Pilot studies of cardiovascular biomarkers, atrial fibrillation and risk stratification in patients with oesophageal cancer undergoing surgery

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Dedication

I wish to dedicate this thesis to my husband Colin and my mum Joy.
Thank you for your love, encouragement and support-you inspire me every day.
Isaiah 40:31

Abstract

Peri-operative medicine is an area that is increasingly important in terms of increasing population size and therefore increasing volume of surgeries completed. The assessment of risk and the communication of this risk to patients and their families is vital to shared decision making which is one of the key considerations for practising realistic medicine. Oesophageal cancer was responsible for 3% of the total number of cancer cases in the UK in 2015 and affected 921 patients in Scotland in 2015 which was 10% of the total number of oesophageal cancer cases in the UK that year. Studies have shown that atrial fibrillation is associated with increased morbidity and mortality and it can occur following operations such as those undertaken in the management of oesophageal cancer. Several cardiovascular biomarkers exist, some of which have been associated with the development of atrial fibrillation and the question I sought to explore was whether it was possible to begin to understand which patients developed atrial fibrillation following their operation for oesophageal cancer and also whether the development of atrial fibrillation, in combination with other cardiovascular biomarkers, in the context of cardiopulmonary exercise testing results would assist in the prediction of the morbidity and mortality of this patient group.

Based on the existing literature, I created the concept for the study and then co-ordinated and implemented the study.

In the work I have presented in this thesis I have shared some of the challenges which I faced during the study and how I dealt with those. I have also shared some of the results including biomarker levels and cardiopulmonary exercise testing results in the context of the onset of AF in patients and the subsequent morbidity and mortality. I have also discussed the challenges I encountered when the study did not go according to plan and the lessons which I have learned as a result.

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Presentations

Oral presentation at the Society of Critical Care Medicine San Francisco January 2014

- Asymmetric dimethylarginine, homoarginine levels and atrial fibrillation in oesophagectomy patients.

Presented at European Society of Intensive Care Medicine Lisbon October 2012

- Haemodynamics, inflammation and mortality in medical patients with first diagnosis atrial fibrillation within a general intensive care unit.
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Presented at Nottingham Association of Cardiothoracic Anaesthesia (ACTA) meeting November 2013

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Preface

I initially undertook this work on a part time basis while working as an honorary anaesthetic registrar attached to the University of Glasgow department of Anaesthesia, Critical Care and Pain. I continued and completed my thesis in my own time as a trainee.

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Author's declaration

I declare that this work is of my own composition and that the research contained within it is entirely my own work, unless otherwise stated.

Katherina Tober
February 2021

List of Abbreviations

AAA: Abdominal Aortic Aneurysm
ACCI: Age adjusted Charlson Co-morbidity Index score
ADMA: asymmetric dimethylarginine
AF: Atrial Fibrillation
AGXT2: alanine glyoxylate aminotransferase 2
ANP: Atrial Natriuretic Peptide
ASS: Argininosuccinate synthetase
ASL: Argininosuccinate lyase
AT: Anaerobic threshold
BHOM: Biochemistry and Haematology Outcome Models
BMI: Body Mass Index
BNP: Brain Natriuretic Peptide
CABG: Coronary Artery Bypass Grafting
CACI: Charlson Age Comorbidity Index
CEPOD: Confidential Enquiry into Peri-operative Deaths: The CEPOD list is a permanently staffed operating theatre that runs on a 24 hour basis for urgent or emergency operations
CPET: Cardiopulmonary Exercise Testing
CRP: C-Reactive Protein
DDAH: Dimethylarginine Dimethylaminohydrolase
DMA: Dimethylarginine
ECG: Electrocardiogram
Echo: Echocardiogram
EDTA: Ethylenediaminetetraacetic acid
GPS: Glasgow Prognostic scoring system
HPLC: High performance Liquid Chromatography
Hs CRP: high sensitivity C-Reactive Protein
ICU: Intensive Care Unit
IL-1: Interleukin-1
IL-6: Interleukin-6
LAD: Left Atrial Diameter
LVEF: Left Ventricular Ejection Fraction
MACE: Major Adverse Cardiac Event
MI: Myocardial Infarction
ml: millilitres
Mmol: millimoles
MPO: myeloperoxidase
NO: Nitric oxide
NOS: Nitric Oxide Synthase
NYHA: New York Heart Association
NT-proBNP: N terminal pro Brain Natriuretic Peptide
POSSUM: Physiological and Operative Severity Score for the enUmeration of Mortality and morbidity
P-POSSUM: Portsmouth-Physiological and Operative Severity Score for the enUmeration of Mortality and morbidity

POAF: Post-operative Atrial Fibrillation
PoAF: Post oesophageal Atrial Fibrillation
PRMT: Protein Arginine Methyltransferases
QoL: Quality of Life
RCRI: Revised Cardiac Risk Index
SDMA: symmetrical dimethylarginine
TNF: Tumour Necrosis Factor
TGF: Transforming Growth Factor
VO2 peak/max: maximum rate of oxygen consumption during incremental exercise

1 CHAPTER 1

1.1 INTRODUCTION

Cancer of the oesophagus is the eighth most common cancer worldwide with an estimated 462,000 new cases (4.2% of the world total) in 2002[1]. It is also the sixth most common cause of death from cancer with 386,000 deaths (5.7% of the world total). It affected 8900 people in 2014 in the UK with approximately twice as many cases occurring in men as in women. For people diagnosed in England and Wales in 2010-11, the 5 year survival rate was only 15%. Oesophageal cancer is frequently diagnosed at an advanced tumour stage and this in addition to co-morbidity means that only 30-40% cases are suitable for curative resection[2].Although early post-operative mortality rates have decreased during recent years due to the development of surgical technique and peri-operative care, they are still as high as 4-14%[3,4]. Operations involving patients with oesophageal cancer are amongst those with the highest morbidity and mortality.

Oesophageal surgery, whether for benign or neoplastic disease is a major undertaking associated with significant morbidity and mortality. Stratifying and assessing the patients pre-operatively so that the appropriate level of intervention is offered is important in helping reduce these risks.

1.2 Peri-operative medicine and clinical risk scoring systems

Peri-operative medicine describes the practice of multidisciplinary, integrated, patient-centred medical care of patients from the contemplation of surgery until full recovery[5].Surgery is an important treatment option for a wide range of acute and chronic diseases and for most patients it is a success, however; the population is changing and so must our current services. The peri-operative care of patients undergoing major surgery is recognised as an area that significantly affects public health and one in which needs are unmet. Predicting and preventing morbidity and mortality peri-operatively is of vital importance as populations age and the number of procedures performed worldwide each year increases. Approximately 250

million major surgical operations are performed worldwide each year[6] and approximately 8 million of those are in the UK[7]. For many years the care received by patients undergoing surgery of major importance has been titrated to the operation and the disease treated by the procedure as opposed to the patient and their co-morbidities. The Confidential Enquiry into Perioperative Deaths (CEPOD) was published in 1987 in response to professional concern about peri-operative deaths[8]. Following publication of the work the Department of Health announced that it would fund the National Confidential Enquiry (NCEPOD) to repeat the work and it published its first report in 1989[9]. The name was subsequently changed and in 2011, the National Confidential Enquiry into Patient Outcome and Death (NCEPOD) entitled ' Peri-operative care-Knowing the Risk' highlighted the process care of patients aged 16 years and over who underwent elective and emergency inpatient surgery and evaluated their outcome at 30 days. It was an observational study of over 16,000 surgical patients in the Isle of Man, Jersey, Guernsey, England, Wales and Northern Ireland[10] and it is estimated that 20,000-25,000 deaths per year occurred in hospital after a surgical procedure. Approximately 80% of these deaths occurred in a small population of patients who were deemed to be high risk. These high-risk patients are estimated to comprise about 10% of the overall inpatient surgical workload and are a major source of morbidity, mortality and resource utilisation. There are concerns that UK outcomes may be poorer than in other countries as when compared with centres in similar sized hospital and patient populations in the United States of America (USA), the NHS has poorer outcomes as a whole[11,12]. The 2011 report led to pre, peri and post-operative recommendations. Pre-operatively, it suggested that all elective high-risk surgical patients should be seen and fully investigated in pre-assessment clinics. It advised that there should also be greater assessment of a patient's nutritional status and consideration of requirement of fluid optimisation. Peri-operatively there should be consideration of enhanced recovery pathways and post-operatively there should be

availability of high dependency and intensive care beds. Overall, the report revealed that there was a lack of consensus as to what constituted high peri-operative risk.

Accurately predicting peri-operative risk is an ideal goal, which would allow informed consent for patients prior to surgery and guide clinical decision-making during the peri-operative period. Factors to consider include the interplay between the inflammatory response to the tissue injury during surgery, the type and quality of the surgery and the patient's physiological reserve as these have been shown to contribute to the outcome[13]. Optimal assessment and management of patients peri-operatively will ideally lead to reduction in post-operative complications and improved long term survival. Methods already exist which assess peri-operative risk for individual patients.

1.3 Risk stratification tools

One method of assessing risk peri-operatively is by using **risk stratification tools**. These are models or scoring systems that predict or adjust for mortality or morbidity after surgery, based on different risk factors. These risk stratification tools complement investigations to identify high-risk patients such as cardiopulmonary exercise testing and biomarker assays[14].Functional testing such as cardiopulmonary exercise testing is not routinely available and is not practical in urgent or emergency cases[10,15]. The role of biomarkers such as NT-proBNP and BNP in the identification of high-risk patients is still emerging[16,17,18]. Risk stratification tools remain the most readily available means of determining peri-operative risk.

Risk stratification tools can be divided into risk scores and also risk prediction models.

1.4 Risk scores

These assign a weighting to factors identified as independent predictors of outcome. The sum of the weightings in the risk score reflects increasing risk. These scores are easier to use in the clinical setting, but they do not provide an individualised risk prediction of an adverse outcome despite scoring a patient on a scale on which other patients can be compared. Risk

scores include the Lee Revised Cardiac Risk Index and the American Society of Anaesthesiologists Physical Status score (ASA-PS).

The original Cardiac Risk Index or the Goldman Index was developed in 1977 by Goldman and included nine variables associated with an increased risk of peri-operative complications. The Revised Cardiac Risk Index (RCRI) by Lee et al 1999[19] is a tool that is used for predicting a patient's risk of peri-operative cardiac complications following major non-cardiac surgery. It uses six independent variables, which cumulatively increase a patient's risk for peri-operative cardiac complications. It has been found that its predictive power could be improved significantly by adding CRP and NT-proBNP to it (adjusted RR 4.6 p<0.001).

Table 1.1 Revised Cardiac Risk Index (RCRI) [19]

Revised Cardiac Risk Index
History of ischaemic heart disease
History of congestive heart failure
History of cerebrovascular disease (Stroke or Transient Ischaemic Attack)
History of diabetes requiring pre-operative insulin use
Chronic kidney disease (Creatinine > 2mg/dL)
Undergoing suprainguinal, vascular, intraperitoneal or intrathoracic surgery
Risk for cardiac death, non fatal MI, non fatal cardiac arrest 0 predictors: 0.4%, 1 predictor 0.9%, 2 predictors 6.6%, >/- 3 predictors > 11%

The RCRI has been incorporated in a modified form into the 2007 pre-operative cardiac risk evaluation guideline from the American Heart Association (AHA) and the American College of Cardiology (ACC).

American Society of Anaesthesiologists Physical Status score (ASA-PS)

The ASA-PS is a five point classification system that was developed in 1963 to assess fitness of patients before surgery. A sixth class was added later to denote brain dead patients whose organs were being removed for donation purposes[20]. ASA-PS shows good correlation with post-operative outcome for patient populations in a number of surgical settings but does not describe individual patient operative risk. It does not account for the physiological impacts

that the procedure will cause, the experience of the doctors caring for the patient or the peri-operative optimisation that may occur.

1.5 Risk prediction models

These estimate an individual probability of peri-operative risk by entering the patient's data into the multivariable risk prediction model. These models are more accurate when compared to risk scoring, however they are more complex to use in the daily clinical setting.

A qualitative systematic review published in October 2013[21] looked at summarising the available risk prediction methods, to report on their performance and to identify their strengths and weaknesses. Twenty-seven studies evaluating thirty-four risk stratification tools were identified which met inclusion criteria.

1.6 Examples of Risk prediction models

The 2014 pre-operative cardiac risk evaluation guideline from the American Heart Association (AHA) and the American College of Cardiology (ACC) stated that two newer risk prediction models; the American College of Surgeons National Surgical Quality Improvement Programme (N

SQIP) Myocardial Infarction or Cardiac Arrest (MICA) risk prediction calculator and the NSQIP surgical risk calculator[22] had been created by the American College of Surgeons (ACS).

Other risk prediction models include the Donati Surgical Risk Score, Charlson Age Co-morbidity Index (CACI), Surgical Risk Scale and Physiological and Operative Severity Score for the EnUmeration of Mortality and Morbidity (POSSUM). These have been validated in multiple studies. The surgical outcomes risk tool (SORT) and the Nottingham hip fracture score are also examples of risk prediction models. The Surgical Risk Scale and the Surgical Risk Score contain the ASA-PS.

The NSQIP MICA risk prediction tool includes adjusted odds ratios for different surgical sites, with inguinal hernia as the reference group. Target complications were defined as

cardiac arrest or MI. The NSQIP surgical risk calculator uses procedure specific risk assessment for a diverse group of outcomes. Limitations to the NSQIP calculator include the lack of validation in an external population and the use of the Anaesthesiology Physical status classification which has poor inter-rater reliability. NSQIP also has definitions of functional status, with which clinicians would need to familiarise themselves[23].

Donati Surgical Risk Score

The surgical risk score was published by Donati et al in 2004[24] and was introduced as a model to evaluate pre-operative risk. The endpoint of the study was death or survival at hospital discharge in 1936 surgical patients. It included patients having all types of surgery apart from cardiac surgery and Caesarean sections. The model was validated in a further 1849 patients. It incorporates ASA-PS, age, type of surgery (eg. elective) and degree of surgery (eg. major). In estimating the degree or severity of surgery the score uses the modified Johns Hopkins surgical classification system[25] which was adapted from five original levels to three. Levels one and two were combined to produce minor surgery and levels four and five combined to produce level three; major surgery.

Charlson Co-morbidity Index (CCI) and Charlson Age Comorbidity Index (CACI)

The Charlson Co-morbidity Index (CCI) is a medical risk stratification tool which was developed for risk adjustment and prediction in non-surgical settings. It is a weighted score assigned to 17 co-morbidities based on the risk of 1 year mortality. In the tool's original validation, increasing CCI scores were significantly correlated with increased 10 year mortality in medical patients[26]. It is the most extensively validated measure of the prognostic impact of multiple chronic conditions. The Charlson Age Comorbidity Index (CACI) which is a combined age-comorbidity score was validated for the prediction of long-term mortality in patients undergoing non-cardiac elective surgery who had essential hypertension or diabetes mellitus[27]. It accounts for the patient's age and 16 conditions and uses the

International Classification of Diseases (ICD) diagnosis codes. The CACI has been validated in a number of surgical cohorts and it has been found to be a moderately accurate tool[28]. In a colorectal surgery cohort, it was found to be a predictor of in hospital morbidity, duration of hospital stay and mortality[29]. It has predictive ability but its disadvantages include lack of information regarding the surgical procedure and also the subjectivity involved in defining patients' co-morbidities. Since 1984, when the original Charlson weighted scoring was developed, there have been improvements in treatments and advances in technology and consequently patients survive longer than they did previously. The Charlson Index has been updated to include 12 co-morbidities instead of 17. The updated weight was shown to be lower than the Charlson weighting for AIDS/HIV, diabetes with complications and renal disease and higher for dementia, liver disease and congestive heart failure. This increased weighting may be related to an ageing population. The updated index has shown a good ability to discriminate outcome with regard to hospital mortality in 6 developed-country databases but needs validation in other developing-country settings in disease specific or procedure specific cohorts[28].

Surgical Risk Scale

The Surgical Risk Scale (SRS) was published by Sutton in the British Journal of Surgery in 2002. It is a cumulative score which adds the Confidential Enquiry into Perioperative Deaths (CEPOD) category to the American Society of Anesthesiologists Physical Status (ASA-PS) score and the British United Provident Association (BUPA) category scores. This means that a patient undergoing emergency complex major surgery, with an ASA of 5 dictated the highest rate of in hospital mortality. The benefit of this scoring system is that it can be used at the bedside prior to surgery[30]. However, previous validation studies are limited to two analyses from the same collaborators[30,31] and an external validation that included urgent or emergency surgery in a single hospital[32].

Physiological and Operative Severity Score for the EnUmeration of Mortality and Morbidity (POSSUM)

To address the limitations and the poor ability of the ASA-PS to identify individuals at risk of complications post-operatively, Copeland et al[33] developed the Physiological and Operative Severity Score for the EnUmeration of Mortality and Morbidity (POSSUM) scoring system. It was developed as a post hoc audit tool for surgery. It uses 12 pre-operative physiological parameters and 6 surgical or operative variables. Morbidity and mortality is calculated by using the sum of the physiological and surgical variables and entering them into two mathematical equations.

Table 1.2 A summary of the physiological and surgical variables used in the original POSSUM score[33].

Physiological Parameters	Operative Parameters
Age	Operation type
Cardiac	Number of procedures
Respiratory	Operative blood loss
ECG	Peritoneal contamination
Systolic BP	Malignancy status
Pulse rate	CEPOD
Haemoglobin	
White cell count	
Urea	
Sodium	
Potassium	
Glasgow Coma Scale	

(CEPOD = defines the urgency of surgery)

POSSUM was further developed when researchers identified a need to adjust the logistic regression analysis used in POSSUM scoring and weighting to better predict inpatient mortality. This resulted in the Portsmouth version (P-POSSUM) which uses the same 18 physiological and operative parameters as the original but a different calculation is used to determine predicted mortality[34]. It also includes some subjective variables such as interpretation of a chest x-ray, which means inter-observer variability may affect its accuracy.

Several researchers have found the predictive ability of P-POSSUM to be more accurate than POSSUM. While some studies have found that POSSUM AND P-POSSUM predict risk of morbidity and mortality for individual patients, others have found overestimation of mortality using this score, especially in low risk patients[35]. Other risk models have been developed including V-POSSUM (vascular surgery) CR-POSSUM (colorectal surgery) and O-POSSUM (oesophagogastric surgery)[36]. O-POSSUM has been found to be superior to other POSSUM models for predicting mortality in oesophageal-gastric surgery[37]. It has 16 parameters and has performed better than P-POSSUM when predicting post-operative mortality. P-POSSUM and its variations remain the scoring systems which have been globally validated for predicting individual risk. Both POSSUM and P-POSSUM have had variable success of being incorporated into clinical practice. Difficulty using them may be due to complexity or accessibility if a blood test is required. In 2013, a qualitative systematic review of risk stratification tools for predicting morbidity and mortality, validated in heterogeneous patient cohorts was published. The P-POSSUM and Surgical Risk Scale (SRS) were found to be the most widely validated and accurate risk stratification tools available, based on published data but both were noted to have limitations[22].

Surgical Outcome Risk Tool (SORT)

SORT was developed using data from an observational study of over 16,000 patients conducted by the NCEPOD 'Knowing the Risk' study[7]. It is based on six pre-operative variables and does not require blood tests. It gives a 30 day mortality risk for adult inpatients undergoing non-obstetric, non-cardiac, non -transplant and non-neurological surgery and has been validated for use in predicting post-operative morbidity when used pre-operatively in addition to clinical judgement, for major elective surgical patients[38]. In the validation cohort, the SORT demonstrated better discrimination than the ASA-PS and Surgical Risk Scale. Its validity has been assessed in hemi-hepatectomy patients and it has been validated in a single

centre cohort of adult patients undergoing major elective surgery[39]. A SORT App is now advertised on the NCEPOD website[40].

1.7 Models based on biochemical and haematological data

The Glasgow Prognostic scoring system (GPS) and the Modified Glasgow Prognostic scoring system (mGPS) are the most extensively validated of the systemic inflammation-based prognostic scores with more than 60 studies having examined their use[41]. The GPS evaluates C-reactive protein (CRP) and albumin levels and assigns a score. It has independent prognostic value in patients with cancer and an incrementally worsening prognosis is suggested by an increasing score. The mGPS weighs the inflammatory component more heavily and does not assign a score for an isolated low albumin. Another model, the Biochemistry and Haematology Outcome Model (BHOM) includes, age, sex, mode of admission, physiological parameters such as haemoglobin, white cell count, British United Provident Association (BUPA) operative severity score and 30 day mortality. The BHOM has the advantage of having variables that are mostly available pre-operatively, apart from operative severity. The Vascular BHOM has also been shown to be feasible after index arterial operations. The BHOM has been shown to have similar predictive accuracy to P-POSSUM in one study[32] however, it has not been validated in multicentre cohorts.

1.8 Functional Testing: Cardiopulmonary exercise testing (CPET) and surgery

Cardiopulmonary exercise testing is a non invasive clinical tool that assesses an individual's functional capacity or fitness and also the performance of the cardiorespiratory system. It can help to identify risk in patients pre-operatively and as a result, the stratification of patients pre-operatively using CPET in cardiopulmonary and non-cardiopulmonary surgery is increasingly widespread. It allows the objective assessment of exercise capacity pre-operatively and identifies the causes of exercise limitation by means of the calculation of a number of diagnostic and prognostic variables. It can identify pathophysiology not apparent at rest and distinguishes patients who may be less able to meet the increased oxygen delivery

demands of major surgery. It can inform peri-operative planning, including the use of invasive monitoring, pre-operative optimisation and allocation of post-operative critical care resources. CPET therefore has the capacity to identify patients at risk of adverse outcome before a range of non-cardiopulmonary surgical procedures[42]. In the mid 1990's, Older et al[43] completed an Australian study of 548 elderly patients who were undergoing major non cardiac surgery and assessed the functional capacity of their heart and lungs by carrying out pre-operative CPET. There were no deaths attributable to cardiopulmonary causes in any patient with adequate ventricular function, defined as an anaerobic threshold (AT) above $11ml.kg^{-1}.min^{-1}$ even if myocardial ischaemia was present. The conclusion was that the functional capacity of the heart and lungs as defined by CPET influences morbidity and mortality by determining the ability to deal with increased post-operative oxygen demand after major surgery. Patients with an AT below $11ml.kg^{-1}.min^{-1}$ were deemed to be high risk. With the uptake of CPET it is important that there is standardisation to ensure valid, reproducible results to inform clinical decision-making. Recently the Peri-operative Exercise Testing and Training Society (POETTS) has been established, which promotes high standards for patients undergoing training and testing in the peri-operative period. Guidelines have been developed by consensus following a systematic literature review[44]. There are many physiological variables obtained from CPET testing but three have been shown to identify high risk patients; VO2 peak or max, anaerobic threshold (AT) and ventilator equivalent for carbon dioxide (VE/VCO2)[43,44]. VO2 max is also known as the maximal oxygen consumption and is the maximum rate of oxygen consumption measured during incremental exercise. It can be expressed as an absolute rate eg. litres of oxygen per minute or it can be expressed as a relative rate in millilitres of oxygen per kilogram of body mass per minute (ml/kg/min). The AT is the point at which aerobic metabolism is no longer adequate and anaerobic supplementation begins. After the AT has been reached, aerobic metabolism does

not cease, but anaerobic metabolism supplements aerobic production of adenosine triphosphate (ATP) as the work rate increases[45].

