?

Not at all A little bit Moderately Quite a bit Extremely

5. These questions are about how you have been feeling during the **past 4 weeks**.
For each question please give the one answer that comes closest to how you are feeling.

How much of the time during the **past four weeks**...

	All	Most	A good bit	Some	A little	None
...have you felt calm and peaceful?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
...did you have a lot of energy?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
...have you felt downhearted and blue?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

6. During the **past 4 weeks**, how much of the time has your physical health or emotional problems interfered with your social activities (like visiting friends, relatives etc.)?

All of the time Most of the time Some of the time

A little of the time None of the time

APPENDIX II: MYOCARDIAL BIOPSY AND HISTOLOGICAL ANALYSIS

This analysis was undertaken by Dr Jacek Kwieciński and is detailed in the Supplementary Material of the published manuscript (Anand A et al. *Heart*. 2018;104:1101–1108).

Tissue Sampling

At the time of open heart surgery myocardial biopsies were obtained from the basal segment of the septum using a Tru-Cut needle biopsy gun. In order to minimise the chance of missed biopsies at least two samples per patient were collected. Immediately after collection, tissue was placed in buffered 10% formalin and subsequently embedded in paraffin.

Tissue processing

For apoptosis 7µm thick sections were deparaffinised in xylene, rehydrated through a graded series of alcohols and subsequently the DeadEnd™ Fluorometric TUNEL System (Promega Co, US) was applied according to the manufacturer's guidelines. The system labels fragmented DNA which is a hallmark of apoptosis. After cell membrane permeabilisation the 3' OH ends of the cleaved DNA multimers were "tailed" with labelled fluorescein-12-dUTP by the Terminal Deoxynucleotidyl Transferase enzyme. In addition all specimens were stained with 4,6-diamidino-2-phenylindole (DAPI) to adequately visualise nuclei which was necessary for proper image analysis.

For autophagy and oncosis formalin-fixed, paraffin-embedded 4µm thick tissue sections were cut. Slides were dried for 24 hours in a 45°C oven, deparaffinised in xylene and rehydrated through a graded series of alcohols. Slides were then loaded into a Celerus Riptide de-cloaking chamber (Celerus Diagnostics Carpinteria, CA, United States) where heat-induced epitope retrieval was performed using Novocastra Epitope Retrieval solution Ph6 (Leica Microsystems GmbH, Ernst-Leitz-Straße, Wetzlar, Germany). Slides were then loaded onto Leica Bond-Max automated immunostainer (Leica Microsystems GmbH, Ernst-

Leitz-Straße, Wetzlar, Germany). For autophagy and oncosis ubiquitin and C9 mouse monoclonal antibodies at 1:3000 and 1:2000 dilution (Life Technologies, Carlsbad, CA, United States and AbD Serotec, Kidlington, Oxford, UK respectively) were applied to sections at room temperature for 2 hours. The specificity of these antibodies was verified by omission. The presence of antigen was visualized using a 3,3'-diaminobenzidine (DAB) based Bond Polymer refine detection kit (Leica Microsystems GmbH, Ernst-Leitz-Straße, Wetzlar, Germany). Slides were counterstained using haematoxylin (in order to enable nuclei identification), removed from bond max, dehydrated, cleared and mounted with permanent mounting media (Pertex).

Histological image analysis

The TUNEL stained tissue samples were analysed using confocal microscopy with FITC and UV filter cubes. All measurements have been performed using 40x objectives. Apoptotic cells (co-positive for both DAPI and TUNEL) were counted manually on entire tissue sections. The total cell number present on each slide was derived from two sets of data: the total sample area and the number of cells positively stained with DAPI which was evaluated manually in three random areas of interest. Eventually the number of apoptotic cells was expressed as a percentage of the total cell number.

All C9 and ubiquitin stained slides images were acquired on the AxioScan Z1 (Carl Zeiss, Oberkochen, Germany) and analysed using Image-Pro Premiere 9.1 (MediaCybernetics, Rockville, MD, USA). In the first step the number of oncotic/autophagic cells was calculated using the counting toll after manual protocol adjustment. Cut off values of signal intensity, object size (area) and a roundness criterion was used to distinguish myocytes positively stained with DAB from artefacts. For Oncosis a pixel intensity of 0-163 on the Mono scale together with an object area range of 400-3000 square pixels and roundness criterion of 1-1.7 was applied. For autophagy the settings were as follows: a pixel intensity of 0-60 and an object area range of 200-7000 square pixels. In the second step the average cell area and the

total tissue area was measured using a threshold of 0-238 on the Mono pixel intensity scale. Finally the number of oncotic or autophagic cells was expressed as a percentage of the total cell count.

Cell counts

The median number of cells examined per patient was 115,161 [IQR 78,427 – 193,585] for oncosis, 109,355 [IQR 90,821 – 169,453] for autophagy and 216,320 [IQR 196,640 – 284,500] for apoptosis.

Example apoptotic cell

As demonstrated by confocal microscopy and immunofluorescence. A: DAPI; B: TUNEL; C: fused image demonstrating co-staining.