1.9 CPET and Oesophageal surgery

The link between upper gastrointestinal (GI) surgery and CPET derived variables and outcomes initially was investigated by two studies in Japan which looked at thoracolaparotomy for thoracic oesophageal cancer and the other which looked at 91(3 of which were female) patients who had undergone oesophagectomy with lymphadenectomy for squamous cell carcinoma (SCC)[46]. They revealed that a VO2 max of 800ml/min/m^2 was the optimal threshold to discriminate those at a high risk of post-operative cardiopulmonary morbidity. Locally in Scotland, 108 patients were studied from 2008-2010 with oesophageal cancer and gastric cancer and had CPET as a formal fitness assessment. This showed that patients with a lower AT and a cut-off value of 9ml/min/kg had a higher risk of developing cardiopulmonary complications. One of the limitations of the study was its size and the resultant need for the results to be validated in a larger cohort and also the heterogeneity of the study population with some patients undergoing oesophagectomy and some undergoing gastrectomy[47]. Another CPET study prospectively evaluating 78 patients suggested that an association may exist between V02 peak and outcome following oesophagectomy, although capacity to predict those at increased risk is low and again study size was a limitation in this cohort[48]. Pre-operative cardiopulmonary exercise testing is routinely carried out for patients requiring oesophagectomy. Studies have shown that pre-operative VO2 max and anaerobic threshold measurements from CPET have been used to help predict risk of post-operative morbidity and mortality in non cardiopulmonary surgery patients, however, further larger studies are needed[49].

1.10 Oesophageal Cancer

There are two main types of oesophageal cancer in the UK; adenocarcinoma, which accounts for 60% cases and squamous cell carcinoma (SCC), the rest.

Squamous cell carcinomas mostly arise in the middle third of the oesophagus and the majority of adenocarcinomas arise in the lower third of the oesophagus. There are several risk factors for developing the disease including smoking tobacco which is causally associated. However, despite a reduction in smoking, the incidence of this cancer has risen in England and Wales. This is thought to be due to the additive effects of alcohol consumption and smoking tobacco. In Western countries, the main risk factors for SCC are smoking and alcohol consumption, however, adenocarcinoma predominantly occurs in patients with chronic gastro-oesophageal reflux disease. This risk is correlated with the patient's body mass index (BMI), with a higher risk for obese people[50,51].

Treatment for oesophageal cancer depends on the size, location and extent of the tumour and these are described by Tumour Node Metastasis (TNM) staging. Management often involves surgery, chemotherapy and sometimes radiotherapy. Resection of smaller tumour size in oesophageal cancer at stage T1-2 results in long-term survival but prognosis for oesophageal surgery alone is poor for stages T3-4 where the cancer has spread further. Neoadjuvant chemotherapy or chemoradiotherapy improved the overall survival of patients with advanced carcinomas of the oesophagus by about 10% over 5 years, according to Cochrane analysis and the prospective randomised Chemoradiotherapy for Oesophageal cancer followed by Surgery Study (CROSS) trial. In a study of 78 patients, neoadjuvant chemotherapy did not produce any significant differences in the incidence of cardiopulmonary and noncardiopulmonary complications, unplanned ICU admission rates or length of hospital stay[48]. The patient’s nutritional status and history of weight loss should also be assessed, according to The European Society for Clinical Nutrition and Metabolism (ESPEN) guidelines[52]. More than half of patients lose >5% of their body weight before admission for oesophagectomy and 40% lose >10%. This weight loss, independent from the BMI, confers an increased operative risk, worsens a patient's quality of life and is associated with poor

survival in advanced disease. Nutritional support according to the ESPEN guidelines[53] is an important part of the medical care for patients with oesophageal cancer in the curative and in the palliative setting. The five-year survival rate from all stages of oesophageal cancer remains 20-25%. A US study involving Surveillance, Epidemiology and End results (SEER) database analysis of 62,523 patients with cancer of the oesophagus suggested that 10 year survival is only 14%. Patients younger than 65 years and those receiving surgical therapy have the highest chance of survival[54]. The presence of a systemic inflammatory response is a prognostic indicator in patients with this type of cancer. This inflammatory response is linked to the development of atrial fibrillation in up to 30% of patients undergoing oesophagectomy[55]. This was a large retrospective study, however it is difficult to say whether the findings in a US population, can be fully extrapolated to a UK population due to differences in deprivation, ethnicity and access to healthcare.

1.11 Atrial Fibrillation

A normal heart pumps blood in a synchronised manner from the atria to ventricles. This regular or sinus rhythm leads to electrical activity that can be recorded in the form of an electrocardiogram (ECG). An abnormal rhythm or arrhythmia results when the synchronicity of the atria and ventricles is impaired. Many arrhythmias exist which affect both the atria and ventricles but the most commonly occurring rhythm disturbance in the general population is atrial fibrillation (AF). At a consensus conference in Scotland in 2012 it was stated that in our society, the incidence of AF is greater than 6% in those over 65 years of age[56]. It is a significant public health issue as it is associated with an increase in rates of stroke, other thrombo-embolic events, heart failure, hospitalisations and death[57].

AF is an abnormal heart rhythm which is characterised by rapid and irregular beating of the atria. It is defined as having three main characteristics including irregular RR intervals, no distinct P waves on the ECG and when the atrial cycle length is visible, it is usually variable;

less than 200ms or greater than 300bpm. Figure 1.4 is an ECG illustrating AF and Figure 1.5, is an ECG demonstrating sinus rhythm.

Fig 1.1 & 1.2 Normal cardiac conduction pathways and ECG trace and abnormal conduction pathways in atrial fibrillation[58]

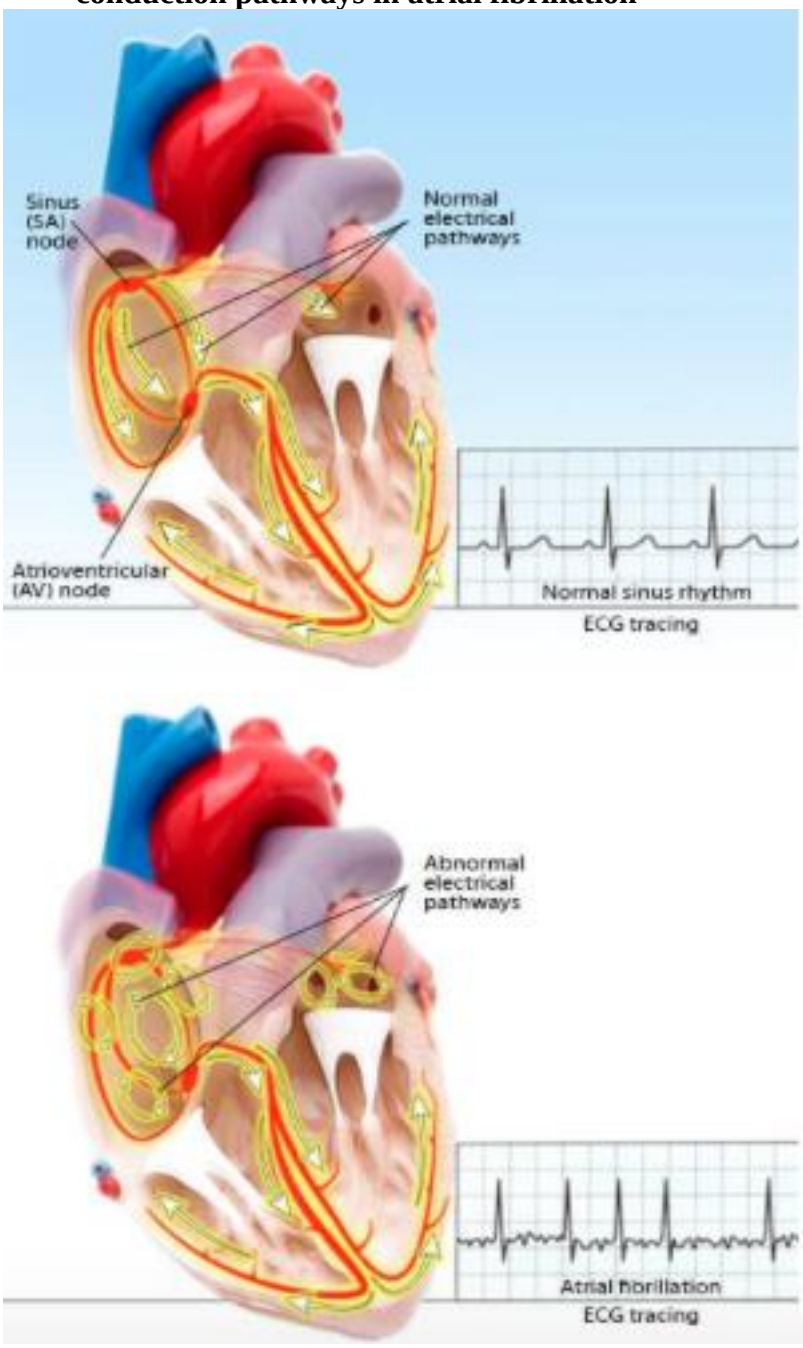


Figure 1.3: ECG waves and R-R interval[59]

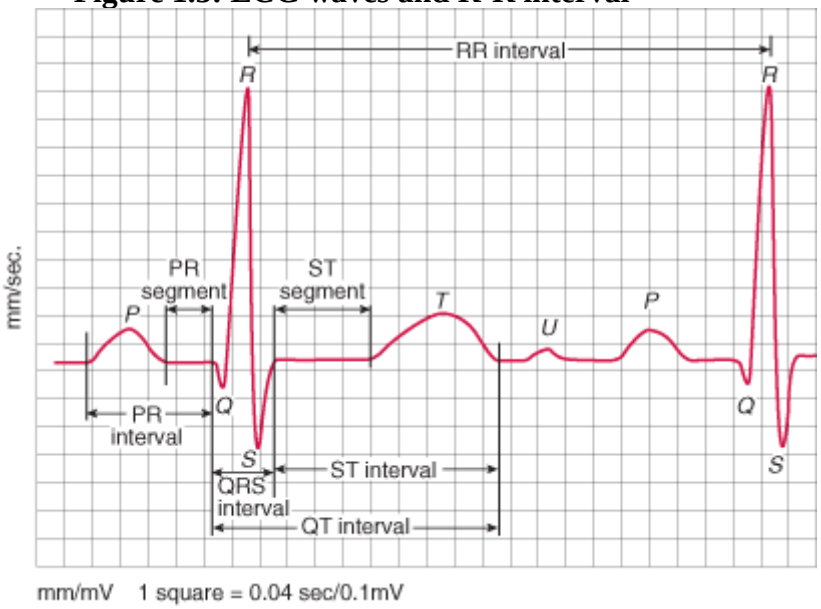


Figure 1.4: 12 lead ECG demonstrating AF rate of approximately 150 bpm[60]

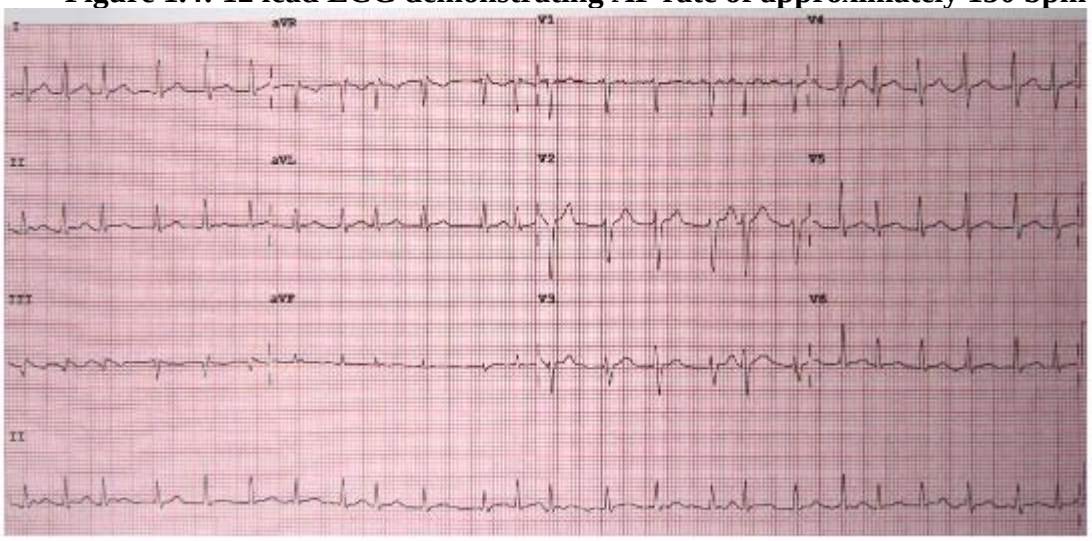
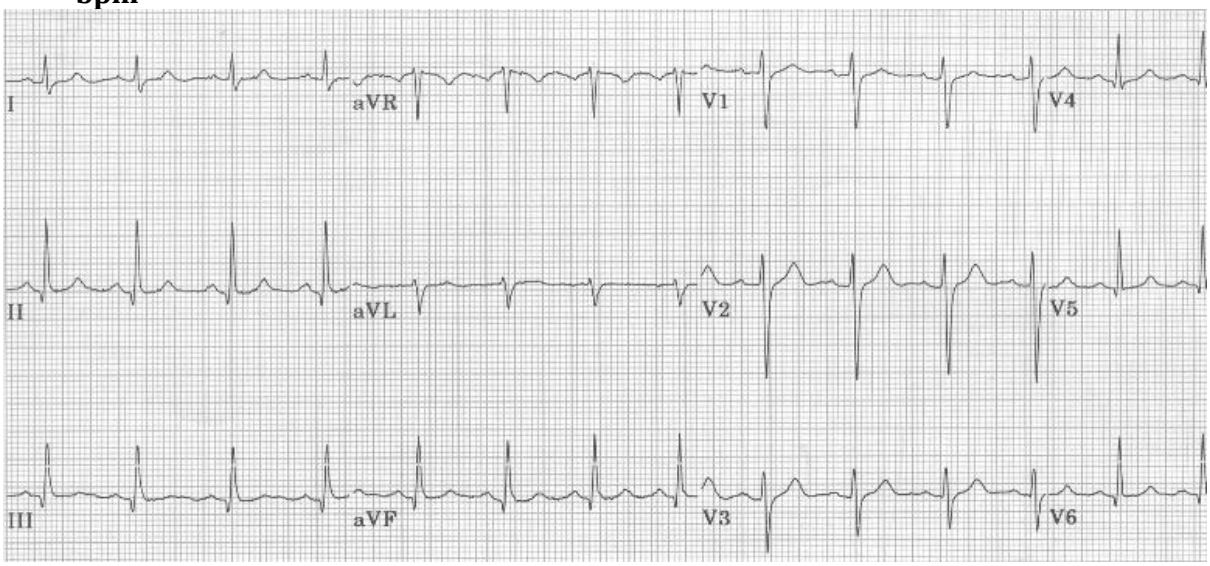


Figure 1.5: 12 lead ECG demonstrating sinus rhythm rate approximately 75 bpm[61]



There are five types of AF based on the presentation and duration of the arrhythmia. These include permanent, long-standing persistent, persistent, paroxysmal and first diagnosed[57]. Patients often do not have symptoms with atrial fibrillation, however, some may suffer from palpitations, lightheadedness, chest pain and shortness of breath.

1.12 Risk factors for atrial fibrillation

AF is linked to cardiovascular disease but also can occur in hearts that are otherwise normal. Risk factors for development of chronic or paroxysmal atrial fibrillation in the general population include obesity, having a pericardial fat pad,[62] being male, white and being older. Cardiovascular factors associated with AF include hypertension, coronary artery disease, pericarditis, previous cardiac surgery, mitral stenosis, mitral valve regurgitation and ischaemic heart failure. In terms of risk factors for cardiovascular disease, which may precede AF, the data is not consistent for the association of triglycerides, high and low-density lipoprotein (LDL) cholesterol and cholesterol with AF. A recent study in the community noted that higher concentrations of LDL and total cholesterol were associated with lower incidence of AF[63].

Experimental studies suggest that long-chain n-3 polyunsaturated fatty acids (n-3 PUFAs) may reduce the risk of AF. The n-3 PUFAs are eicosapentaenoic acid and docosapentaenoic acid and docosahexaenoic acid. As part of the Cardiovascular Health Study which involved 3326 US men and women > 65 years old and AF free at baseline, higher circulating total long-chain n-3 PUFA and docosahexaenoic acid levels were associated with a lower risk of incident AF when evaluated non parametrically[64]. Respiratory diseases such as lung cancer, pulmonary embolus and pneumonia also play a role in its aetiology. Endocrine disorders including diabetes and hyperthyroidism are also associated with an increased risk of developing AF[65]. Renal problems such as Chronic Kidney Disease (CKD), regardless of severity have been associated with an increased prevalence of AF[66]. Patients who are admitted to Intensive Care Units (ICU) with sepsis are at risk of developing new onset AF. The development of this rhythm is independently associated with increased mortality[67,68].

1.13 Prevention of development of atrial fibrillation

Most clinical efforts have been aimed at preventing the complications of AF. As we have a limited understanding of the pathophysiology of atrial fibrillation and lack of preventive strategies in the general population, up to this point we have concentrated efforts at modifying cardiovascular risk factors as much as possible. This has proved useful for explaining some of the population attributed risk factors, but novel risk factors are still being studied. A better understanding of the other factors that underlie the development of AF is required and genomics and monitoring of electrocardiographic precursors of AF are evolving areas of research. Genome-wide association studies have successfully identified 3 genetic loci, amongst others (4q25 near transcription factor *PITX2* and 16q22 near *ZFHX3* and 1q21 at the small-conductance calcium-activated potassium channel *KCNN3*)[69]. The truth is that knowledge of risk prediction in AF and primary prevention is still limited[65].

1.14 Atrial fibrillation, inflammation and surgery

As mentioned previously, patients can develop atrial fibrillation post-operatively which can significantly affect morbidity and mortality. Out of 370,447 patients in the USA who underwent major non-cardiac surgery at 375 US hospitals in 2008, those that developed post-operative atrial fibrillation (POAF) had significantly increased length of stay and significantly increased mortality rates of 14.1% compared with 2.1% in those who did not develop AF ($p < 0.01$)[70].

Following cardiothoracic surgery, AF occurs in up to 60% of patients following Coronary Artery Bypass Grafting (CABG) procedures, whereas in non-cardiac thoracic surgery, the incidence of atrial arrhythmias ranges from 9 to 46%[71,72]. The morbidity and mortality following the development of AF in patients may be explained by the decrease in cardiac output and blood pressure that occurs in many patients due to reduced filling of the left ventricle. The presence of rapid ventricular response rates can result in haemodynamic compromise and heart failure. AF has also been associated with the development of intra-cardiac thrombi, which can pose a subsequent risk of systemic embolisation and stroke[73].

Biochemically, AF is associated with inflammatory activation and impairment of nitric oxide signalling[74]. Investigations have reported associations between AF, circulating levels of cytokines, CRP, complement and the activation state of leucocytes. However, whether inflammation is the cause or consequence of AF and which inflammatory mediators may increase the atria's susceptibility to fibrillation remain elusive.

The link between inflammation and AF was initially made by observing an increased frequency of AF after coronary artery bypass surgery. The peak incidence of AF occurred on the second and third post-operative day, coinciding with the peak elevation of CRP[75,76].

To support the link between inflammation and AF, when right atrial appendages have been taken from patients in permanent AF who are undergoing cardiac surgery and compared with those from patients in normal sinus rhythm, there was evidence of inflammatory infiltrates

within the atrial tissue. Additionally, there was oxidative damage and endothelial dysfunction in those with AF. Surgery causes a depletion of plasma antioxidants and this, combined with the associated acute inflammatory process is known to affect the electrophysiological properties of the myocytes in the atrium[77]. A retrospective observational study of 583 patients published in 2016 showed that development of POAF in oesophagectomy patients independently predicted increased mortality a year following hospital discharge[78]. There are multiple factors involved in the inflammatory process and a relatively small retrospective study is subject to confounding and due to its nature, some risk factors associated with increased inflammation may not have been measured. Additionally, retrospective studies can only determine association and not causation.

Table 1.3 Risk factors for atrial fibrillation post-operatively[79]

System	Aetiology	Assessment
Respiratory	Hypoxia, pneumonia,pleural effusions, thromboembolic disease	Pulse oximetry, arterial blood gas (ABG), CXR
Cardiovascular	Underlying ischaemic heart disease,valvular heart disease, sinus node disease	12 lead ECG, Echocardiogram, stress testing
Electrolyte disturbances	Low potassium, magnesium and calcium levels and high potassium levels	Blood biochemistry testing
Metabolic	Acidosis, thyrotoxicosis, diabetes mellitus	ABG, thyroid function tests,blood glucose levels, liver function
Others	Hypovolaemia, increasing age	Fluid status

1.15 Peri-operative prevention of atrial fibrillation

A Cochrane review of data from 58 trials including over 8500 patients acknowledged that beta blockers, amiodarone and atrial overdrive pacing all reduce the risk of post-operative AF in patients undergoing cardiac surgery[80]. A trial published in 2005 called Prophylactic Oral Amiodarone for the Prevention of Arrhythmias That Begin Early After Revascularization, Valve Replacement, or Repair (PAPABEAR) reduced the post-operative risk of AF by 50%

in young and old patients. The problem was that nearly a week of pre-treatment with amiodarone was specified. Additionally, amiodarone was given to patients with a variable risk of developing AF[81]. The problem with this is exposing a significant number of patients to the adverse effects of this therapy, which can include peripheral neuropathy and pneumonitis. This reinforces the need for pre-operative stratification of patients at risk of developing AF.

1.16 Oesophageal cancer surgery and atrial fibrillation

In patients with oesophageal cancer who undergo oesophagectomy, cardiac complications can occur in up to 40%. Atrial arrhythmias were the most frequent complication and occurred in 33% of patients in one study which looked at 146 patients over a 16 year period undergoing either Minimally Invasive Oesophagectomies (MIE) and also 'open' procedures[82]. The most frequently occurring atrial rhythm is atrial fibrillation which can occur in up to 30% of patients. Several factors have been found to be associated with the development of post-operative arrhythmias and the pre-operative risk factor that is persistently identified for post-oesophagectomy atrial fibrillation (poAF) is advanced age. Age can lead to chronic, progressive re-modelling of the heart including changes such as left atrial enlargement. Other factors associated with increased risk of poAF include being male, having pre-operative chemoradiotherapy, a history of COPD and a history of cardiac disease. Acute peri-operative factors include inflammation, sympathetic activation and oxidative stress. Additionally, damage to the vagus nerve can also be a factor[55]. Stawicki and colleagues found that in the multivariate analysis of a retrospective cohort of 156 oesophagectomy patients, anastomotic leaks, pulmonary complications and a number of other complications were significantly associated with AF[83]. It is useful if we briefly consider different types of surgery and their association with onset of POAF. In the majority of studies looking at cardiac surgery, POAF peaks during the second post-operative day and then declines. In this setting there is clearly a role for surgical trauma, inflammation and structural heart disease in its development which is associated with prolonged ICU and hospital

admissions and an increase in hospital mortality[83,84]. As mentioned, a retrospective study of 583 patients has shown that the development of POAF in an oesophagectomy patient group independently predicted increased mortality a year following discharge from hospital[78]. The development of POAF has also been shown to independently predict long term survival following non-oesophageal thoracic surgery such as coronary bypass, lung transplantation, aortic valve replacement and pulmonary lobectomy[55].

Following oesophagectomy most patients develop POAF within the first 72 hours. This is reinforced by a retrospective review of 156 patients undergoing oesophagectomy over an 11 year period in the USA[83]. It follows that it might be useful to monitor markers of inflammation in patients during this period and ascertain whether there is any relationship between these and the development of POAF. Echocardiographic studies of atrial size and specifically increased left atrial diameter in relation to AF are associated with an increase in markers of inflammation[77]. Correlating echocardiogram findings with evidence of inflammation in oesophagectomy patients with AF may serve to enhance understanding of the pathophysiological processes involved in its development. Despite this, until recently, there has been little evidence regarding longer-term prognosis in oesophagectomy patients who develop this rhythm.

It may be that POAF also predicts longer-term survival in patients undergoing oesophagectomy for oesophageal cancer. This was suggested by a recently published 21 year retrospective observational study from New Zealand in 89 patients undergoing Ivor Lewis oesophagectomies for cancer. Ivor Lewis oesophagectomies are performed by removing the oesophageal tumour through an abdominal incision and a right thoracotomy. The study suggested that post-operative AF may be an independent predictor of poorer longer-term survival. This may be because new onset atrial fibrillation is a marker of severity of disease[55]. The retrospective study has limitations, one of which is its size and therefore difficulty

eliciting what the implications of a small study should be. If new onset AF is indeed a predictor of poorer long-term survival, then studying markers of inflammatory change peri-operatively in patients undergoing oesophagectomy may help to shed light on some of the ways in which these biomarkers may pre-dispose them to developing POAF. Ultimately, an increased understanding regarding the onset of atrial fibrillation in this group of patients could potentially lead to not only decreased morbidity but also decreased mortality by means of contributing to an optimal strategy to help prevent this arrhythmia.

1.17 Previous departmental studies regarding atrial fibrillation

In the Intensive Care Unit (ICU) of Glasgow Royal Infirmary, which is part of a tertiary teaching hospital in Scotland, a retrospective observational cohort study has been carried out with respect to the incidence of first diagnosis and pre-existing AF and outcomes. Over a 41-month period, 193 patients with atrial fibrillation were admitted to the ICU and data was analysed according to their peak inflammatory markers and outcomes. A sub-group analysis of 100 surgical patients with AF assessed haemodynamic factors, inflammatory markers and their association with outcomes. Hospital mortality in the surgical patients with first diagnosis AF was 44.16% compared with 21.74% in those with pre-existing AF. The AF patients that died in hospital spent a longer period of time at a systolic BP ≤ 90mmHg compared with those patients with AF who survived. They also tended to have a heart rate that was greater than 100 beats per minute (bpm) for a longer period of time. Additionally, the AF patients that died had a higher peak CRP than those that survived. Overall hospital mortality in patients with new onset AF was 54.1% compared with 30.4% in those patients with pre-existing AF. Limitations of this study included the variation in the cohort studied, which included surgical and medical patients and potential confounding factors that had not been fully accounted for such as age, sex and medications that patients were taking. However, despite these limitations, this study reinforced the need for further work regarding

investigation into why patients were developing AF and how this arrhythmia might be predicted and prevented.

1.18 Biomarkers, inflammation and atrial fibrillation

Biomarkers are characteristics of an organism that reflect a particular physiological state and in medicine they can be compounds which are isolated from serum, urine or other fluids that can be used as an indicator of the presence of a particular disease state. Biomarkers hold great promise for personalised medicine as information can be used to titrate treatment for the individual. In the setting of AF they could be used as novel instruments to enhance prediction and also to help understand the pathophysiology of the disease[85]. In a community-based population of 5445 older adults NT-proBNP was a strong predictor of incident AF, adjusting for other risk factors including age, sex, medication use and blood pressure (adjusted HR, 4.0; 95% CI, 3.2-5.0; p <0.001)[86].

Schnabel et al[87] chose a panel of 10 candidate AF biomarkers which represented pathophysiological processes including inflammation (CRP and fibrinogen), endothelial dysfunction (homocysteine) and neurohormonal activation (BNP and NT proatrial natriuretic peptide). In 3120 Framingham heart study patients[65] they found that the panel was associated with incident AF (p <0.0001). After multivariable adjustment, CRP (p=0.004) and log transformed BNP (p <0.0001) remained associated with AF. Adding BNP and CRP separately and together to an AF risk score based on clinical covariates demonstrated that only BNP improved risk stratification beyond clinical risk factors that were well established.

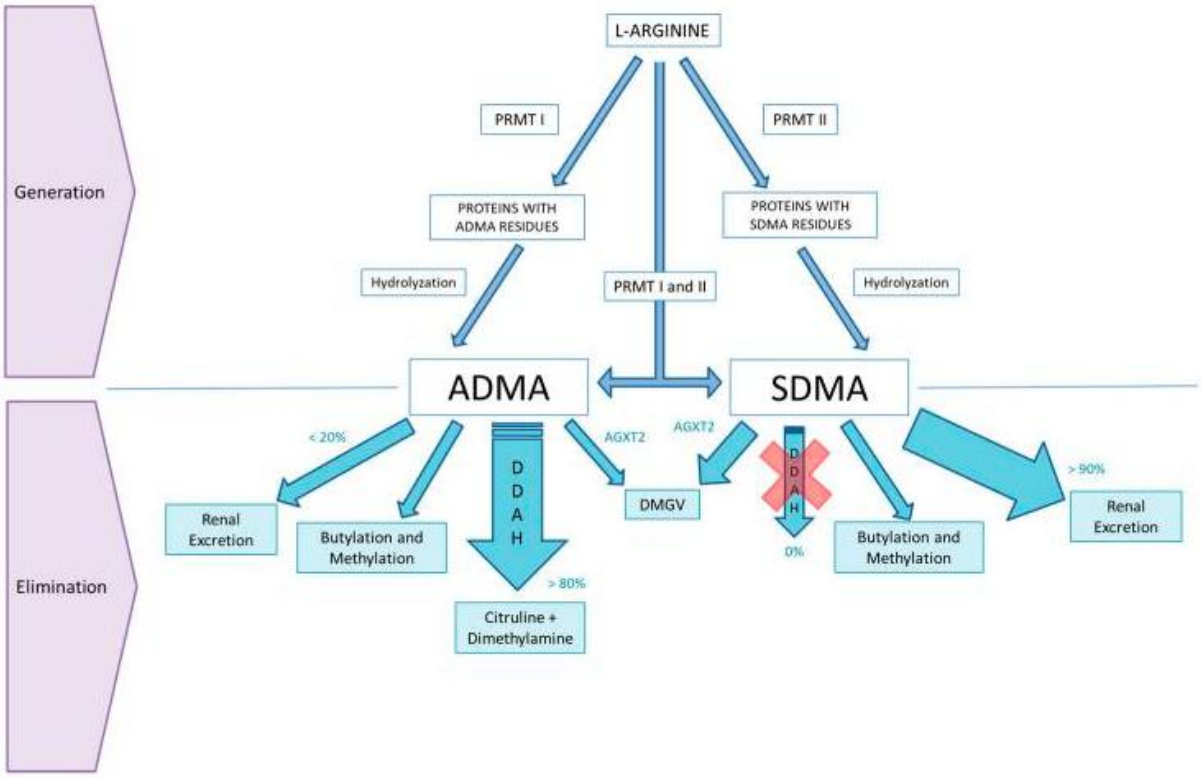
Asymmetrical dimethylarginine (ADMA), Symmetrical dimethylarginine (SDMA), homoarginine, myeloperoxidase (MPO), interleukin 6 (IL-6) and BNP are biomarkers which have been associated with cardiovascular outcomes. An elevated pre-operative BNP measurement is an independent predictor of cardiovascular events in the first 30 days after

noncardiac surgery. BNP has been investigated as a biomarker for incident AF and elevated levels have been found in patients with AF.

1.19 Asymmetrical dimethylarginine (ADMA)

ADMA is a derivative of arginine which is a semi essential, naturally occurring amino acid that is found in all protein containing foods. Arginine is a precursor for the synthesis of nitric oxide. Increased serum levels of ADMA have been associated with AF recurrence in patients with persistent AF. Plasma concentrations of ADMA have been shown to be elevated in patients with heart failure, diabetes, hypertension, renal impairment, cardio-embolic cerebrovascular disease and in persistent AF[88,89].

Figure 1.6 Outline of dimethylarginine synthesis and metabolism[90]



1.20 Arginine, dimethylarginines, nitric oxide and nitric oxide synthase

Arginine is a semi-essential amino acid and in addition to dietary intake it is released endogenously as a result of protein degradation. It is also synthesised from citrulline by the sequential action of the cytosolic enzymes argininosuccinate synthetase (ASS) and

arginosuccinate lyase (ASL). It exists in two isomeric forms, dextro (D) and laevo (L)arginine. L-arginine is the form that is of interest. Arginine is used as a substrate in metabolic pathways including degradation by the enzyme arginase to urea and ornithine and also in the production of nitric oxide (NO). In healthy volunteers intravenous arginine infusion decreased systolic and diastolic blood pressure and increased heart rate and plasma catecholamine levels[91].

Nitric oxide is a key regulator of cardiovascular endothelial function. It inhibits the adhesion of inflammatory cells to the vascular wall, the aggregation of platelets and the proliferation of smooth muscle cells. Reduced synthesis and reduction in availability of NO has been recognized in combination with risk factors for cardiovascular disease and may promote hypertension, thrombus formation, atherogenesis and atrial fibrillation. NO and citrulline are synthesized from L-arginine by nitric oxide synthase (NOS). Mechanisms that may lead to endothelial dysfunction include reduced nitric oxide synthase (NOS) expression and activity, decreased NO bioavailability and increased production of oxygen radicals and endogenous NOS inhibitors[92]. The breakdown products of NO are found in the urine of patients.

There are three different isoforms of NOS; neuronal NOS (nNOS), inducible NOS (iNOS) and endothelial NOS (eNOS). Cardiac tissue normally expresses nNOS and eNOS, whereas iNOS is inducible and is only expressed in inflammatory or pathological states such as hypertrophy or heart failure[93].A reduction in endothelial nitric oxide synthase (eNOS) in the cellular region with platelet adhesion and thrombus formation suggests endothelial dysfunction and this may contribute to thrombogenesis in AF.

Symmetric dimethylarginine (SDMA) and asymmetric dimethylarginine (ADMA) are formed from the methylation of L-arginine residues in proteins by enzymes called protein arginine methyltransferases (PRMT) see figure 1.6. Both PRMT 1 and PRMT 2 are associated with the production of SDMA and ADMA.

In forming SDMA and ADMA, N monomethyl L-arginine (NMMA) is formed as an intermediate product as a result of protein incorporated arginine being methylated. They also originate from protein degradation.

SDMA has been shown to have weak inhibitory potency towards nNOS and may compete with L-arginine for cellular uptake, therefore limiting NO production[94]. NMMA and ADMA are endogenous inhibitors of NOS, however ADMA is thought to be most significant physiologically as only small amounts of NMMA exist in plasma[95,96]. ADMA competitively inhibits nNOS and eNOS and in prospective studies it has been confirmed to be an independent risk factor for cardiovascular disease. Evidence suggests that the amount of NO produced may be indicated by the ratio between arginine and ADMA[97,98].

The inhibition of NOS by ADMA is an intracellular process, however, despite clinical studies frequently reporting on plasma ADMA levels, there are few studies concerning the relationship between plasma and intracellular levels of ADMA. ADMA competes with L-arginine and homoarginine for NOS binding and causes NOS uncoupling which leads to impairment of NO synthesis. Uncoupling of NOS means electron transfer is shifted from L-arginine to a produce a superoxide anion (O_2^-). Tetrahydrobiopterin (BH4) is a co-factor for NOS activity and in times of low BH4 and arginine bioavailability relative to NOS or BH4, (O_2^-) is produced and leads to NOS uncoupling to 7,8 dihydrobiopterin (BH2). This superoxide reacts with NO to produce peroxynitrite ($ONOO^-$) which continues to propagate NOS uncoupling. Depletion of BH4 and NOS uncoupling contribute to hypertension, heart failure and atrial fibrillation[99]. Peroxynitrite formation in human atrial fibrillation has been examined and 3-nitrotyrosine, which is a marker for $ONOO^-$ formation is increased in atrial tissues from patients with persistent AF[77]. There was also induction of iNOS and increased 3-nitrotyrosine expression in the right atrium in patients with permanent AF compared with those with normal sinus rhythm[100].An additional reversible pathway contributing to NOS

uncoupling that has recently been described is S-glutathionylation of eNOS, where glutathione is bound to a protein thiol[101].Acute inhibition of endogenous nitric oxide production may lead to vasoconstriction and contribute to the thrombotic events by causing platelet activation[102,103].

ADMA is mainly metabolized by the enzyme dimethylarginine dimethylaminohydrolase (DDAH) and to a lesser extent alanine glyoxylate aminotransferase 2 (AGTX2). Renal excretion represents a less important route of excretion for ADMA. SDMA is metabolised by AGTX2 to (α-keto-δ-(N,N-dimethylguanidino) valeric acid (DMGV) and its major route of excretion is by the kidneys. DDAH has two isoforms; DDAH 1 and DDAH 2. DDAH 1 is located mainly in the proximal renal tubules and liver and studies have shown significant elimination occurring via these organs. DDAH 2 is found in smooth muscle cells and vascular endothelial tissue, where it is probable that it has an effect on vascular responses and ADMA concentrations. DDAH has been estimated to metabolize approximately 80% of the 300 micromoles of ADMA generated daily in humans. In animal studies, on 7 mongrel dogs, increased PRMT-1 protein expression localized to the left atrium and left atrial appendage and decreased DDAH activity in the atria in these dogs who had AF induced may cause an elevation of plasma ADMA level [104]. Impaired activity of DDAH has been related to inflammation, oxygen free radicals and endothelial injury. The breakdown of ADMA by DDAH produces citrulline and dimethylamine. Due to the liver and kidneys being largely responsible for clearance of ADMA, dimethylamine, which is the major metabolite, can be measured in the urine[105].Plasma ADMA can accumulate due to increased synthesis, reduced renal clearance or inhibition of its degradation by dimethylarginine dimethylaminohydrolase (DDAH) and alanine-glyoxylate aminotransferase 2 (AGXT2). Due to the distribution of ADMA and SDMA between compartments being governed by system y+ which is one of the cationic amino acid (CAA) transport systems or transporters (CAT) the extracellular and

intracellular compartments are not in equilibrium and cellular concentrations of ADMA can be ten or twenty times greater than those in plasma. Elevated ADMA plasma levels have been found in critically ill patients and a reduced ratio of L-arginine to ADMA is associated with atherosclerotic disease and all-cause mortality[95].

A double-blinded randomised placebo controlled study in 12 healthy volunteers demonstrated that those having an intravenous infusion of ADMA (3mg/kg up to 250mg) had a reduced heart rate ($p< 0.001$), cardiac output ($p< 0.001$) an higher mean blood pressure ($p< 0.005$) and systemic vascular resistance ($p< 0.001$) compared with those having a placebo infusion (sterile physiological saline)[106].

In terms of effects of an operation on dimethylarginine levels, a study of 38 patients undergoing an elective knee arthroplasty demonstrated that plasma ADMA concentration decreased rapidly during the first 48 hours following surgery and SDMA level remained unchanged. These findings question whether this is due to the intracellular movement of ADMA via CAT and an increased partitioning of ADMA within cells. Also, an increase in SDMA levels in the urine from day 1 post-operatively, suggests increased net production or mobilization from cells. A decrease in ADMA and arginine concentrations was associated with a decrease in urinary nitrate levels (used as a surrogate marker of NO metabolism) and a transient decrease in urinary dimethylarginine (DMA). In light of the increased SDMA levels in the urine and the concurrent reduction in urinary nitrate levels, this may support the hypothesis that SDMA decreases NO availability[94]. In a study of 32 male Wistar rats, who underwent either left anterior descending coronary artery ligation to induce myocardial infarction or sham operation, ADMA concentration within the left ventricle was increased one week after infarction. This was unexpected, considering the increase in DDAH levels in the left ventricle. The reason for the increase in ADMA levels was not accounted for by an increase in local synthesis, as the expression of the PRMT enzyme was not elevated. It

suggests that NOS activity will be partially inhibited by ADMA in vivo. Plasma ADMA was also increased after MI however the cause of this was unclear and further studies are required to confirm the source of ADMA. Some limitations of this study include small study numbers and the fact that it involved animal models[105].

Endothelium-dependent vasodilatation is improved after L-arginine administration in patients with congestive heart failure who had elevated ADMA concentrations. However, L-arginine did not affect endothelium-dependent vasodilation in healthy human subjects who had low ADMA concentrations.

In a Scandanavian study of 258 healthy blood donors, methylarginine levels were analyzed using high performance liquid chromatography (HPLC). A reference range for plasma ADMA levels was 0.4-0.77 micromoles/ litre for the whole population. In women aged greater than 45 years the range was 0.41-0.84 micromol/L compared with 0.38-0.73 micromol/L in those less than 45 years. This age related difference was not present in men[107].

1.21 Asymmetric dimethylarginine and AF

In a study of 42 patients with atrial fibrillation, single sample ADMA levels were higher in those with first diagnosis AF for < 24hr (n=17), compared with those who had pre-existing AF for > 1yr (n=25) (p=0.002). ADMA concentration was evaluated in a prospective cohort study of 138 patients with persistent AF who had blood taken 1 day prior to catheter ablation and were monitored for up to 258 days assessing recurrence of AF (58%). In the multivariable Cox regression model higher serum ADMA, Hazard Ratio (HR): 4.59 (95% CI, 1.81–11.62; p=0.001) and increase in left atrial diameter HR 1.35 (95% CI, 1.18–1.55; p<0.001) were independent factors associated with AF recurrence post catheter ablation[108]. This was another small study and despite a long follow up duration there are multiple markers of inflammation that were unable to be measured which inevitably would have an effect on the development of AF. Another study which was a double blinded, placebo controlled study of 171 patients, ensured that patients with persistent AF were randomised to

receive candesartan 8 mg once daily or placebo for 3–6 weeks before cardioversion and candesartan 16 mg once daily or placebo for 6 months after cardioversion. Plasma levels of *L*-arginine and ADMA were measured at baseline and at the end of the study. It demonstrated that AF patients who remain in sinus rhythm post cardioversion have increased L-arginine to ADMA ratios after 6 months compared with those who have recurrent AF and that candesartan treatment has no effect($p=0.008$)[103].

To help understand what effect the acute inflammatory response following an operation has on daily biomarker levels in the absence of the development of AF, two recent studies have found that ADMA declines initially and then begins to rise[105]. One single centre prospective German study investigated the changes in plasma L-arginine, ADMA, C-reactive Protein (CRP) and IL-6 in 24 healthy living kidney donors. ADMA levels decreased compared with baseline (0.488 ± 0.075 vs. 0.560 ± 0.060 μmol/l, $p < 0.05$). One day after the operation the change was more significant compared with baseline levels (0.478 ± 0.083 μmol/l, $p < 0.01$). This correlated with a peak in IL-6 levels and CRP. L-arginine levels decreased 1 hour after nephrectomy compared with baseline (97.5 ± 22.5 μmol/l $p< 0.01$). Changes in L-arginine were similar to changes in ADMA after this point. After 168 hours when the inflammatory markers were returning to normal, ADMA and L-arginine levels were both elevated compared with baseline ($p< 0.001$). It was unclear whether the reduction in ADMA after unilateral nephrectomy could be the reduction of ADMA synthesis and proteolysis pathways. Limitations of this study include its small size and the lack of data regarding urine concentration of the compounds studied[109].

1.22 Homoarginine, arginine and nitric oxide

Homoarginine is an endogenous amino acid and low circulating concentrations of it have been reported to be a prognostic marker for mortality and cardiovascular events in a variety of diseases, including heart failure, stroke and CKD[110,111]. Homoarginine has an additional methylene group in the carbon chain compared with arginine. In an observational cohort of

1818 patients, 1649 of whom were studied at 3 German tertiary care centres with chest pain, low plasma homoarginine was identified as a risk marker for incident major adverse cardiovascular events (MACEs) including all cause death, myocardial infarction or stroke. Impaired homoarginine was also associated with prevalent AF[112]. It was unclear whether homoarginine levels were lower because of the presence of AF or whether it preceded the development of AF. In two large cohorts of patients, levels of homoarginine were independently associated with cardiovascular and all cause mortality in patients referred for coronary angiography and those undergoing haemodialysis. In one cohort, 3305 patients who were undergoing coronary angiography had levels of homoarginine taken at baseline and were followed for a median of 9.9 years. In total, 991 patients died, including 258 sudden cardiac deaths, 148 heart failure deaths and 105 fatal myocardial infarctions[113].

Homoarginine may also have a role to play in increasing the availability of NO by acting as a competitive substrate of NOS, along with L-arginine. Conversely, by competing with arginine for cellular uptake, high homoarginine levels may limit the amount of intracellular arginine and therefore production of NO may be reduced at a high homoarginine to arginine ratio.

1.23 B type Natriuretic Peptide (BNP) and N-Terminal Pro B Type Natriuretic Peptide (N-T proBNP)

Brain or B type natriuretic peptide is one of a family of cardiac peptides which is secreted by the myocardium of the left ventricle in response to wall stress in association with ventricular dilatation and pressure overload[114]. Small amounts of a precursor protein (pre pro-BNP) are continuously produced by the heart and after a volume or pressure overload, it is cleaved into an 108 amino acid (AA) pro BNP. Pro BNP is then cleaved to release the active hormone BNP (32 AA) and an inactive fragment NT-pro BNP (76 AA) into the blood. BNP has physiological effects on the heart, kidneys, adrenal glands and adipose tissue. At a renal level, it inhibits renin and aldosterone which leads to an increase in natriuresis (sodium excretion)

and diuresis (increased urination). In the central nervous system the inhibition of salt and water intake, in addition to vasopressin secretion takes place which leads to a decrease in systemic vascular resistance via relaxation of vascular smooth muscle and a decrease in cardiac output. Due to the association of BNP with myocardial stress, it has been developed as a biomarker of cardiovascular failure, with levels of BNP being directly related to left ventricular mass and inversely related to ventricular ejection fraction. In a study looking at determinants of NT pro BNP; consecutive patients were recruited in an Emergency department or an outpatient clinic of the same hospital. There were 45 patients with paroxysmal AF, 41 patients with permanent AF and 48 controls. Plasma NT-pro-BNP levels were significantly higher in patients with paroxysmal and permanent AF compared to those with sinus rhythm in the setting of preserved left ventricular systolic function. NT pro-BNP levels were independently predicted by left ventricular ejection fraction (LVEF) and left atrial diameter (LAD) on echocardiogram, in patients with paroxysmal and permanent AF[115].
The relation between N-terminal pro-B-type natriuretic peptide (NT-proBNP) and AF was studied in 5,445 Cardiovascular Health Study (CHS) participants. The CHS was a longitudinal study of 5,888 men and women aged 65 and older, who were randomly selected from 4 communities in the United States and enrolled during two time periods; 1989–1990 and 1992–1993. It showed that elevated peri-operative plasma NT pro-BNP level is an independent predictor of AF in the community independent of any other previously described risk factor [116]. In a prospective observational cohort study of 142 patients it was demonstrated that elevated peri-operative NT-proBNP plasma levels in oesophagectomy patients are an independent predictor of post-operative AF[117]. Again this is a small study, but one of the advantages of prospective cohort studies, is that they reduce the possibility that the results will be biased by selecting subjects for the comparison group ie. the patients who develop AF

vs ones that do not, because in a cohort study the outcome is not known at baseline when exposure status is established.

There have been recommendations from the European Society of Cardiology and Anaesthesiology regarding the use of BNP in pre-operative testing for high-risk cardiac patients undergoing non-cardiac surgery. However, further research is required to determine the benefits of BNP guided management as evidence is lacking[118,119].

There are several causes of raised BNP, see table below.

Table 1.4 Potential causes of raised BNP levels[120]

Cardiac	Non cardiac
Heart failure	Acute Pulmonary Embolus
Diastolic dysfunction	Pulmonary hypertension
Acute coronary syndromes	Sepsis
Hypertension with Left Ventricular hypertrophy	Chronic obstructive pulmonary disease
Valvular heart disease	Hyperthyroidism
AF	Acute or chronic kidney injury

1.24 Potential uses of BNP and NT-proBNP measurement and laboratory testing

BNP assays are a potential aid in the diagnosis of heart failure, as levels correlate closely with the New York Heart Association (NYHA) classification of heart failure. BNP testing allows a rapid assessment for those patients warranting an echocardiogram and also has the potential to enable rapid changes in therapy for those receiving treatment for failure. In a pilot study, BNP levels were the single most reliable variable in predicting short term outcomes in patients with heart failure. In a prospective study of 3346 people without heart failure, increased levels of BNP have been shown to be associated with an increased risk of death, atrial fibrillation and cardiovascular events in people after adjustment for traditional risk factors. Excess risk was apparent at levels well below thresholds used to diagnose heart failure. The strengths of this study include the large, community-based sample, the use of ongoing surveillance for multiple outcomes according to standardized criteria and the use of

high-sensitivity assays. Another consideration is that the Framingham Study cohort is of predominantly white ethnicity and the results may not be generalizable to other populations. Due to the size of the study there was limited statistical power to perform separate analyses of high-risk subgroups, such as people with diabetes. As with other studies, the findings should be confirmed in other cohorts[121].

Brain natriuretic peptide may help in identifying people at risk of stroke and atrial fibrillation. In a representative population of 958 men (46-65 years) from Finland who were followed up for 9.6 years, the multivariable adjusted risks for any stroke or ischaemic stroke for log-transformed standard deviation (SD) (0.237 pmol/l) increment in N-terminal fragment of proB-type natriuretic peptide, the respective risks were 1.36-fold (95% CI 1.05 to 1.76, p = 0.010) and 1.50-fold (95% CI 1.12 to 2.02, p = 0.007). The multivariate adjusted risks for future atrial fibrillation were 1.68-fold (95% CI 1.38 to 2.07, p< 0.001) for each log-transformed SD increment in N-T pro B-type natriuretic peptide. This suggested that N-terminal fragments of pro B type natriuretic peptide might help in identifying subjects at risk for stroke and AF[122].

To give some background context regarding BNP values with respect to patients who have heart failure; the threshold value for this is different in various laboratories but the suggested decision threshold for the Abbott AxSYM® platform assay is 100pg/ml for acute symptoms[123].A study was performed to compare the Abbott ARCHITECT® platform BNP assay to the AxSYM® BNP assay in 171 individuals with and without heart failure. The results showed a significant correlation between the two methods[124].More recently the ARCHITECT® BNP assay was compared with other automated BNP assays including AxSYM® with which it correlated well[125].Comparatively the expected BNP values in the non-heart failure population based on 465 females and 425 males with diabetes, hypertension, and chronic obstructive pulmonary disease (COPD) and renal disease, who were not on

dialysis, were not statistically significant compared with BNP values in a population of apparently healthy individuals.

1.25 NT-proBNP, BNP and AF

The relationship between NT-proBNP and AF was studied in 5445 Cardiovascular Health study participants. Of these, 5021 participants had no history of AF. A baseline NT pro-BNP > 290pg/ml (5^{th} quintile) had an AF prevalence of 11.75% at baseline, compared with those with a baseline of < 50pg/ml who had a prevalence of 0.1%. The unadjusted hazard ratio was 5.2 (95% CI 4.3-6.4; $p< 0.001$) for the development of AF in the highest quintile compared with the lowest. Following adjustment for covariates (including age, sex, medication use, blood pressure, diabetes and heart failure), NT-proBNP remained the strongest predictor of incident AF with an adjusted HR of 4 (95% CI, 3.2-5.0; $p< 0.001$) for the highest versus the lowest quintile. As mentioned earlier, it follows that NT-proBNP predicts AF in a community-based population of older adults[116].

As part of the Framingham study, it was confirmed that BNP levels predict the development of AF. The study included 3120 AF free participants, 54% of whom were women with an average age of 58 years. They were extensively investigated in 1995-1998 and were followed up for a median of 10 years. Ten biomarkers which might be important in development of AF were studied, including CRP and BNP. At the end of a median of 9.7 years, 209 (6.7%) of study participants had been diagnosed with AF. In addition to confirming the conventional risk factors for AF including age, sex, increased BMI and cardiovascular disease, the researchers noticed that those who developed AF had significantly higher baseline levels of CRP, BNP and Atrial Natriuretic Peptide (ANP). CRP was statistically significantly associated with the outcome of AF but did not markedly improve risk prediction beyond BNP. Correlates of BNP are left ventricular size and ejection fraction[115] but even after adjusting for interim cardiac disease and measures of left atrial diameter and systolic

function, BNP retained its strength of association with AF. The conclusion was that BNP is a useful predictor of future AF[87].

1.26 N-T Pro BNP and high sensitivity CRP (hs CRP) for cardiac risk stratification

A study of 592 patients listed for non-cardiac vascular surgery recorded the cardiac history, hs CRP and NT-proBNP, pre-operatively. Hs-CRP levels of at least 6.5mg/L and NT proBNP of 350pg/ml at least were defined as the optimal cut off values for the prediction of post-operative cardiac events. The end point was the composite of 30 day cardiovascular death, Q wave myocardial infarction (MI) and troponin T release. Troponin is a protein in the blood which is released into the bloodstream when the heart muscle has been damaged. Multivariable regression analysis evaluated the association between hs-CRP, NT-proBNP and the end point. Elevated levels of hs-CRP (OR 2.54; 95% confidence interval 1.50-4.30) and NT-proBNP (OR 4.78; 95% CI 2.71-8.42) remained independent risk factors for post-operative cardiac events. When hs CRP and NT proBNP were added to the cardiac risk score the C statistic improved from 0.79 to 0.84. This suggests that hs CRP and NT-proBNP have additional value in predicting post-operative cardiac events in vascular patients[126].BNP and NT-proBNP are useful pathophysiological markers of neurohormonal activation.

1.27 BNP, NT-proBNP and non-cardiac surgery

Many studies have examined the peri-operative role of BNP in elective non-cardiac surgery and there is an association between higher levels of BNP and NT-proBNP and risk of post-operative events[127] which suggest they could be a useful tool to stratify risk. However, various thresholds have been quoted for BNP and this is likely due to differences in age, gender, co-morbidity, BMI (lower levels are often seen in obese patients) and degree of pre-existing cardiac failure. Several studies have looked at determining the optimal values which could assist prediction of post-operative cardiac events including haemodynamic compromise from cardiac arrhythmias, heart failure, non fatal MI and cardiac death but the identification of a single universally applicable BNP point remains elusive. A meta-analysis including data

from 15 publications including 4,856 patients revealed that pre-operative BNP elevation was associated with an increased risk of short-term major adverse cardiovascular event (MACE) (OR 19.77; 95% confidence interval [CI] 13.18-29.65; p < 0.0001), all-cause mortality (OR 9.28; 95% CI 3.51-24.56; p < 0.0001), and cardiac death (OR 23.88; 95% CI 9.43-60.43; p < 0.00001). Results were consistent for both BNP and NT-proBNP. Pre-operative BNP elevation was also associated with an increased risk of long-term MACE (OR 17.70; 95% CI 3.11-100.80; p < 0.0001) and all-cause mortality (OR 4.77; 95% CI 2.99-7.46; p < 0.00001)[128].

1.28 BNP and vascular surgery

A Glasgow based study regarding MACE following elective Abdominal Aortic Aneurysm (AAA) repairs, suggested that BNP might provide valuable information regarding risk stratification in patients undergoing this procedure. The median (interquartile range) BNP concentrations in 16 patients (15%) who suffered immediate post-operative MACE was 206 (118-454) vs 35 (17-61) pg/ml in the remainder (p=0.001). ROC analysis indicated a BNP concentration of 99.5 pg/ml optimally predicted MACE (area under the curve 0.927), with sensitivity of 88% and specificity of 89%. The BNP in patients who suffered cardiac death was significantly higher than in those that did not (median BNP 496 {280-881} vs 38 {18-84} pg/ml, p=0.043). ROC analysis revealed a cut-off of 448 pg/ml (AUC 0.963), with sensitivity 80%, specificity 100%, positive predictive value 100% and negative predictive value 99%. Not only did higher values of BNP predict MACE, but it was also found to predict all-cause mortality in the immediate (median BNP 100 [84-521] vs 35 [17-81], p=0.028), intermediate (median BNP 201 [97-496] vs 35 [17-73], p< 0.001) and long-term (median BNP 98.5 [58-285] vs 32 [17-71.5], p< 0.001) post-operative periods[129].

1.29 BNP, NT-proBNP and thoracic surgery

In a study involving 55 patients undergoing major thoracic surgery, levels of NT pro-BNP were measured at baseline and also on the first post-operative day. The patients were

monitored to detect the occurrence of AF. Baseline NT-proBNP was more than two fold higher in patients that went on to develop AF (506.1+/-108.4 pg/mL versus 197.7+/-54.9 pg/mL; p=0.001). Patients with NT-proBNP level above the median (113.0 pg/mL) had an 8-fold increased risk of developing AF[130].

In patients with lung disease, NT-proBNP or BNP is independently associated with low peak VO2. A score combining blood gases, NT-proBNP or BNP and spirometry has a high accuracy for prediction of a peak VO2 < 15ml/kg/min which is the cut-off indicating an increased risk of complications during lung resection surgery[131]. As previously mentioned, a systematic review and meta-analysis of observational studies showed that pre-operative BNP or NT-proBNP measurement is an independent predictor of adverse outcomes within 30 days of non-cardiac surgery[132].

1.30 BNP, thoracic and oesophageal surgery

As mentioned previously, first diagnosis AF is a common arrhythmia following oesophagectomy and the incidence varies from 9-46%[71,72].

A study which looked at 415 patients aged 60 years or older, who had undergone lung or oesophageal surgery during a 1-year period, compared pre-operative BNP levels between patients who developed post-operative atrial fibrillation during hospitalisation and those who did not. After anatomic lung resection or oesophagectomy, 46 of the 135 patients with BNP levels ≥ 30 pg/mL developed post-operative atrial fibrillation compared with only 12 of 134 patients with BNP levels less than 30 pg/mL (p < 0.0001). The rates of post-operative atrial fibrillation in patients undergoing other thoracic procedures were low and not associated with the BNP levels. Of note, post-operative atrial fibrillation complicating general thoracic surgery is a marker of increased morbidity and stroke risk. Multivariate logistic regression analysis showed that in patients undergoing oesophagectomy or anatomic lung resection, older age (5-year increments, odds ratio [OR], 1.28; 95% confidence interval [CI], 1.01-1.61:

p = 0.04), male gender (OR, 2.61; 95% CI: 1.12-4.17: p =0.02), and BNP level 30 pg/mL or greater (OR, 4.52; 95% CI:2.19-9.32: p < 0.0001) were independent risk factors for post-operative atrial fibrillation. The length of hospital stay was significantly increased in patients who developed post-operative atrial fibrillation compared with those who did not (p < 0.0001). In patients undergoing lung resection or oesophagectomy, male gender, increased age and a pre-operative BNP level of 30 pg/mL or greater were significant risk factors for the development of post-operative atrial fibrillation[133]. The decision regarding approach for oesophagectomy depends on factors such as the location of the tumour, prior operations, radiation treatment, body habitus and surgical preference. There appears to be no difference in incidence of post-operative AF in trans-hiatal oesophagectomies compared with the Ivor Lewis method, however, when thoracic dissection is restricted below the level of the carina and an anastomosis placed in the low chest, the incidence of AF is lower. It is unclear why this is the case but compared with the Ivor Lewis method, less time is spent on single lung ventilation and there is relative lack of dissection of vagal nerves which lie cephalad to the inferior pulmonary veins using this technique. These could both contribute to a lower incidence of AF[134].

1.31 Myeloperoxidase

Myeloperoxidase (MPO) is a haemoprotein bactericidal enzyme abundantly expressed in polymorphonuclear neutrophils and secreted during leukocyte degranulation. MPO kills bacteria by generating hypochlorous acid (HOCl) from hydrogen peroxide (H_2O_2) and physiological chloride (Cl^-) concentrations. It also has an oxidative function which activates latent forms of pro matrix metalloproteinases (MMP) 8 (collagenase 2) and 9 (gelatinase B) and inactivates Tissue Inhibitors of Metalloproteinases (TIMPs). Matrix metalloproteinases (MMPs) are a large multigene family of structurally and functionally similar calcium dependent zinc containing endopeptidases that play an important role in tissue development

and remodelling in addition to pathological processes. They are classified into substrate specificity including the collagenases, gelatinases, stromelysins, matrilysins, Membrane-Type MMPs and seven other unclassified groups[135]. Matrix metalloproteinases are implicated in a wide variety of cardiovascular diseases[136]. They have been regarded as potential etiologic agents in atrial remodelling and studies in animal models have demonstrated effects of MMPs on the course of cardiac dilation and heart failure[137]. MPO appears to play an important role in structural remodelling of the myocardium and is mechanistically linked to atrial fibrosis and fibrillation and it has been associated with a wide variety of cardiovascular diseases. MPO catalyses the generation of hypochlorous acid which affects intracellular signalling in various cells and deposition of atrial collagen which results in atrial arrhythmias[138].It consumes nitric oxide and supporting its association with AF, a study of MPO deficient mice pre-treated with angiotensin II to promote leucocyte activation, showed lower atrial tissue abundance of 3-chlorotyrosine, a MPO by-product, and reduced incidence of atrial fibrosis, as compared to normal mice. Myeloperoxidase deficiency decreases atrial fibrosis and protects mice from AF, which was reversed after restoring myeloperoxidase. In the same study, human patients with AF had higher plasma concentrations of MPO and more MPO in their right atrial tissue compared with patients without AF[139]. Elevated plasma MPO levels have been associated with a variety of clinical conditions including systemic inflammation, severity of coronary artery disease and vascular endothelial dysfunction. High levels of myeloperoxidase have also been linked to an increased risk of AF recurrence in patients who have undergone catheter ablation. In a study, there was adjustment for age, sex, left atrial diameter and hs CRP. Findings suggested that there was a higher risk of AF in subjects with the highest MPO quartile compared with those with the lowest quartile (hazard ratio, 3.18; 95% confidence interval, 2.12–5.23; p=0.024). MPO was also an independent predictor of recurrence of AF (hazard ratio, 2.12; 95% confidence interval, 1.71–3.27; p = 0.032)[139, 140].

Lymphomononuclear cells are found to infiltrate the myocardium of patients in those with structural heart disease and in those with AF and co-morbidities. This would suggest that these patients have chronic inflammation. These cells secrete high levels of tumour necrosis factor (TNF), transforming IL-6 and transforming growth factor (TGF)-beta 1, which lead to atrial fibrosis and electrical remodelling[141]. A study has also shown that significantly increased levels of MPO independently increased risk for major adverse cardiac events including myocardial infarction, reinfarction, need for revascularisation. It also increased the risk of death at 30 days or 6 months[138].

1.32 C-Reactive Protein (CRP), Interleukin-6 (IL-6) and AF

CRP is synthesised primarily by the liver in response to IL-1 and IL-6 which both have pro inflammatory and cyto-protective effects. The association between IL-6 and AF is less consistent compared with CRP and AF[142].

In the ARMYDA-3 study, which looked at the use of atorvastatin in trying to reduce post-operative AF in patients undergoing cardiac surgery, high post-operative C-reactive protein levels were linked to an increased risk of AF. On the second day following Coronary Artery Bypass Graft (CABG) surgery, CRP levels which have reached their peak are associated with development of AF (R^2=0.41, p=0.0037)[76]. A systematic review and meta-analysis were conducted to examine the association between baseline CRP and recurrence of AF after successful cardioversion. Seven prospective studies with 420 patients were analysed. This suggested that CRP levels were greater in patients who experience a recurrence of AF[143]. The limitations included the significant heterogeneity across the studies which could likely be attributed to the differences in CRP assays. The use of CRP levels in predicting sinus rhythm maintenance appeared promising but requires further study. In the Cardiovascular Health Study, a population-based longitudinal study of coronary heart disease and stroke in 5,806 patients adults aged 65 years and older, who were followed up for a mean period of 6.9 years;

higher CRP levels (>3.41 mg/l) were associated with the presence of AF compared with lower levels (<0.97 mg/l; adjusted OR 1.8, 95% CI 1.2–2.5). Baseline CRP could be used to predict the risk of developing AF (adjusted HR 1.24 for 1 SD increase, 95% CI 1.11–1.40). A large Danish study tested whether the association of C-reactive protein with increased risk of atrial fibrillation is a robust and perhaps even causal association. It looked at 10,276 individuals from the prospective Copenhagen City Heart Study, including 771 individuals who had atrial fibrillation during follow-up, and another 36,600 people from the cross-sectional Copenhagen General Population Study, including 1,340 cases with atrial fibrillation. Individuals were genotyped for 4 CRP gene polymorphisms and had high-sensitivity CRP levels measured. It found that genotype combinations of the 4 CRP polymorphisms were associated with up to a 63% increase in plasma CRP levels ($p < 0.001$), but not with increased risk of atrial fibrillation. This suggests that an elevation in CRP levels as such, does not increase the risk of AF development. A CRP level in the upper versus lower quintile was associated with a 2.19 times (95% confidence interval [CI]: 1.54 to 3.10) increased risk of atrial fibrillation. Risk estimates after multifactorial adjustment decreased to 1.77 (95% CI: 1.22 to 2.55), and after additional adjustment for heart failure and plasma fibrinogen level to 1.47 (95% CI: 1.02 to 2.13) and 1.63 (95% CI: 1.21 to 2.20), respectively. Elevated plasma CRP was robustly associated with increased risk of atrial fibrillation; however, genetically elevated CRP levels did not. The study concluded that elevated plasma CRP as such did not increase atrial fibrillation risk, however, the study had several limitations. These included potential selection bias and misclassification of plasma CRP levels, CRP genotype, and atrial fibrillation. Potentially misclassifying a diagnosis of atrial fibrillation would result in an underestimation of the risk estimates, and therefore more conservative estimates for the association of plasma CRP levels with risk of atrial fibrillation than observed in the study[144]. Pre-operative CRP levels are prognostic markers of survival in

patients undergoing oesophagectomy for cancer and albumin and C-reactive protein are indicators of a systemic inflammatory response. The modified Glasgow Prognostic score combines these into a risk stratification score for predicting clinical outcome in patients with cancer of the lung, gastric tract and colorectal cancer. The potential value of pre treatment mGPS in patients with squamous cell carcinoma of the oesophagus who are undergoing chemoradiotherapy was evident in one study where higher mGPS scores correlated with lower overall 3 year survival[145].

IL-6 is a pleiotrophic cytokine which means it acts on multiple cell types. Many cells express IL-6 including endothelial cells (under the influence of endothelins), sympathetic neurons, cerebral cortex neurons, adrenal medulla chromaffin cells, retinal pigment cells, mast cells, Langerhans cells, neutrophils, monocytes, eosinophils, colonic epithelial cells and pancreatic islet beta cells. IL-6 production is generally correlated with cell activation and is normally kept in control by glucocorticoids, catecholamines, and secondary sex steroids. The value of measuring cytokines in patients undergoing coronary artery bypass grafts in relation to the development of AF has been confirmed in several studies[146]. Patients that undergo off-pump CABG surgery, have levels of IL-6 and IL-8 which are elevated immediately after surgery (IL-6 from 0 to 435pg/ml). The increase in the level of IL-6 after surgery is associated with post–operative AF. Logistic regression analysis indicated that the highest quartile of IL-6 level immediately after the surgery (if IL-6 >401pg/ml) (odds ratio 7.63; 95% CI, 1.06-54.9; p=0.04) and age independently predict post-operative AF. (OR 7.63, p=0.04)[147]. Cytokines such as IL-6, which are pro-inflammatory and can induce platelet activation, are associated with adverse cardiovascular outcomes in patients with AF. In clinical studies, high levels of CRP, and IL-6, and low levels of IL-18, are associated with increased atrial size, which further supports a role for inflammation in atrial fibrillation[148]. In patients with AF who have high levels of IL-6 due to a genetic polymorphism, the IL-6 levels

modulate the pathogenesis of AF[149]. The association between IL-6 and AF is less consistent compared with CRP and AF[142].

1.33 Cardiopulmonary Exercise Testing (CPET) and the Modified Glasgow Prognostic Score (mGPS) pre and post-operatively

Cardiopulmonary exercise testing is routinely carried pre-operatively out for patients requiring oesophagectomy. Studies have shown that pre-operative VO2 max (maximal oxygen consumption) and AT measurements from CPET can be used to help predict risk of post-operative morbidity in oesophagectomy patients[48].As mentioned previously, in a local study, patients with a lower pre-operative AT with a cut-off value of 9ml/min/kg had a higher risk of developing cardiopulmonary complications but this study required validation in a larger cohort[47].It has been shown that all post-operative cardiopulmonary deaths occur in patients with an anaerobic threshold (AT) of <11ml/min/kg and/or with significant myocardial ischaemia on CPET[150]. A literature search revealed no studies that have quantified post-operative fitness in this population. Quality of life (QoL) is a factor that is very important to patients when considering the possible outcomes of an operation and it has been named the 'missing axis' in morbidity risk prediction and information provided for patients. Whilst QoL pre-operatively and post-operatively have been compared in this population, comparing post-operative QoL with post-operative fitness in patients undergoing oesophagectomy has not been studied before.

1.34 Improving prognostication in patients with oesophageal cancer having surgery

It is clear that for patients who undergo oesophageal cancer surgery, knowledge about mechanisms leading to the development of AF and increased mortality is still lacking. A Swedish population based cohort study which included 609 patients undergoing surgical resection for oesophageal or gastro-oesophageal junctional cancer over a 4 year period found that cardiac disease and a Charlson comorbidity score of ≥2 appeared to increase the risk of severe and early post-operative complications in patients with oesophageal cancer while hypertension, pulmonary disorders, diabetes and obesity do not. Additionally, a larger study

of 1822 patients in Sweden undergoing oesophageal cancer surgery over a 23 year period suggested that overall cause mortality was increased in patients with a Charlson score of 2 or more (HR 1.24, 95% CI 1.08-1.42) and those with a history of MI (HR 1.2, 1.01-1.49) or congestive cardiac failure (HR 1.31, 1.04-1.67). Exposure to peripheral vascular disease, chronic pulmonary disease, connective tissue disease, peptic ulcer disease, diabetes mellitus and liver disease did not increase mortality[151].

1.35 Aims and research questions

I propose to evaluate the mechanisms associated with the development of AF and increased mortality following oesophagectomy. I wish to evaluate the CACI, in addition to biomarkers including dimethylarginines, CRP, BNP, myeloperoxidase, homoarginine and IL-6 in the context of pre-operative Cardiopulmonary Exercise Testing (CPET) results. This may aid prognostication in this patient group.

In this thesis the lead researcher hopes to address some of the following questions. In chapter 3 the question the aim of the study was to ask; Are pre-operative plasma concentrations of dimethylarginines and homoarginine associated with the development of atrial fibrillation in adult patients following oesophageal surgery? Are low levels of homoarginine associated with increased levels of BNP?[152,153]. In chapter 4, the aim was to evaluate whether BNP, NT-pro BNP, left atrial diameter is associated with development of atrial fibrillation and outcomes in patients undergoing oesophageal surgery? What association do myeloperoxidase, IL-6 and CRP have with the development of atrial fibrillation and outcomes in patients undergoing oesophageal surgery?

Chapter 5 will ask does the use of the modified Glasgow Prognostic scoring system (mGPS) pre-operatively and post-operatively (poGPS) assist in determination of associations with morbidity and mortality in oesophagectomy patients and if the results of Cardiopulmonary exercise (CPET) tests and the results of Quality of Life (QoL) questionnaires are associated with each other. In chapter 6 we can apply the Charlson Comorbidity Index (CACI) to the

cohort and use this in addition to biomarkers, CPET results and development of AF to help a better understanding of factors influencing the development of AF and associated morbidity and mortality in patients undergoing oesophagectomy.

In chapter 7 the challenges with conducting the study and the limitations of the study will be addressed.

In chapter 8 conclusions from the study will be drawn and future plans will be discussed.

1.36 Key messages

- New-onset AF is a complication in patients following oesophagectomy.

- New-onset AF is independently associated with a risk of stroke, a prolonged length of stay in the ICU, and increased mortality.

- AF onset in the post oesophagectomy population is potentially preventable and although there are multiple factors in its evolution including genetics, circulating biomarkers have been implicated in its development.

- Early identification of patients who are at increased risk for developing AF may allow for pharmacological interventions to prevent this complication.

- Evaluating the relationship between cardiovascular biomarkers, CPET, onset of atrial fibrillation and outcomes may help to better understand factors influencing increased mortality in patients. As a result of understanding the changes in biomarkers, it may help us to better identify patients at risk for AF, therefore taking a step closer to enabling personalized care.

2 CHAPTER 2

METHODS

2.1 Study design

As a result of a literature review that I completed prior to commencing the study, it appeared that there were no studies examining the associations between plasma and urine levels of dimethylarginines, homoarginine and first diagnosis AF in patients following oesophageal surgery.

An observational prospective cohort study was planned to investigate the initial hypothesis as to whether patients who undergo oesophageal surgery and develop AF post-operatively had higher dimethylarginine levels compared to those who did not and whether these levels could assist in prognostication of outcomes for these patients. Additionally, CACI, mGPS and pomGPS were determined and peri-operative echocardiograms were planned, as well as CPET testing and also, the determination of the peri-operative levels of IL-6, CRP, myeloperoxidase, BNP and homoarginine of patients. Specifically, it was hypothesised that higher CACI, higher pre-operative mGPS and higher pomGPS, lower VO2 max and AT values, higher ADMA, lower homoarginine, higher IL-6 and myeloperoxidase, higher BNP and CRP would be related to the onset of AF and postoperative morbidity and mortality. It was evaluated whether the combination of these factors, in addition to the above would be useful in helping to identify mechanisms related to patient outcomes following oesophageal surgery. As previously mentioned in section1.14, I had a pre-existing interest in atrial fibrillation, having looked at incidence of this dysrhythmia in an ICU cohort at Glasgow Royal Infirmary. This led to further consideration as to how this dysrhythmia could be predicted and potentially prevented. In terms of patient cohorts who are at risk of developing this rhythm, it is widely accepted that patients undergoing oesophageal surgery are at an increased risk of this and the researcher was also aware that the numbers of patients

undergoing oesophageal surgery would be adequate to run a pilot prospective cross sectional and longitudinal observational cohort study.

A prospective observational cohort study was planned to investigate the initial hypothesis as to whether patients who undergo oesophageal surgery and develop AF post-operatively had higher dimethylarginine levels compared to those who did not and whether these levels could assist in prognostication of outcomes for these patients. Additionally, CACI, mGPS and pomGPS were determined and a plan to carry out peri-operative echocardiograms, CPET testing and to determine the peri-operative levels of IL-6, CRP, myeloperoxidase, BNP and homoarginine of patients was made. Specifically, the hypothesis was that higher CACI, higher pre-operative mGPS and higher pomGPS, lower VO2 max and AT values, higher ADMA, lower homoarginine, higher IL-6 and myeloperoxidase, higher BNP and CRP would be related to the onset of AF and postoperative morbidity and mortality. It was evaluated whether the combination of these factors, in addition to the above would be useful in helping to identify mechanisms related to patient outcomes following oesophageal surgery.

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The study was performed according to the Research Governance Framework for Health and Community Care[154].

2.2 Definition of study variables

A diagnosis of atrial fibrillation was defined as irregular and uncoordinated atrial electrical activity on electrocardiogram. Plasma and urine levels of BNP, IL-6, CRP and myeloperoxidase were measured with respect to development of atrial fibrillation, and patient morbidity and mortality. I analysed Charlson Age-Comorbidity Index (CACI), modified Glasgow Prognostic Score (mGPS) and post-operative Glasgow Prognostic Score (po mGPS) results and considered Cardiopulmonary Exercise Testing results in these patients to assist in determining the effects of these combined factors on morbidity and mortality.

2.3 Study Population

Forty patients who had all been deemed by the multidisciplinary team to require an oesophagectomy or oesophagogastrectomy at Glasgow Royal Infirmary (GRI) were consented for the study. This number was chosen founded on the feasibility of recruitment based on the total number of patients undergoing oesophageal surgery each year at the GRI. The pilot study sample size was based on the number of patients that were likely to have oesophageal surgery at the Glasgow Royal Infirmary over a period of 9 months and over that time period, all patients undergoing elective oesophageal surgery were invited to take part in the study.

The list of patients due to undergo surgery was obtained from the secretaries working for the Upper GI consultants at the GRI. The patients involved were referred to the Glasgow Royal Infirmary as it is a specialist centre which deals with this type of surgery. There is inevitable bias present in this process as this cohort is a pre-selected group by definition who have a specific type of cancer and we know that there are certain predisposing factors and exposure variables which lead to this diagnosis being made.

2.4 Identification of participants and consent

I identified patients listed for trans-hiatal or trans-thoracic oesophagectomy at Glasgow Royal Infirmary by means of communications with the consultants' secretarial team. The majority of the target cohort for the pilot study population had all undergone neo-adjuvant

chemotherapy. As mentioned previously, neoadjuvant chemotherapy had been shown to improve the overall survival of patients with advanced carcinomas of the oesophagus over 5 years and so most of the patients recruited into the trial had already undergone neoadjuvant chemotherapy. Most patients underwent the ECX regimen which was epirubicin, cisplatin and capecitabine also known as Xeloda.

We already know that oesophageal cancer affects males more than females and affects most patients in the age range from 65-74 years. The baseline characteristics of my cohort included 30 males and 10 females. The median age was 65.5 years and the range was 27-84 years. 2 patients were not treated with neoadjuvant chemotherapy. There were 20 current or ex-smokers.

The lead researcher sent information regarding the study to the patients via post, along with a cardiopulmonary exercise test appointment (CPET).

Information about the study was sent to the patients and subsequently the lead researcher reviewed the patients when they attended a pre-assessment clinic where they underwent Cardiopulmonary Exercise testing and had a nutritional assessment including referral to a dietician. During this appointment, a full medical history including a list of medications was recorded. At the appointment for CPET testing, the patient had the opportunity to ask the lead researcher more questions prior to the pre-operative visit with the Upper Gastrointestinal surgeon.

The lead researcher obtained written consent from each study participant and explained the nature of the study, by means of provision of a patient information sheet and also verbally. This included reviewing the side effects and risks of participating in the clinical trial. The participants were informed that they were free to withdraw their consent from the study at any stage.

Originally, it was planned that a quality of life questionnaire should be undertaken at this point. Where possible, baseline blood tests including full blood count, urea and electrolyte levels and liver function tests in addition to an electrocardiogram (ECG) were obtained. The baseline blood tests were recorded from the NHS Greater Glasgow and Clyde laboratory results system onto an anonymised encrypted Excel spreadsheet on the lead researcher's University of Glasgow laptop computer. An echocardiogram was done if determined necessary according to the patient's medical history. Reasons for doing an echo included increasing shortness of breath and a history of cardiac problems. Specific to the study, when the lead researcher was able to obtain fasting bloods from the patients they were analysed for L-arginine, homoarginine, ADMA, SDMA, BNP, high sensitivity CRP, myeloperoxidase (MPO) and IL-6. Urinary levels of nitrate in addition to DMA, SDMA and ADMA were taken at same time as the blood tests wherever possible. These specialised samples were sent via porter on rare occasion or hand delivered by the lead researcher to the biochemistry lab and analysed by colleagues using High Performance Liquid Chromatography (HPLC)[155].All the patients received neo–adjuvant chemotherapy in the weeks prior to undergoing transhiatal oesophagectomy, transthoracic oesophagectomy or oesophagogastrectomy. It was difficult to objectively analyse the effect of neo-adjuvant chemotherapy on the patients' inflammatory biomarker levels as levels of biomarkers were only taken pre-operatively and not preceding chemotherapy.

There were several differing exposure variables that affected patients, including the surgeon carrying out the procedure, the type of surgical approach used, the anaesthetic used, the amount of fluid given perioperatively, in addition to medications that the patient was taking pre-operatively. Also, the varying plasma levels of different biomarkers will have an effect on patients.

The operations were performed by one of three consultant surgeons working at Glasgow Royal Infirmary (GRI). All patients had a general anaesthetic delivered by a consultant anaesthetist and stayed on the Intensive Care Unit at the GRI post-operatively. The anaesthetic techniques were standardised for the most part with the use of epidurals for analgesia in most patients and volatile anaesthetic was used for the maintenance of anaesthesia.

2.5 Outcome variables

The main outcome variable in the study was the development of atrial fibrillation or not and its association with various biomarker levels. This outcome was chosen due to the association that developing this arrhythmia has with poorer outcomes for patients.

The haemodynamics of these patients were closely monitored peri-operatively on the Intensive Care Unit and the diagnosis of AF was made on the basis of a single lead printed rhythm strip at the time of onset and confirmed by a 12 lead ECG where possible. Prior to the study starting the lead researcher had ensured that the nursing and medical staff at all stages of the study had been fully briefed about it. They also ensured that there was an information leaflet (see appendix) available for staff with information regarding what investigations needed to be obtained on a daily basis. The lead researcher was also available via phone and email to answer any questions and also made themselves available on the Intensive Care Unit for at least two days every week to check on progress and troubleshoot problems. Plasma levels of arginine, homoarginine, ADMA, SDMA, CRP, MPO and IL-6 and urinary levels of nitrate, DMA, SDMA and ADMA were taken by the nursing staff every morning for 7 days or until the patient was discharged, if that was sooner. These biomarkers were measured according to local protocols, baseline and daily BNP was also done until day 2. Levels of electrolytes such as magnesium and potassium were also recorded by myself, in addition to fluid balance and the requirement for catecholamines, in an anonymised encrypted Excel spreadsheet on the lead researcher's University of Glasgow laptop. The management of atrial

fibrillation in the intensive care unit was standardised by adherence to local protocols in order to minimize inter-patient variation. The protocols involved clinical assessment of the patient and consideration of requirement for fluid supplementation and also whether magnesium and potassium levels required augmentation. There was also optimisation of the patient's heart rate, ultimately aiming for rhythm control. When a patient developed AF, a trans-thoracic echocardiogram was recorded within 24 hours by an intensive care consultant who regularly carried out echocardiography. For the patients that had this investigation the lead researcher was on the intensive care unit at the time and facilitated these procedures.

2.6 Inclusion criteria

- Patients had to be able to give their written informed consent
- Patients had to be males or non-pregnant females >18 years of age
- Patients booked to have trans-hiatal or trans-thoracic oesophagectomy or oesophagogastrectomy.

2.7 Exclusion criteria

- Patients with pre-existing atrial fibrillation
- Patients deemed to be unable to give their consent

2.8 Withdrawal of subjects

Patients who withdrew from the study by their own volition had no further investigations carried out. Patients who withdrew due to adverse events were followed up by the lead researcher and they liaised with the patient to ensure that they had satisfactory medical follow up.

2.9 Study process and timeline

Visit 1: CPET and study information

Patients attended the Respiratory function lab in the Glasgow Royal Infirmary for CPET testing. In terms of equipment, the ZAN® 600 (nSpire Health, Hertford, UK) and the Ergoselect bicycle ergometer (Ergoline, Bitz, Germany) were used for the tests and the patients were exposed to incremental physical exercise to a maximally tolerated level,

determined by pain, shortness of breath or exhaustion. Several parameters were recorded during the exercise, VO2 peak and AT being the readings required for my study.

The CPET appointment was an opportunity for me to engage patients about the study and to give them the patient information sheet, if they had not already received one.

Visit 2: Pre-operative assessment visit

The lead researcher obtained informed consent for the study and patients were given the opportunity to ask them questions. Baseline bloods, ECG and urine were taken by the lead researcher if possible. Patients may also have had an echocardiogram requested as part of their pre-operative assessment by the anaesthetist or surgeon. This visit was after neoadjuvant chemotherapy had taken place.

Visit 3: Admission for procedure

The lead researcher obtained baseline bloods and urine if these had not already been taken. Also, they facilitated the actualization of a pre-operative echocardiogram by an ICU consultant, if this had not already been done.

Originally proposed follow up

Visit 4: Routine surgical follow-up at 3 months post-operatively in collaboration with another investigator who wished to follow patients up with sequential CPET testing and Quality of Life assessments.

The lead researcher planned that the surgeon assessed the patient's general health on the day of the clinic appointment and if they were regarded as fit enough for further CPET testing they would be introduced to them or a colleague as the investigator. There was an opportunity for patients to meet the researchers and ask questions. Importantly, the patients were reminded at this point that they were not obliged to take part in the study and could withdraw at any time. The plan was that the lead researcher would show the patients the consent form, and ask them if they wished to take part and subsequently encourage them to sign it. If they

were agreeable the lead researcher would then organise a CPET appointment. The lead researcher gave their contact details to the patients so that they could contact them by phone if they changed their mind or wished to cancel the CPET.

Visit 5: CPET and Quality of Life assessment at 6 months to coincide with surgical review clinic appointment

The lead researcher planned to review the patient again to ensure that they still wished to participate prior to the CPET.

Visit 6: CPET and Quality of Life assessment at 1 year

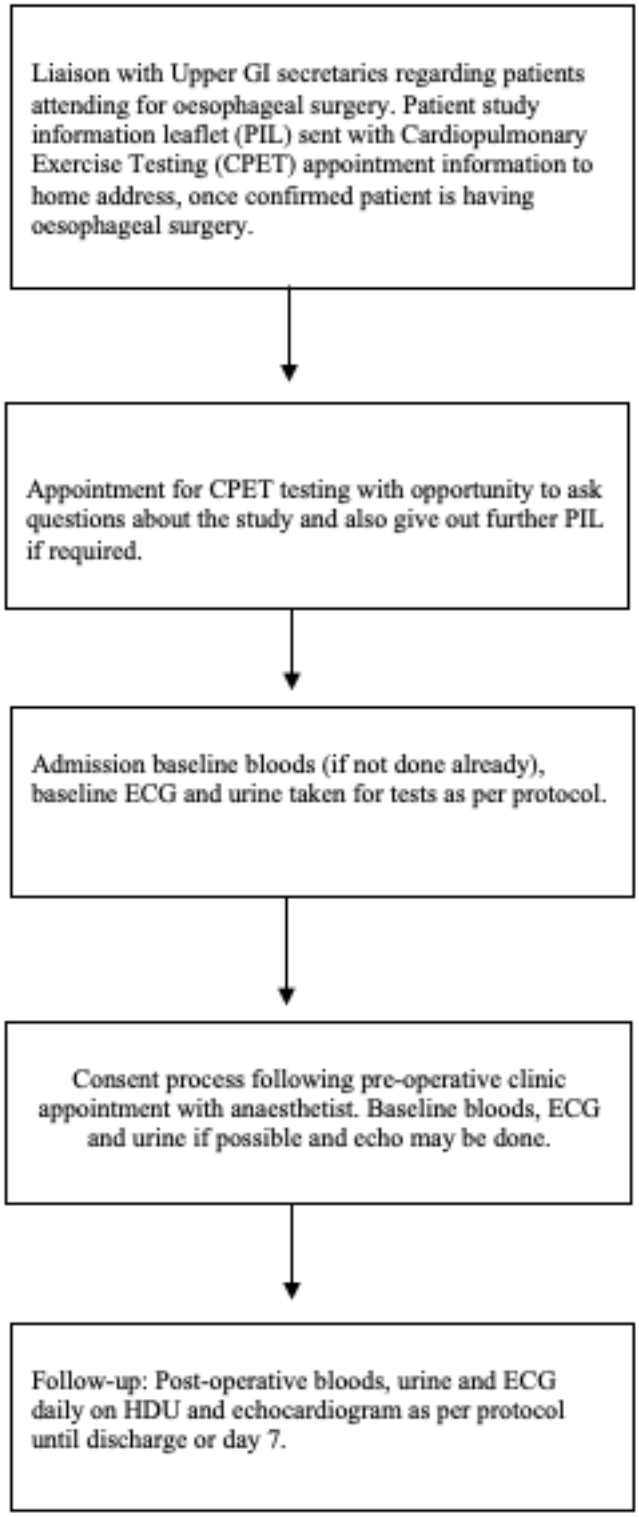
The lead researcher planned to review the patient again at this point to ensure that they still wished to participate prior to the CPET.

Visit 7: CPET and Quality of Life assessment at 2 years

The lead researcher planned to review the patient again to ensure that they still wished to participate prior to the CPET.

Unfortunately, the colleague who was going to take over running the CPET and QoL part of the study was unable to commence due to ill health and this part of the study never reached fruition.

Figure 2.1 Cardiovascular biomarkers, CPET, onset of AF and outcomes study flow chart



2.10 Laboratory analytical methods for methylarginines and homoarginine. Collection and preparation of blood samples.

The Department of Biochemistry at Glasgow Royal Infirmary has a research interest in dimethylarginines, and has well-established methods for conducting affiliated tests. Researchers in the department have published HPLC methods by the use of internal standard monoethylarginine, which is not inherent in human plasma[155,156]. The plasma and urine samples were analysed using high performance liquid chromatography (HPLC).

Venous blood samples (EDTA) were withdrawn from 40 patients on the day of their preadmission (day 0) and further follow-up samples were taken daily between days 1-7 for the analysis of plasma ADMA, arginine, homoarginine and SDMA. Blood samples were centrifuged (500 g, 10 minutes), the plasma removed and stored at -70°C until analysis. All the blood samples which were taken were handled according to established standard operating procedures in the hospital laboratory.

2.11 Laboratory analysis

Blood samples were obtained in EDTA tubes for the determination of ADMA arginine, homoarginine and SDMA were measured by isocratic reverse-phase high-performance liquid chromatography (HPLC) with fluorescence detection with coefficient of variation (CV) <3.0%[156]. Plasma was collected into tubes containing EDTA. Arginine, SDMA, ADMA and homoarginine were extracted from plasma using Isolute PRS cation exchange solid phase extraction (SPE) columns. The extraction columns were placed on Vac Elut extraction system, activated and equilibrated with 2 ml methanol followed by 2 ml of 50 mM borate buffer, pH 8.5.

In a glass tube, 0.2 ml of plasma sample, quality control (QC) or aqueous calibrator was mixed with 80 microlitres of internal standard: monoethylarginine (MEA), 5 micromols) and 720 microlitres of borate buffer, which was then loaded on to the equilibrated SPE column. The column was consecutively washed with borate buffer (1 ml), water (3 ml) and methanol

(3 ml). These steps were performed under gravity, with no vacuum suction required (flow rate was 0.5 ml/min).

Analytes were eluted with 3ml of a solution containing 50% methanol and 10% concentrated ammonia in water. The eluent was then evaporated to dryness at 80 °C under air. Using the Vac Elut system, 20 samples could be extracted within 2 h for overnight analysis by HPLC. After evaporation of the solvent under nitrogen, the amino acids, the dried extract was dissolved in 0.1 ml water and 0.1 ml of the derivatising agent was then added and the samples thoroughly mixed and equilibrated for 15 min. The derivatisation reagent was freshly prepared each week. Briefly, 10 mg of orthophthaldialdehyde (OPA) was dissolved in 0.2 ml of methanol, followed by the addition of 1.8 ml of 200 mM borate buffer (pH 8.5), and finally 10 microlitres of 3-mercaptopropionic acid. The reagent was stable for one week when stored at 4 °C, provided mercaptopropionic acid (5 microlitres) was added every 48 hr to the stock solution. Shortly before use, the stock solution was diluted five-fold in the same borate buffer. The derivatised samples were transferred to autosampler vials, maintained at 10 °C, and 20 microlitres injected onto the HPLC analytical column for chromatography[155].

The derivatives were separated by High-Performance Liquid Chromatography (HPLC). Sodium acetate dissolved in 454 ml water (pH 6.3) containing 48 ml acetonitrile was used as mobile phase at a flow rate of 1.0 ml/min and a column temperature of 30°C. Fluorescence detection was performed at excitation and emission wavelengths of 340 and 455nm, respectively. After elution of the last analyte, strongly retained compounds were quickly eluted by a strong solvent flush with 50% acetonitrile, resulting in a total analysis time of 40 minutes.

Reference values for ADMA, arginine, homoarginine and SDMA were obtained from plasma of healthy laboratory personnel (n=86). The concentration of ADMA, arginine, homoarginine

and SDMA in these individuals is normally distributed with a median value of 0.45 µmol/L, 57.8 µmol/L, 1.9 µmol/L and 0.37 µmol/L respectively[156].

The departmental research team collaborated with me regarding my study into dimethylarginines, homoarginine, BNP and IL-6 levels. The University of Glasgow immunology department collaborated with me and analysed myeloperoxidase levels in patients who developed first diagnosis atrial fibrillation following oesophageal surgery. Echocardiography was performed pre-operatively on patients undergoing oesophageal surgery and post-operatively on the patients that developed AF and their left atrial diameter and volume were measured if possible. I also analysed the data from the cardiopulmonary exercise tests which patients had pre-operatively and I had then planned to repeat these at 6 months, 12 months and 2 years after their operation. I had also planned to get patients to complete a quality of life questionnaire at these time intervals.

2.12 Collection and preparation of blood samples

All patients undergoing oesophagectomy had their blood taken at pre-operative assessment clinic and during their post-operative stay in ICU on days 2 and 3 as the incidence of AF is most likely up to 72 hours following an operation[83].Whole blood samples collected in plastic EDTA tubes were centrifuged to remove the plasma for BNP analysis. Some samples were stored at 2-8 degrees Celsius and were processed within 24 hours of collection, however, not every sample could be processed within this time period and some samples underwent centrifugation and were frozen. Prior to analysis, frozen samples were thawed and thoroughly mixed by low speed vortexing until specimens were visually homogenous to ensure consistency in results.

All the blood samples that were taken were handled according to established standard operating procedures in the hospital laboratory.

2.13 Laboratory analytical methods for BNP. Principles of the BNP assay procedure

The Abbott ARCHITECT® platform BNP assay was used. It is a two-step immunoassay for the quantitative determination of BNP in human EDTA plasma using a Chemiluminescent Microparticle Immunoassay (CMIA) on the ARCHITECT® iSystem[125]. Briefly, the sample and anti-BNP (mouse, monoclonal) coated paramagnetic microparticles are combined. The BNP in the sample binds to the anti-BNP coated microparticles. After washing, the anti-BNP acridinium labeled conjugate is added to create a reaction mixture. The resulting chemiluminescent reaction is measured as relative light units (RLUs). There is a direct relationship between the amount of BNP in the sample and the RLUs detected by the ARCHITECT iSystem optics.

Calibration of Assay

The assay is calibrated using a 5-point calibration curve. The ARCHITECT® BNP assay uses a point-to-point data reduction method to generate a calibration curve. The ARCHITECT® BNP calibrators are traceable to an internal reference standard that has been prepared gravimetrically with synthetic BNP. The internal reference standard correlates to the AxSYM BNP assay with a decision threshold of 100pg/ml. There is no internationally recognized BNP standard currently.

Quality Performance characteristics

Three QC levels (low, medium and high) are used in every assay in order to verify test results and to monitor the performance of the assay. The imprecision (% CV) of the assay is < 12%. The assay has an analytical sensitivity of 10 pg/ml and is linear up to 5000 pg/ml.

Expected values in non-heart failure population

Normal values for BNP using the Abbott BNP kit are < 100pg/ml in patients less than 50 years old[125].Some labs report in ng/L which is equivalent to pg/ml.

Results calculation

The ARCHITECT® BNP assay uses a point-to-point data reduction method to generate a calibration curve.

Interference

Potential interference in the ARCHITECT® BNP assay from various drugs such as simvastatin and lisinopril is ≤ 10%.

2.14 Collection and preparation of blood samples and laboratory analytical methods for MPO

Venous blood samples from patients were taken into EDTA tubes prior to their surgery, on the day of surgery and daily for 7 days or until their discharge from HDU, depending on which came first. These samples were processed by the biochemistry lab at Glasgow Royal Infirmary and stored in freezers at -80 °C. A serum separator tube (SST) was used and samples were allowed to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Samples were aliquoted and stored at -80 °C. Following analysis for BNP, dimethylarginines and homoarginine, the samples were transferred to the immunology laboratory at Glasgow University for MPO analysis. All samples were brought to room temperature prior to immunoassay.

Assay

The Quantikine® Human MPO Immunoassay is a solid-phase Enzyme Linked Immunosorbent Assay (ELISA) designed to measure human MPO in serum, platelet-poor plasma, cell culture supernates, cell lysates, saliva, and urine. The serum samples were assayed at a 1 in 50 dilution as recommended by the kit and a selection of samples was assayed twice for reproducibility. This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human MPO had been pre-coated onto a microplate. Samples were pipetted into the wells and any MPO that was present was bound by the immobilized antibody. Following any unbound substances being washed

away, an enzyme-linked polyclonal antibody specific for human MPO was added to the wells. A wash to remove any unbound antibody-enzyme reagent was followed by a substrate solution being added to the wells. Colour then developed in proportion to the amount of MPO bound in the initial step. The colour development was stopped and the intensity of the colour was measured[184]. For information regarding the precision, sensitivity and specificity of the assay please refer to the Appendix.

Details of MPO assay procedure

Precision

Intra-assay Precision Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision Three samples of known concentration were tested in twenty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

SERUM/PLASMA/URINE ASSAY

	Intra-Assay Precision			**Inter-Assay Precision**		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	20	20	20
Mean (ng/Ml)	1.03	3.13	6.63	1.07	3.29	6.71
Standard deviation	0.024	0.046	0.172	0.12	0.26	0.55
CV (%)	2.3	1.5	2.6	10.8	8.0	8.2

SENSITIVITY

Fifty-four assays were evaluated and the minimum detectable dose (MDD) of human MPO ranged from 0.003-0.062 ng/Ml. The mean MDD was 0.014 ng/Ml.

The MDD was determined by adding two standard deviations to the mean O.D. value of twenty zero standard replicates and calculating the corresponding concentration.

CALIBRATION
This immunoassay is calibrated against highly purified MPO from human leukocytes.

SPECIFICITY
This assay recognizes natural human MPO.

No significant cross-reactivity or interference was observed.

2.15 Laboratory analytical method for IL-6

The Quantikine® Human IL-6 Immunoassay is a 4.5 hour solid phase immunoassay designed to measure human IL-6 in cell culture, serum, and plasma. It contains *E.Coli*-expressed recombinant human IL-6, and antibodies raised against the recombinant protein. Natural human IL-6 showed dose-response curves that were parallel to the standard curves obtained using the Quantikine® kit standards, indicating that this kit can be used to determine relative levels of natural human IL-6.

Collection and preparation of blood samples

Plasma was collected from patients in lithium heparin tubes pre-operatively and then daily for up to 7 days post-operatively. These samples were centrifuged for 15 minutes at 1000 x g within 30 minutes of collection. They were then assayed immediately or aliquoted and stored at ≤ -20 °C.

Assay

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human IL-6 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-6 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human IL-6 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and colour develops in proportion to the amount of IL-6 bound in the initial step. The colour development is stopped and the intensity of the colour is measured[187].

Calibration

This immunoassay is calibrated against highly purified *E. Coli*-expressed recombinant human IL-6 produced at R&D Systems®. The NIBSC/WHO 1st International Standard for IL-6 (89/548), intended as a potency standard, was evaluated in this kit. The NIBSC/WHO standard is a CHO cell-derived recombinant human IL-6.

Details of IL-6 assay precision

Intra-assay Precision (Precision within an assay) Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays) Three samples of known concentration were tested in twenty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

Intra-Assay Precision				**Inter-Assay Precision**		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	20	20	20
Mean (pg/Ml)	16.8	97.7	186	17.2	101	191
Standard deviation	0.7	1.6	3.8	1.1	3.3	7.2
CV (%)	4.2	1.6	2.0	6.4	3.3	3.8

2.16 Laboratory analytical methods for hs CRP

The assay that was used at Glasgow Royal Infirmary biochemistry labs was the MULTIGENT CRP Vario assay which is intended for the quantitative immunoturbidimetric determination of C-reactive protein in human serum and plasma. It has variable assay ranges. This was used with the Abbott ARCHITECT c system[190].

Collection and preparation of blood samples

Plasma was collected from patients in lithium heparin plastic tubes pre-operatively and then daily for up to 7 days post-operatively. These samples were centrifuged for 15 minutes at

1000 x g within 30 minutes of collection. They were then assayed immediately or aliquoted and stored at ≤ -20 °C.

Assay

MULTIGENT CRP Vario is a latex immunoassay developed to accurately and reproducibly measure blood CRP levels in serum and plasma. Agglutination results when an antigen-antibody reaction occurs between CRP in a sample and anti-CRP antibody, which has been adsorbed to latex particles. This agglutination is detected as an absorbance change (572 nm), with the rate of change being proportional to the quantity of CRP in the sample. The MULTIGENT CRP Vario assay should be calibrated using the correct parameters as suggested in the manual. The ARCHITECT c system has an automated dilution procedure. When using the Automated Dilution Protocol, the system performs a dilution of the specimen and automatically corrects the concentration by multiplying the result by the appropriate dilution factor. The dilution for high sensitivity CRP is 1 in 10. To manually dilute the sample 0.9% saline is used. The precision of the MULTIGENT CRP Vario assay is ≤ 6% Total CV. The limit of quantitation or detection limit was 0.1mg/L for hsCRP. At 1000mg/L the observed result was flagged as above the linearity of the assay[190].

2.17 Other laboratory tests

Liver function tests, blood glucose levels and C-reactive protein were measured in accordance with the manufacturer's instructions, using routine laboratory procedures and an automated analyzer. For CRP, the limit of detection was 0.5 mg/L. The interassay co-efficent of variation (CV) was <5% over the sample concentration range for the analytes measured. The CV is defined as the ratio of the standard deviation to the mean and it is the standard deviation divided by the mean. The IL-6 and MPO concentrations were quantified by commercial enzyme assays from R&D Systems Abingdon, UK, measured according to

manufacturers' instructions. BNP in the serum was also measured by immunoassay[157]. The methods relating to the analysis of these biomarkers is detailed above.

2.18 Statistics and data analysis

The sample size was 40 patients. This was based on the number of oesophageal surgeries carried out every year by the Upper Gastrointestinal surgeons at Glasgow Royal Infirmary. The baseline demographics of the cohort were presented using descriptive statistics. The researcher analysed the relationship between dimethylarginines, homoarginines and the onset of AF using an unpaired T test as data was non normally distributed.

The researcher originally used logistic regression to analyse the association between perioperative levels of several biomarkers including myeloperoxidase, CRP, IL-6 and BNP and onset of AF following oesophageal surgery to elicit more about the association between several predictor factors on the outcome of development of AF or no AF. Logistic regression was carried out as there was a binary dependent variable, despite having a small sample size following sample losses. For the original analyses, the researcher used an unadjusted, adjusted modelling strategy. This means that the outcome (AF or no AF) was considered in the context of no covariates and then the researcher used individual covariates such as age and post-operative values of biomarkers and CPET results to address confounding.

The advantages of logistic regression over other tests such as Chi squared or Fischer's exact test are that the researcher could include more than one explanatory variable and also it provided a value for the strength of the association adjusting for other variables.

When completing logistic regression, it is suggested that data should contain at least 10 events for each variable entered into a logistic regression model. This was not possible for the researcher's study, due to lack of complete data. The researcher could have potentially used Firth's logistic regression which has become a standard approach for the analysis of binary outcomes with small samples.

The examiners deemed logistic regression to be incorrect and therefore the statistical analysis was changed by the lead researcher.

As the data demonstrated a non-normal distribution, the researcher chose to use the Mann Whitney U test which is a non parametric test, instead of a parametric test which makes assumptions about a population's parameters such as the mean and standard deviation. Assumptions of a non parametric test include independence of observations, where no participants are in both groups.

Additionally, Mann Whitney U tests are helpful as the sample size was very small and the data had some outliers which could not be removed.

The software the researcher used for statistical analysis was R.[158]

Confounding

Age and sex in addition to electrolyte levels such as magnesium and potassium and also fluid balance are recognised potential confounding factors when looking at the development of AF. Confounding was controlled in the analysis of data by using stratified analysis. Stratification means making a separate table of disease by exposure for each confounder combination. We can compute a weighted average of the estimates of the risk ratios or odds ratios across the strata. So, for example, we can identify the relationship between sex and developing AF or not developing AF in patients in a certain age group (see section 2.22).

Adjusting for confounders meant that the internal validity of the study will be more robust. The loss of samples caused bias by reducing the sample size and this in turn determined validity in terms of the statistical power required to accept or reject the working hypothesis. Information bias can occur as a result of having incomplete data due to loss of information at time of recording data such as ECG recordings of AF being misplaced.

The small size of the pilot study resulted in challenges concerning missing data. During the data collection of the study, the researcher had the opportunity to try and prevent the

incidence of missing data, however this was beyond their control and therefore it was decided that this should be addressed during the data analysis part of the study. Problems with missing data include bias in the estimation of parameters and it can also reduce how representative a sample is. The researcher considered several methods of dealing with missing data including methods that discard data and also those that retain data. These included complete case analysis, available case analysis, mean substitution, regression imputation and multiple imputation[159].

The researcher chose to use a complete case analysis due to data which were missing completely at random (MCAR). This excluded any cases for which the outcome measure was missing. The researcher's data was MCAR because of equipment failure (freezer breakdown) and samples were also lost in transit. The researcher chose to omit the cases with the missing data and analyse the remaining data. There are problems with a complete case analysis as it discards data, also, sometimes the missing values differ from the completely observed cases which could potentially bias the analysis. There was a significant amount of missing data and so there were relatively few cases left for analysis.

Due to small numbers of patients undergoing oesophageal surgery over the time period that the researcher was recruiting patients, they were unable to randomise the patients that they recruited as they had already been listed for oesophageal surgery. They also did not restrict the entry of patients with potential confounding factors into the study as restricting entry would have risked bias in itself. They also knew that as a pilot study it would be too small to match patients to ensure equal distribution of confounders. Within one year, the researcher recruited 40 patients, of whom, they predicted between 5 and 10 would develop AF. The researcher originally used logistic regression to determine the relationship between pre-operative and day 0-day 7 values of biomarkers. The lead researcher used logistic regression to test whether there is an association between age of the patient and developing AF in their

cohort. The internal validity of a study is determined by multiple factors that can lead to errors or biases. Bias can be caused by several sources such as the sample selection, data collection and analysis. As such, bias can originate when patients are enrolled in the study. In the study the selection criteria were that patients had to be awaiting trans-hiatal, trans-thoracic oesophagectomy or oesophagogastrectomy, be males or non-pregnant females > 18 years old, not have AF and have capacity to give their written consent. This automatically creates selection bias. Ideally, studying a group that was matched by age and gender to the group undergoing oesophageal surgery, would have helped to address bias, however, this was not possible in this study.

The researcher could also have trialled linear regression to estimate missing values, by using the best predictors of the variable as independent variables and the variable with the missing data as the dependent variable in a regression equation. However, when replaced values are predicted from other variables they fit together too well and standard error is deflated. Also, the assumption is that there is a linear relationship between the variables used in the regression equation when there may not be one.

2.19 Study closure and definition of the end of the trial

The study ended when the planned sample size was achieved. When the results were collated, data from 40 patients was not present, however there was no time remaining for the researcher to extend the trial.

2.20 Data Handling

Data from patients participating in the trial was anonymised, encrypted and managed in an Excel spreadsheet on a University of Glasgow computer by the researcher. The computer that was used to hold the data was in an office that was locked when not in use and was password protected. Access to the department building itself was by entry code during office hours. All visitors were required to sign in and out of the building using the visitor's book at reception.

No identifiable data was released for off-site working and when non-identifiable data was taken off-site it was transported by me using an NHS approved encrypted USB stick. Data has been kept securely in password-protected, encrypted electronic format by the researcher and will be for at least 10 years after publications have been completed.

2.21 Record Retention

The researcher agreed to keep records including the identity of all participating subjects (with sufficient information to link records) and all original signed consent forms and source documents. Data will be retained for a minimum of 5 years.

2.22 Routine management of trial

The researcher co-ordinated the trial at Glasgow Royal Infirmary.

All aspects of the conduct and progress of the trial were monitored by the researcher and they ensured that the protocol was adhered to and that the quality of the trial and safety of participants was maintained.

2.23 Study monitoring and auditing

NHS Greater Glasgow and Clyde audit 10% of studies on a random basis per annum. This study has not been audited.

2.24 Protocol amendments

After further reading and consideration the researcher decided that it would be useful to monitor patient's progress by means of sequential CPET testing at 6,12 and 24 months following their operation. At these intervals the researcher planned to conduct Quality of Life questionnaires to get a subjective evaluation of the patient's progress. This change in the study protocol required an amendment and the proposed protocol amendment was initiated by the researcher following discussion with the Principal Investigator, Professor Kinsella. The researcher submitted the requisite amendment forms to the ethics committee and sponsor. The researcher also liaised with the study sponsor and determined that the amendment was deemed to be substantial. All amended versions of the protocol were signed by the researcher and the Sponsor representative. Before the amended protocol was implemented, the

researcher sought approval from the original reviewing REC and Research and Development (R&D) office. The outcome of the amendment is included in the appendices. It was decided that the CPET testing and QoL questionnaires should be carried out as part of a sub-study.

2.25 Ethical considerations

Ethical conduct of the study and approval

The study was carried out in accordance with the World Medical Association Declaration of Helsinki (1964) and its revisions (Tokyo [1975], Venice [1983], Hong Kong [1989], South Africa [1996] and Edinburgh [2000]). This reinforces that the health of patients was the researcher's first consideration.

The researcher sought favourable ethical opinion from the West of Scotland Research Ethics Service (WoS REC) before patients were entered into this clinical trial. Patients were only allowed to enter the study once they had provided the researcher with written consent. The meeting was convened on the 27th September 2012 and approval was granted via letter on 5th October 2012, reference 12/WS/ 0232: see appendices.

The researcher requested an Amendment (AM02) to the study with regard to carrying CPET testing and completion of Quality of Life questionnaires. This was considered by the WoS REC and a decision was made to undertake this element as a sub-study with the requirement that another consent form should be completed. The researcher was responsible for updating the Ethics committee of any new information related to the study.

3 CHAPTER 3

ARE PRE-OPERATIVE PLASMA CONCENTRATIONS OF DIMETHYLARGININES AND HOMOARGININE ASSOCIATED WITH THE DEVELOPMENT OF ATRIAL FIBRILLATION IN ADULT PATIENTS FOLLOWING OESOPHAGEAL SURGERY? ARE LOW LEVELS OF HOMOARGININE ASSOCIATED WITH INCREASED LEVELS OF BNP?

3.1 Setting; SDMA, ADMA, atrial fibrillation and outcomes

Nitric oxide (NO) is important for vascular health as it mediates vascular homeostasis and has antithrombotic and anti-inflammatory effects. As previously mentioned, NO is synthesized from L-arginine via endothelial NO synthase (e NOS). Research has indicated that increased presence of arginine in the circulation may improve endothelial function and contribute to delayed development of atherosclerosis[160]. ADMA and SDMA are the result of modifications to arginine residues. A growing number of studies have associated elevated concentrations of ADMA and SDMA to cardiovascular disease (CVD) and mortality. A recent systematic review and meta-analysis quantified associations of ADMA and SDMA with the risks of all-cause mortality and incident cardiovascular disease accounting for different populations and methodological approaches of prospective studies identified in PubMed until February 2015. ADMA and SDMA have overlapping as well as unique biological effects. Both may alter transport of arginine but only ADMA appears to directly inhibit NOS. Elevated ADMA may represent impaired DDAH activity while elevated SDMA may reflect impaired renal function and impaired alanine glyoxylate aminotransferase2 (AGXT2) activity. For ADMA a total of 34 studies (n=32,428) investigating associations with all-cause mortality events (n=5035) and 30 studies assessing the association with incident CVD events (n=3,396) were included. Summary Relative Risk (RR) (95% CI) for all-cause mortality were 1.52 (1.37-1.68) and for CVD 1.33 (1.22-1.45) contrasting high versus low ADMA concentrations. For SDMA, 17 studies (n=18,163) were included for all cause mortality events (n=2903) and 13 studies (n=16,807) for CVD events (n=1534) high as compared to low levels of SDMA, were

associated with increased risk of all-cause mortality summary RR (95% CI): 1.31(1.18-1.46) and CVD summary RR (95% CI): 1.36 (1.10-1.68). The strongest associations were observed in general population samples. The conclusion was that ADMA and SDMA are independent risk markers for all-cause mortality and CVD across different populations and methodological approaches[161]. AF is postulated to be related to pathways of oxidative stress, nitric oxide availability and arginine derivatives. As part of the Gutenberg health study, arginine in addition to NMMA, ADMA, SDMA levels were determined in association with ECG, and echocardiographic variables and onset of AF. ADMA (odds ratio [OR] 1.21, 95% confidence interval [CI] 1.11-1-32; p= 0.013) and NMMA (OR 1.17, 95% CI 1.09–1.26, p= 0.014) were related to prevalent AF. The arginine/ADMA ratio was inversely associated (OR 0.8, 95% CI 0.71–0.90, p=0.0082). Results were similar after adjustment for creatinine. SDMA was related to left atrial diameter and in Bonferroni-corrected multivariable-adjusted regression analyses, moderate inverse associations were observed for arginine, SDMA, and arginine/ADMA ratio and ventricular heart rate, and for arginine and arginine/ADMA ratio with QTc interval[162].

ADMA and SDMA modulate vascular function, partially by inhibiting nitric oxide generation and as such, may modulate the risk of thromboembolism and also cardiac death that patients with AF are exposed to. In 3,310 individuals from the community-based Framingham study levels of arginine, SDMA, ADMA and ratio of arginine/ADMA to incidence of AF were studied prospectively. Using proportional hazards age and sex adjusted regression models, ADMA and related arginine derivatives were not associated with incident AF in the community after accounting for other clinical risk factors and confounders[163]. As part of the 2011 ‘Apixaban for Reduction in Stroke and Other Thromboembolic Events in Atrial Fibrillation’ (ARISTOTLE) trial, 4951 patients had ADMA and SDMA plasma levels determined by HPLC at entry. Outcomes were determined by using Cox proportional hazards

models and were adjusted for factors including age, sex, heart failure, diabetes and previous stroke. Results showed that in patients with AF, ADMA and SDMA levels provided prognostic information, independent of other risk factors, for major bleeding, cardiac mortality and stroke or systemic embolism. This may suggest that impairment of nitric oxide generation may contribute to the risk of complications. ADMA may be useful as a biomarker for risk stratifying patients with AF[164].

3.2 Homoarginine levels, atrial fibrillation and outcomes

Over the past seven years, studies have suggested that low concentrations of homoarginine predict a risk of adverse cerebrovascular and cardiovascular outcomes and also mortality[113,114]. Due to homoarginine being similar in structure to L-arginine, it is thought that it interferes with the nitric oxide (NO) pathway. It does this as it is a weak alternative NO synthase substrate and competes with arginine for cellular transporters and so it improves arginine availability and inhibits its metabolism by arginases[165].

In a German cohort of 1649 patients with chest pain, low plasma homoarginine was identified as a risk marker for incident major adverse cardiovascular events (MACEs) including all cause death, myocardial infarction or stroke. When correlated with other biomarkers, circulating homoarginine had the strongest correlation with BNP; low homoarginine levels were associated with elevated levels of BNP. This finding correlates with previous studies[152,153,166]. In 288 patients with AF, significantly more patients had homoarginine concentrations below or at the median compared to above the median (n=168 vs 120; p=0.0023). In two large cohorts of patients, levels of homoarginine were independently associated with cardiovascular and all cause mortality in patients referred for coronary angiography and those undergoing haemodialysis[113].

In support of the association between homoarginine and endothelial function, low homoarginine concentrations have been associated with phenotypes of subclinical atherosclerosis such as brachial intima-media and aortic wall thickness[167]. Homoarginine

levels have been inversely associated with prevalent AF and low homoarginine levels have been associated with cardiovascular outcomes in patients with acute chest pain.

Laboratory analytical methods for methylarginines and homoarginine and collection and preparation of blood samples is addressed in chapter 2.

3.3 RESULTS

Table 3.1a Demographic data for study patients who developed AF compared with those who did not develop post-operative AF

	Pats developed AF(n=8)	Pats who did not develop AF (n=13)	Tot no of pats (n=21)
Age	63.5(11)	64(15.5)	64(14)
Male:Female	5:3	13:0	18:3
Type of cancer : Adenocarcinoma	7	12	19
Type of cancer: Squamous cell	1	1	2
Pre-op chemotherapy: Yes	8	11	19
Pre-op chemotherapy: No	0	2	2
Type of surgery :Transthoracic	2	3	5
Type of surgery : Transhiatal	3	5	8
Oesophagogastrectomy	3	5	8
60 day mortality n(%)	1(12.5%)	1(7.7%)	2(9.5%)
Hospital length of stay (LOS) days	23(16)	16(20.5)	21(19.5)

Age and hospital LOS figures are Median (Interquartile range).

Table 3.1b Gender and developing AF or not, in patients aged younger than 64 years old or people aged ≥ 64 years old.

	AGE <64 years				AGE >/= 64 years		
	AF	No AF	Total		AF	No AF	Total
Male	3	5	8	Male	2	8	10
Female	1	0	1	Female	2	0	2
Total	4	5	9	Total	4	8	12

The risk ratio (RR) for the total combined sample is 0.27, which is unadjusted for potential confounding factors.

The stratum specific risk ratios are;

Among those age < 64 years: 0.375

Among those ≥ 64 years: 0.2

The Cochran-Mantel-Haenszel estimate for the risk ratio is: 0.26.

This demonstrates that there is evidence of confounding due to age in the overall sample. Despite very small numbers, the table suggests that older females had a higher risk of developing AF than younger females.

The Cochran-Mantel-Haenszel method produces a single summary measure of association which provides a weighted risk ratio across the different strata of the confounding factor.

An Odds Ratio was unable to be calculated due to zero being in the calculations.

Limitations of stratified analysis include the inability to control simultaneously for multiple confounding variables. As you increase the number of strata you reduce the number of people in each stratum, so sample size will become a major problem.

Table 3.2a Comparison of mean preoperative levels of ADMA, SDMA and homoarginine levels in patients that developed AF post-operatively and those who did not and the 95% confidence interval comparing the means of these 2 groups.

Biomarkers	Levels in control gp	Mean preop plasma levels in pats who developed AF (total n=8)	Mean preop plasma levels in pats who did not develop AF (total n=13)	95% Confidence interval for difference between means of the 2 groups
ADMA (micromol/L)	0.46 (0.08)	0.50 (n=2)	0.59 (n=7)	0.09+/-0.009
SDMA (micromol/L)	0.38(0.07)	0.66 (n=2)	0.58 (n=7)	0.08+/-0.04
Homoarginine (micromol/L)	1.34(0.5)	0.92 (n=2)	1.87 (n=7)	0.956+/-0.87

Table 3.2b Comparison of mean perioperative levels of potassium (K^+) in patients that developed AF and those that did not and the 95% confidence interval comparing the means of these 2 groups.

	Patients who developed AF		Patients who did not develop AF		
Serum potassium levels (K+) (mmol/L)	no of patients (n)	Mean K+ level (mmol/L)	no of patients (n)	Mean K+ level (mmol/L)	95% CI for difference between the means of the 2 groups
Pre op K+	4	4.175	10	4.14	0.035+/-0.07
Day 0 K+	4	5.075	10	4.81	0.265+/-0.316
Day 1 K+	4	4.775	10	4.53	0.245+/-0.22
Day 2 K+	3	4.9	10	4.29	0.61+/-0.17
Day 3 K+	3	4.4	10	4.01	0.39+/-1.0
Day 4 K+	3	4.43	10	3.81	0.62+/-0.144
Day 5 K+	3	3.86	10	3.71	0.15+/-0.08
Day 6 K+	3	3.8	10	3.81	0.01+/-0.28
Day 7 K+	3	4.23	10	3.99	0.24+/-0.12

Table 3.2c Comparison of mean perioperative levels of magnesium (Mg) in patients that developed AF and those that did not and the 95% confidence interval comparing the means of these 2 groups.

	Patients who developed AF		Patients who did not develop AF		
Serum magnesium levels (Mg) (mmol/L)	no of patients (n)	Mean Mg level (mmol/L)	no of patients (n)	Mean Mg level (mmol/L)	95% CI for difference between the means of the 2 groups
Preop Mg	4	0.865	11	0.879	0.014 +/-0.008
Day 0 Mg	2	0.695	6	0.628	0.067+/-0.015
Day 1 Mg	3	0.743	10	0.7	0.43+/-0.03
Day 2 Mg	3	0.926	8	0.85	0.07+/-0.04
Day 3 Mg	3	0.96	10	0.848	0.12+/- 0.04
Day 4 Mg	2	0.925	8	0.857	0.07 +/- 0.62
Day 5 Mg	2	0.95	10	0.825	0.13+/- 0.005
Day 6 Mg	3	0.753	7	0.782	0.03+/- 0.005
Day 7 Mg	3	1.09	10	0.79	0.302+/- 0.02

3.4 Discussion

With reference to table 4.2a; in patients who developed AF, the mean preoperative ADMA level was lower compared with the mean preoperative ADMA level in those patients that did not go on to develop AF. We can conclude that there is a statistically significant difference in mean ADMA levels between the 2 groups as the confidence interval does not pass through zero.

Also, in patients who developed AF, the mean preoperative SDMA level was higher compared with the mean preoperative SDMA level in those patients that did not go on to develop AF. The confidence interval infers that there is a statistically significant difference in mean SDMA levels between the 2 groups as it does not pass through zero. Mean preoperative homoarginine levels were lower in patients that developed AF, compared with those who did not and again the confidence interval does not pass through zero, suggesting a statistically significant difference between the mean homoarginine levels in both groups.

The limitations include a very small sample size, which will give an imprecise estimate of the difference in mean ADMA, SDMA and homoarginine levels between the 2 groups.

The researcher has chosen not to include p values in the results as confidence intervals are equally valid.

The control group consisted of age range 42.5 yr +/-10.1years and comprised 42 females and 43 males from a healthy population. However, we are unable to draw any comprehensive conclusions from these results as the sample size is so small and data was missing. The unexpected lower pre-operative plasma ADMA levels in patients who went on to develop AF may represent increased cellular uptake or degradation. We also may have missed asymptomatic AF episodes and therefore the values of biomarkers may be influenced by clinically undetected paroxysmal AF. This is a potential source of bias. Limitations of this pilot observational study included the variation in the cohort studied and potential confounding factors that had not been fully accounted for such as age, sex, co-morbidities and medications that patients were taking could have an effect on whether patients in the study develop AF or not. Data concerning the patients including electrolyte levels such as magnesium and potassium and also fluid balance and electrolyte requirements could also be potential confounding factors when looking at the onset of AF.

In tables 4.2b and 4.2c the researcher compared mean perioperative levels of potassium and magnesium in patients that developed AF and those that did not develop AF.

In these small samples, patients who develop AF have higher mean potassium (K+) levels compared with those who do not develop AF apart from on day 6. Based on the mean values we can conclude that there is a statistically significant difference in mean K+ values between the 2 groups, apart from on days 0, 3 and 6, when the 95% CI for difference between the means includes the null value of zero, therefore meaning we don't have enough evidence to conclude that there is a difference.

The limitations include a small sample size, which will give an imprecise estimate of the difference in mean K+ levels between the 2 groups.

Patients who develop AF have higher mean magnesium (Mg) levels compared with those who do not develop AF apart from on day 6. Based on the mean values we can conclude that there is a statistically significant difference in mean Mg values between the 2 groups, apart from on day 4, when the 95% CI for difference between the means includes the null value of zero, therefore meaning we don't have enough evidence to conclude that there is a difference. The limitations include a small sample size, which will give an imprecise estimate of the difference in mean Mg levels between the 2 groups.

As a pilot study it was acknowledged that it would be too small to match patients to ensure equal distribution of confounders. Confounding can have a significant impact on internal validity and so it was decided that logistic regression should be used as a way of addressing potential confounders. In the results, due to the small sample size following sample losses doing logistic regression was limited. The entry of patients with potential confounding factors into the study was not restricted, as restricting entry would have risked bias. The external validity of the results was evidently dependent on the internal validity of the study which was limited due to patient recruitment being restricted to patients that were due to undergo oesophageal surgery at Glasgow Royal Infirmary.

Of note, in hospital mortality was 1 patient out of 8 patients (12.5%) who developed post-operative AF. This patient died on day 30 post-operatively. The two patients who died in the group who did not develop AF died on day 58 and 6 months after their operation. The hospital stay was longer in the group that developed AF post-operatively but this was not statistically significant. Mann Whitney U test $p > 0.05$.

3.5 Key message

- There may be a potential role for dimethylarginines and homoarginine as biomarkers for highlighting groups at risk of developing AF.

4 CHAPTER 4

ARE BNP, NT-PRO BNP, LEFT ATRIAL DIAMETER ASSOCIATED WITH DEVELOPMENT OF ATRIAL FIBRILLATION AND OUTCOMES IN PATIENTS UNDERGOING OESOPHAGEAL SURGERY? WHAT ASSOCIATION DO MYELOPEROXIDASE, IL-6 AND CRP HAVE WITH THE DEVELOPMENT OF ATRIAL FIBRILLATION AND OUTCOMES IN PATIENTS UNDERGOING OESOPHAGEAL SURGERY?

4.1 Setting : BNP and NT-pro BNP

As previously mentioned, NT-proBNP has been associated with an increased risk of death, atrial fibrillation and cardiovascular events in people after adjustment for traditional risk factors. Excess risk was apparent at levels well below thresholds used to diagnose heart failure[121].A Finnish study of 958 men suggested that N-terminal fragments of pro B type natriuretic peptide might help in identifying subjects at risk for stroke and AF[122].The relationship between NT-proBNP and AF was studied in the Cardiovascular Health study and the results suggested that NT-proBNP predicted AF in a community-based population of older adults[116].A systematic review and meta-analysis of observational studies showed that pre-operative BNP or NT-proBNP levels are an independent predictor of adverse outcomes within the first 30 days following non-cardiac surgery[132].A study of 142 patients demonstrated that elevated peri-operative plasma levels of NT-proBNP in oesophagectomy patients are an independent predictor of post-operative AF. Multivariable logistic regression adjusted for age, gender, COPD, history of cardiac disease, hypertension, diabetes mellitus, site of anastomosis, post-operative hypoxia, thoracic-gastric dilatation and plasma NT-proBNP showed NT-proBNP levels were associated with the highest risk factor for post-operative AF (OR= 4.711, 95% CI = 1.212-7.644, P = 0.008)[117]. In a systematic review and meta-analysis of 742 patients who participated in 5 observational studies, where BNP or NT pro BNP were measured up to a month before non cardiac thoracic surgery, the combined incidence of postoperative AF was 14.5% (n = 108/742), and the natriuretic peptide thresholds used to predict AF varied among studies. An elevated preoperative natriuretic

peptide measurement was associated with an OR of 3.13 (95% CI 1.38-7.12; I2 = 87%) for postoperative AF, with the sensitivity analysis reporting an OR of 9.51 (95% CI 4.66-19.40; I2 = 0) [168].

A best evidence topic regarding whether plasma level of BNP could predict the onset of post-operative AF in patients undergoing non cardiothoracic surgery was written on the basis of evidence from fourteen papers that were identified; five of which represented best evidence. The findings suggested that if NT-pro BNP or BNP levels were increased during the pre-operative period, then it identifies patients at risk for the development of post-operative AF after anatomical major lung resection or oesophagectomy[169]. Due to local protocols we were unable to test NT-proBNP levels in the laboratory which was dealing with the study samples and so we measured BNP levels[170].

4.2 AF and left atrial (LA) diameter

In a retrospective analysis of patients undergoing cardiac surgery for correction of mitral regurgitation, Kernis et al found that anteroposterior LA dimension to be related to POAF[171]. However, subsequent studies have found the LA diameter to underestimate left atrial volume (LAV)[172]. Cardiac diastolic dysfunction as measured by LA size has been found to be associated with adverse cardiovascular events in patients undergoing stress echocardiography. It can also indicate existing cardiac disease and can predict cardiovascular outcomes[173,174,175]. AF is a confounding factor in diastolic dysfunction as it is known to affect left atrial remodelling and geometry. LA dilation and geometric distortion may be primary or can be secondary to left ventricular diastolic dysfunction. The LA is usually enlarged in patients with AF and in a multicentre trial involving over 3,400 patients, the mean LA diameter was 6mm greater in those with AF compared with those in sinus rhythm at the time of echocardiography. The estimated independent contribution of atrial rhythm to LA diameter was 2.5mm. Independent predictors of LA diameter included prolonged duration of AF, LV

dilatation and hypertension[176]. In a study of 15 people, left and right atrial dimensions were measured at two different time points in patients who had normal atrial sizes initially and had AF diagnosed by two-dimensional echocardiography or by history. There were significant increases in calculated left atrial (45.2 to 64.1cm^3 p< 0.001) and right atrial volume (49.2-66.2cm^3, p < 0.001)[177]. This study suggested that left atrial enlargement could occur as a result of AF. The population Framingham and Strong Heart studies show that increased LA size is an independent risk factor for AF[173,174,175].Osranek's historical cohort study of 46 patients with lone AF observed that the patients who were diagnosed with normal atria and AF had a benign clinical course and patients with increased left atrial volume (>32ml/m^2), based on blinded analysis of echocardiographic videotape recordings of left atrial volume at diagnosis or during follow up, experienced adverse events such as myocardial infarction, congestive cardiac failure and cerebral infarction[178].The remodelling effect is present and independent of loading conditions within the LA and occurs in chronic and paroxysmal AF. It carries a degree of risk and therefore the maintenance of sinus rhythm may assist the risk reduction.

Post-operative AF and LA size

Atrial arrhythmias such as AF are encountered frequently after cardiac and other surgeries and are associated with increased incidence of systemic thromboembolism and stroke, haemodynamic instability and prolonged hospital stay[179]. More recently LA volume has been thought to be a more accurate measure of LA size than LA diameter although both parameters have been shown to be markers of cardiovascular disease[175]. Echocardiographic LA volume has been estimated by the biplanar method and has previously been used as a risk predictor of AF in elderly outpatients[180] and as a predictor of cardiovascular outcomes in patients with sinus rhythm. The relationship between LA size in AF patients and cardiovascular complications was not able to be fully determined in a study of 423 patients of

whom 106 had paroxysmal or permanent AF at baseline[181].Osranek completed a study assessing incidence of post-operative atrial fibrillation and LA volume (LAV). In 205 patients undergoing cardiac surgery, 84 patients developed AF and pre-operative LA volume was an independent predictor of AF. Patients with LAV > 32ml/m^2 had an almost 5-fold increased risk of POAF independent of age and clinical risk factors (Adj HR 4.84 95% CI 1.93-12.17, p= 0.001)[182]. We decided to determine LA diameter and left atrial volume pre-operatively in patients and to evaluate whether they went on to develop AF. We also decided to evaluate whether this measurement correlated with an increased risk of morbidity and mortality. Consideration was given as to whether this could assist with enhancing pre-operative risk stratification. Laboratory analytical methods for BNP are covered in chapter 2.

4.3 RESULTS

Figure 4.1 Flow chart of patients undergoing oesophageal surgery and their BNP data

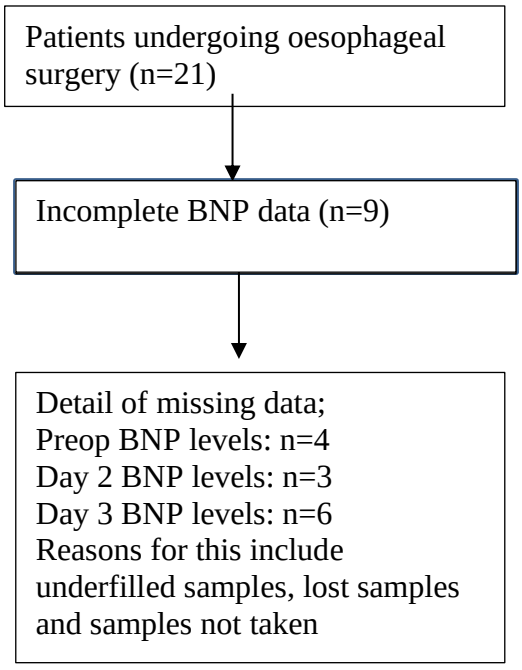


Table 4.1 Mean and Median (IQR) of pre-operative BNP values in patients who did not develop AF. (total n=13)

Pre op BNP values in pts who do not develop AF (pg/ml) n=12
Mean 44.45
Median 32 (63.05)

Table 4.2 Mean and Median (IQR) of pre-operative BNP values in patients who did develop AF (total n=8)

Pre op BNP values in pts who do develop AF (pg/ml) n=5
Mean 44.45
Median 35 (34.6)

A Mann Whitney U test was conducted to compare the 2 groups above. Mann Whitney U value: 28 and p value: 0.873.

Table 4.3 Mean and Median (IQR) of BNP values on day 2 in patients who did not develop AF total n=13 and also those who did develop AF total n=8

BNP values day 2 post op in patients without AF (pg/ml) n=11	BNP values day 2 post op in patients who develop AF (pg/ml) n=7
Mean: 140.02	Mean=199.69
Median: 75.6 (126.7)	Median=109.2

A Mann Whitney test revealed a U value of 31and p-value: 0.53.

Table 4.4 Mean and Median (IQR) of BNP values on day 3 in patients who did not develop AF total n=13 and also those who developed AF total n=8

BNP values day 3 post op in patients without AF (pg/ml) n=10	BNP values day 3 post op in patients who develop AF (pg/ml) n=5
Mean=107.39	Mean=219.48
Median=43.85 (86.65)	Median=105.7 (178)

Mann Whitney U test revealed a U value of 11 and p-value was 0.098.

Table 4.5 Difference in mean left atrial diameter (cm) for patients who developed AF post-operatively compared with those who did not.

Timing of Echo	Mean left atrial diameter (cm) on echo in pats who developed AF (n=3)	Mean left atrial diameter (cm) on echo in pats who did not develop AF (total n=6)
Pre-operatively	3.6	3.9
Post-operatively	3.9	3.8 (n=2)

The BNP values are non normally distributed and therefore Mann Whitney U tests were conducted on the data. In tables 4.1 and 4.2, the median pre-op BNP value in the group that went on to develop AF was 35 pg/ml and in the group that did not develop AF it was 32

pg/ml. The difference in the pre-op BNP values was not statistically significant. U= 28, p: 0.873 (two tailed).

Out of interest, I decided to study the day 2 and 3 BNP values in patients who developed AF and also in those who did not. In table 4.3 the median day 2 BNP value in the group that developed AF was 109.2 pg/ml and in the group that did not develop AF it was 75.6 pg/ml. The difference in the day 2 BNP values between groups was not statistically significant. Mann Whitney U=31, p: 0.53.

In table 4.4 median day 3 BNP values in the group that developed AF was 105.7 pg/ml and in the group that did not develop AF it was 43.85 pg/ml. The difference in the day 3 BNP values between groups was not statistically significant. Mann Whitney U=11, p: 0.098.

The reason for this could be that the sample sizes are too small. Also, although Mann Whitney U tests can be used for groups of different sizes, their ability to detect a difference between groups diminishes as the group sizes become more unequal.

4.4 Discussion

Pre-operatively there were three BNP values that were registered as <10 pg/ml and these were included in the analysis. On day 2 there was one BNP result which registered as <10pg/ml and on day 3 there was another BNP result <10pg/ml and both were included in the analysis by recording them as values of 5. The limitations were that the numbers of patients were small and some BNP samples were missing due to blood samples being underfilled, misplaced in the labs and also BNP levels not being taken post-operatively due to the work pressures on the nursing staff in ICU.

Due to small numbers of patients having an echocardiogram pre-operatively and post-operatively it was difficult to draw any conclusions regarding left atrial diameter measurements. The echocardiograms were done by one experienced consultant but

unfortunately due to lack of this expertise being available when patients went into AF, an echocardiogram was not always possible. Left atrial volume was not able to be calculated. In future, larger studies with a wider scope and resources could carry out multivariable logistic regression to analyse details of cardiac structure and function and levels of BNP in the context of developing AF. In addition to not only controlling for age and sex but also sedentary lifestyle, smoking, obesity, diabetes mellitus, hypertension, heart failure and myocardial infarction.

4.5 Key messages

- Peri-operative BNP values may be a useful adjunct in clinical risk stratification for patients undergoing oesophagectomy however this was a small pilot observational study and data were missing so conclusions could not be drawn.
- Left atrial diameter and left atrial volume may be of use in clinical risk stratification for patients undergoing oesophagectomy but larger studies are required.

4.6 Setting: Role of myeloperoxidase in onset of atrial fibrillation in oesophagectomy patients.

The phagocytic enzyme myeloperoxidase (MPO) has been strongly associated with ongoing inflammation, vascular endothelial dysfunction and the development of atrial fibrillation. We know that nitric oxide is a key regulator of cardiovascular endothelial function and it is thought to inhibit the adhesion of inflammatory cells to the vascular wall, the aggregation of platelets and the proliferation of smooth muscle cells. Reduction in synthesis and availability of NO has been recognised in combination with risk factors for cardiovascular disease and may promote atrial fibrillation. Research has suggested that MPO catalytically consumes nitric oxide (NO) in the presence of hydrogen peroxide (H_2O_2) and that MPO may act on NO at sites of inflammation, influencing its bioavailability[183].In patients undergoing oesophagectomy we hypothesized that patients who develop atrial fibrillation and adverse cardiovascular outcomes will have higher levels of myeloperoxidase associated with vascular endothelial dysfunction and inflammation. Laboratory analytical methods for MPO are covered in more detail in chapter 2.

4.7 RESULTS

Figure 4.2 Flow chart of patients undergoing oesophageal surgery and their MPO data

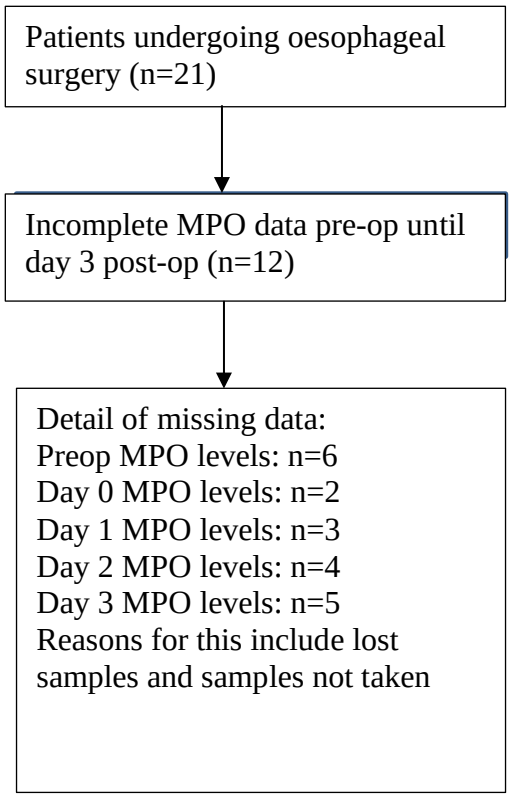


Table 4.6 Mean and Median (IQR) of preop myeloperoxidase (MPO) values in patients who develop AF total n=8 and also those who do not develop AF total n=13

Preop MPO AF (ng/ml) n=5	Preop MPO no AF (ng/ml) n=11
Mean=380.5	Mean= 445.8
Median=365.9(339.4)	Median=248(407.85)

Comparing both groups, the Mann Whitney U value is 21. The p value: 0.496

Table 4.7 Mean and Median (IQR) of Day 0 (day of operation) myeloperoxidase (MPO) values in patients who develop AF total n=8 and also those who do not develop AF total n=13

MPO Day 0 AF (ng/ml) n=8	MPO Day 0 no AF (ng/ml) n=12
Mean=735.7	Mean=685.4
Median=909.1(465.5)	Median=785.3(608.4)

The Mann Whitney U-value is 45. The p-value: 0.8493.

Table 4.8 Median (IQR) of Day 1 myeloperoxidase (MPO) values in patients who develop AF total n=8 and also those who do not develop AF total n=13

MPO Day 1AF (ng/ml) n=7	MPO Day 1 no AF (ng/ml) n=11
Median=499.9(270.6)	Median=1000(456)

The Mann Whitney U-value is 21. The p-value: 0.12356.

Table 4.9 Median (IQR) of Day 2 **myeloperoxidase (MPO) values in patients who develop AF total n=8 and also those who do not develop AF total n=13**

MPO Day 2 AF (ng/ml) n=8	MPO Day 2 no AF (ng/ml) n=9
Median=1000(140.45)	Median=1000(38.4)

The Mann Whitney U-value is 34. The p-value: 0.88866.

Table 4.91 Median (IQR) of Day 3 myeloperoxidase (MPO) values in patients who develop AF total n=8 and also those who do not develop AF total n=13

MPO Day 3 AF (ng/ml) n=6	MPO Day 3 no AF(ng/ml) n=10
Median=622.2(524.03)	Median=1000(0)

The Mann Whitney U-value is 11. The p-value: 0.04444.

Figure 4.3 MPO levels (ng/ml) in patients pre-operatively and up to day 7 in patients that developed AF, identified by their age.

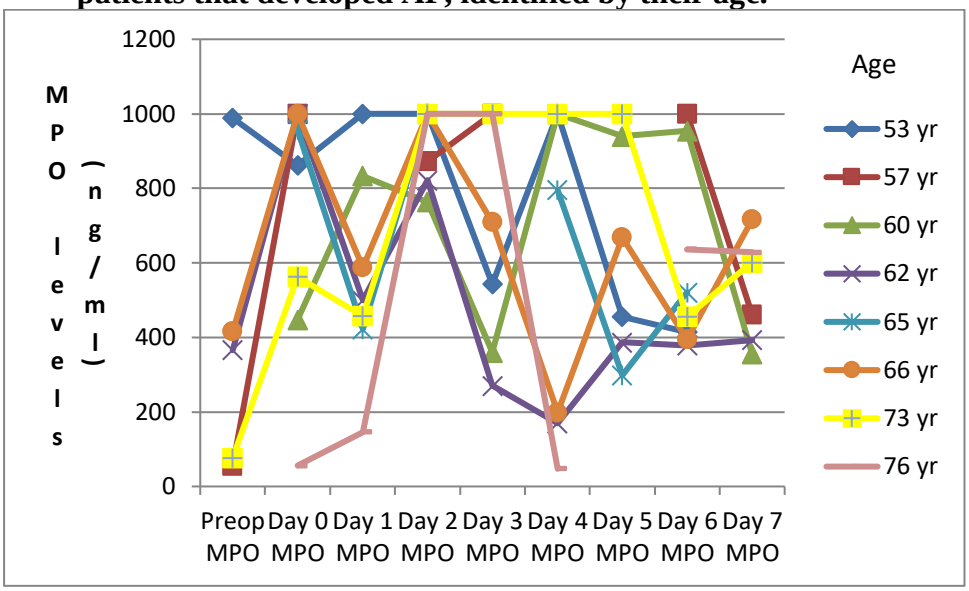
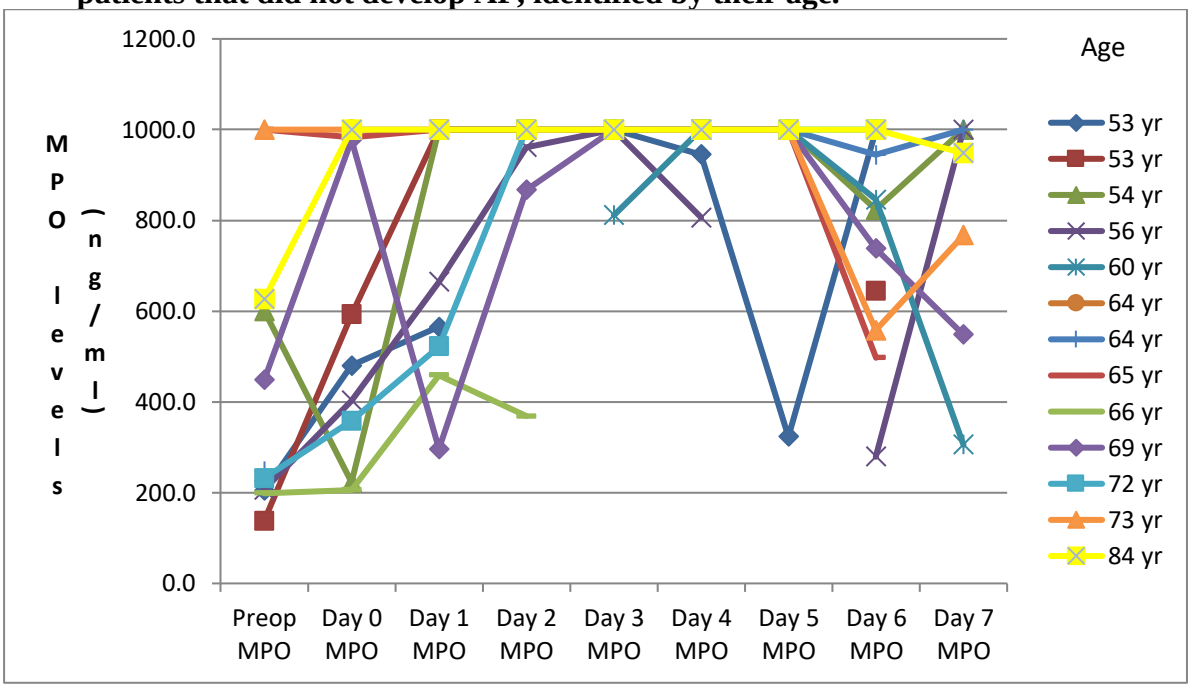


Figure 4.4 MPO levels (ng/ml) in patients pre-operatively and up to day 7 in patients that did not develop AF, identified by their age.



The MPO values are non normally distributed and therefore Mann Whitney U tests were conducted on the data. In table 4.6 the median pre-op MPO value in the group that went on to develop AF was 365.9ng/ml and in the group that did not develop AF it was 248ng/ml. The difference in the pre-op MPO values was not statistically significant. U value: 21 p-value:

0.496 (two tailed). I also decided to study the pre operative, day 0,1 2 and 3 MPO values in patients who developed AF and also in those who did not to determine whether MPO had a role in the onset of AF or not. In table 4.7 the median day 0 MPO value in the group which developed AF was 909.1ng/ml and in the group that did not develop AF it was 785.3ng/ml. The difference in the day 0 MPO values between groups was not statistically significant. Mann Whitney U= 45. The p-value: 0.8493. In table 4.8 median day 1 MPO values in the group that developed AF was 499.9 ng/ml and in the group that did not develop AF it was 1000 ng/ml. The difference in the day 1 MPO values between groups was not statistically significant. Mann Whitney U= 21. The p-value: 0.12356.

In table 4.9 median day 2 MPO values in the group that developed AF was 1000 ng/ml and in the group that did not develop AF it was 1000 ng/ml. The difference in the day 2 MPO values between groups was not statistically significant. Mann Whitney U= 34. The p-value: 0.88866. In table 4.91 median day 3 MPO values in the group that developed AF was 622.2 ng/ml and in the group that did not develop AF it was 1000 ng/ml. Mann Whitney U= The U-value is 11. The p-value: 0.04444 which is significant. Therefore, the difference in the day 3 MPO values between groups was statistically significant, with the MPO levels being higher in the group that did not develop AF compared with the group that did.

4.8 Discussion

Some of the MPO values were capped at 1000 nanograms per ml, as the values were high. The reason for doing this is that samples with high MPO values were above the validated range that the kit measured and assigning a sensible value allowed them to be included in the statistical analysis. Ideally these samples should be re-assayed at a higher dilution, but that was not possible. The limitations were that the numbers of patients were small and some MPO samples were missing due to blood samples being misplaced in the labs and also MPO levels not being taken post-operatively due to the work pressures in ICU. Another factor that

could have played a part in lack of sample taking was that nursing staff working on ICU had not all managed to attend the meetings that were hosted by the lead researcher, which briefed staff on the study and introduced them to the paperwork and how to identify which blood tube to use. The significant p value for the day 3 MPO values, despite small numbers, suggests that this warrants larger numbers and further investigation. Some of the reasons for this result could be that theoretically short episodes of AF might have been undetected in the group that did not develop AF, which could have caused the rise in MPO levels. A rise in MPO levels could be reflective of a post operative inflammatory process and there might be other patient factors that may have acted as protective factors. Due to the small numbers the lead researcher was unable to draw definitive conclusions based on the sample size.

4.9 Key messages

- Peri-operative MPO values and day 3 MPO levels in particular may be a useful adjunct in clinical risk stratification for patients undergoing oesophageal surgery, however the numbers in the cohort were small and a larger study is required.

4.10 Setting: The role of Interleukin-6 (IL-6) and C-reactive protein (CRP) in patients who develop atrial fibrillation following oesophagectomy for oesophageal cancer and evaluation of effect on outcome.

Interleukin-6 (IL-6)

IL-6 is a pleiotropic cytokine which means it affects the activity of multiple cell types. It acts in the acute phase reaction and is also involved in inflammation, haematopoiesis, bone metabolism, and cancer progression[185]. IL-6 production is generally correlated with cell activation and is normally kept under control by glucocorticoids, catecholamines, and secondary sex steroids. Normal human circulating IL-6 is in the 1 pg/ml range, with modest elevations in certain cancers and large elevations after surgery. After IL-6 is synthesised in a

local lesion in the initial stage of inflammation, it moves to the liver through the bloodstream, followed by the rapid induction of an extensive range of acute phase proteins such as C-reactive protein (CRP), serum amyloid A (SAA), fibrinogen and haptoglobin[186]. When dysregulated it contributes to chronic inflammation in conditions such as obesity, insulin resistance, inflammatory bowel disease, arthritis, and sepsis. IL-6 contributes to atherosclerotic plaque development and destabilization. Inflammatory biomarkers, including CRP and interleukin IL-6 are associated with the presence, persistence and outcome of AF in the general population. In my cohort, I wanted to determine whether there was any association between IL-6, AF and outcomes. Laboratory analytical methods for IL-6 are detailed in chaper 2**.**

4.11 RESULTS

Figure 4.4 Flow chart of patients undergoing oesophageal surgery and their IL-6 data

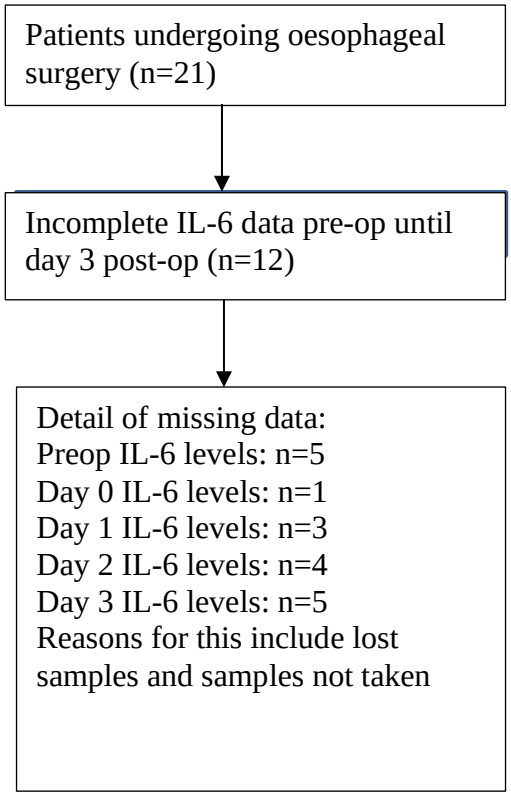


Table 4.92 Pre-op IL-6 values in patients who develop AF total n=8 and also those who do not develop AF total n=13

Preop IL6 (ng/ml) no AF n=11	Preop IL6 (ng/ml) AF n=5
2.8	5.4
6.1	2.1
1.6	2.0
2.2	2.0
2.0	592.6
1.0	Mean=120.8
2.7	Median=2.1
2.0	
2.0	
4.2	
2.1	
Mean=2.6	
Median=2.1	

Conparing both groups preoperatively, the Mann Whitney U-value is 21.5. The p-value is .53526.

Table 4.93 Day 0 IL-6 values in patients who develop AF total n=8 and also those who do not develop AF total n=13

IL-6 Day 0 (pg/ml) no AF n=12	IL-6 Day 0 (pg/ml) AF n=8
548.3	600.0
255.3	597.1
600.0	478.3
600.0	600.0
277.1	583.6
600.0	549.3
600.0	304.1
600.0	600.0
600.0	
600.0	
600.0	
600.0	

Day 0 The Mann Whitney U-value is 35.5. The *p*-value is .35238.

Table 4.94 Day 1 IL-6 values in patients who develop AF total n=8 and also those who do not develop AF total n=13

IL-6 Day 1(ng/ml) no AF n=11	IL-6 Day 1(ng/ml) AF n=7
296.9	600.0

264.8	458.4
600.0	600.0
295.2	239.3
89.3	65.7
600.0	425.7
370.1	600.0
144.7	
600.0	
379.8	
372.3	

Day 1 IL-6 The Mann Whitney U-value is 30.5. The p-value is .4965.

Table 4.95 Day 2 IL-6 values in patients who develop AF total n=8 and also those who do not develop AF total n=13

IL-6 Day 2 (ng/ml) no AF n=9	IL-6 Day 2 (ng/ml) AF n=8
92.2	600.0
600.0	92.9
303.6	600.0
493.6	62.3
100.0	122.3
261.6	112.4
280.8	37.1
322.4	600.0
473.6	

Day 2 The Mann Whitney U-value is 30.5. The p-value is .63122.

Table 4.96 Day 3 IL-6 values in patients who develop AF total n=8 and also those who do not develop AF total n=13

IL-6 Day 3(ng/ml) no AF n=10	IL-6 Day 3(ng/ml) AF n=6
29.1	600.0
160.2	600.0
96.3	173.3
62.9	50.8
528.1	35.1
71.2	600.0
36.4	
214.7	
374.5	
181.7	

Day 3 The Mann Whitney U-value is 21. The p-value is .35758.

Table 4.97 Median IL-6 levels (pg/ml) in patients that did develop AF post-operatively (total n=8)

	Median IL-6(pg/ml)	IQR	Total patients n=8
Pre-op	2.1	(2-299)297	n=5
Day 0	590.35	(513.8-600)86.20	n=8
Day 1	458.4	(239.3-600)360.70	n=7
Day 2	117.35	(77.6-600)522.40	n=8
Day 3	386.65	(50.8-600) 549.20	n=6
Day 4	284.2	(49.5-600) 550.50	n=7
Day 5	287.4	(112-600)488	n=7
Day 6	166.95	(581.4-148) 433.40	n=8
Day 7	397.4	(84.1-600)515.90	n=6

Table 4.98 Median IL-6 levels (pg/ml) in patients that did not develop AF post-operatively (total n=13)

	Median IL-6(pg/ml)	IQR	Total patients n=13
Pre-op	2.1	(2-2.8)0.80	n=11
Day 0	600	(574.2-600)25.80	n=12
Day 1	370.1	(280-600) 320	n=11
Day 2	303.6	(180.8-483.6)302.80	n=9
Day 3	128.25	(62.9-214.7)151.80	n=10
Day 4	73.7	(35.9-145.6)109.70	n=12
Day 5	70.1	(45.4-144.8)99.40	n=10
Day 6	50.9	(39-139)100	n=11
Day 7	60.1	(32.6-77.4)44.80	n=7

Figure 4.6 IL-6 levels (pg/ml) in patients pre-operatively and up to day 7 in patients that developed AF, identified by their age (total n=8)

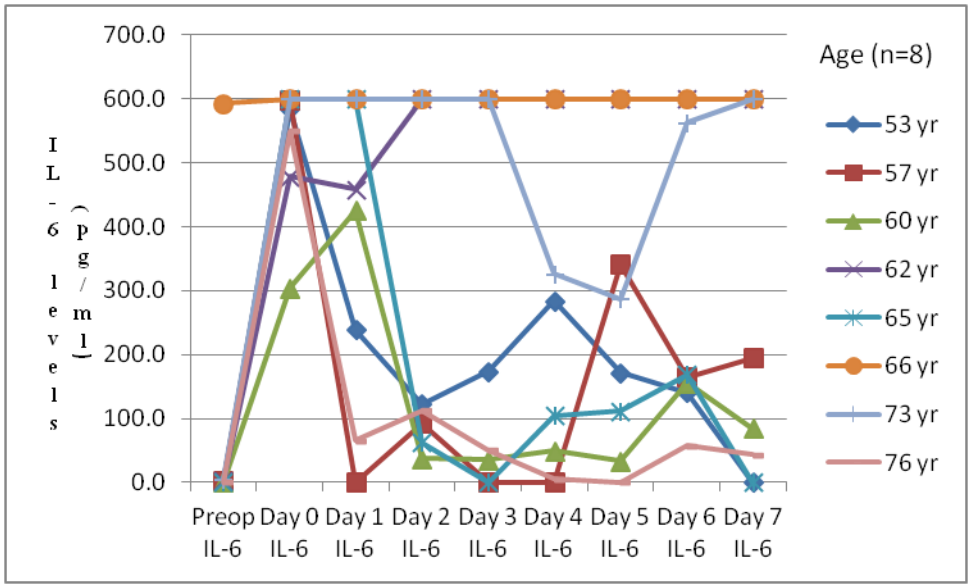
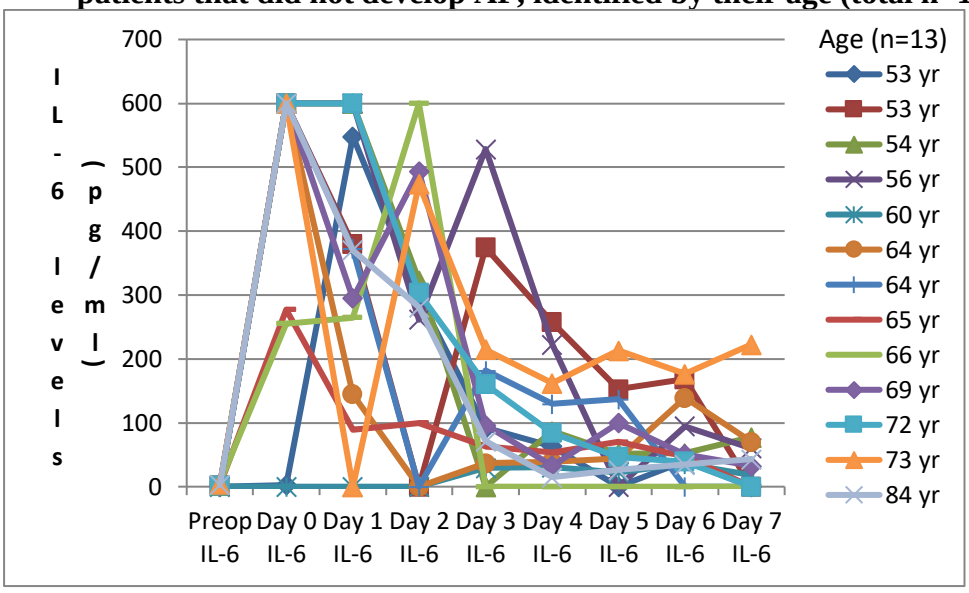


Figure 4.7 IL-6 levels (pg/ml) in patients pre-operatively and up to day 7 in patients that did not develop AF, identified by their age (total n=13)



4.12 Setting: The role of Hs (high sensitivity) CRP in patients who develop AF following oesophagectomy for oesophageal cancer and evaluation of effect on outcome

The large Danish study that was previously mentioned in the thesis found that elevated plasma CRP was robustly associated with increased risk of atrial fibrillation, although genetically elevated CRP levels were not. This suggested that elevated plasma CRP as such, does not increase atrial fibrillation risk. However, the study did not exclude a potential causal

association between inflammation and atrial fibrillation[144].CRP is produced by hepatocytes in response to inflammatory cytokines such as IL-6[188].CRP has been associated with cardiovascular, cerebrovascular and all-cause mortality. Conventional CRP assays that can accommodate very large increases have not been optimized for the highly sensitive detection of the low-level increases in values. CRP levels in apparently healthy individuals can be below 0.2 mg/L which requires high sensitivity or hs-CRP assays[189]. Laboratory analytical methods for hs CRP are detailed in chapter 2.

4.13 RESULTS

Figure 4.8 Flow chart of patients undergoing oesophageal surgery and their CRP data

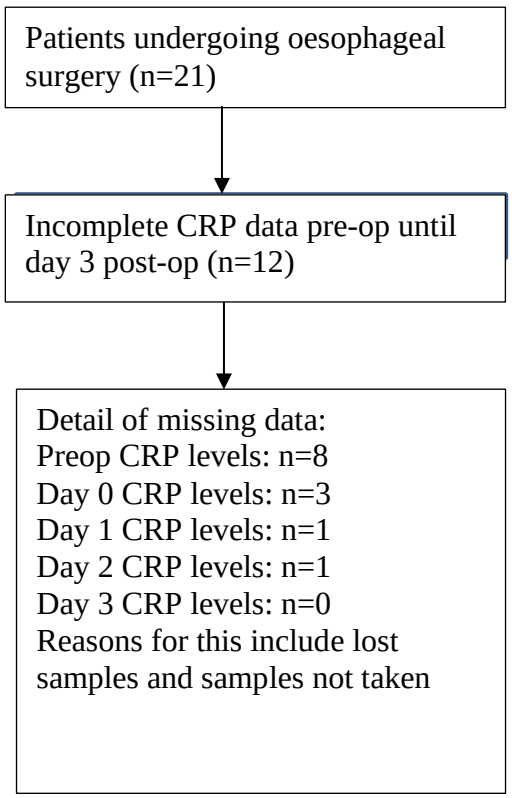


Table 4.99 Pre-op CRP values in patients who develop AF total n=8 and also those who do not develop AF total n=13

Preop CRP (mg/L) AF n=5	Preop CRP (mg/L) no AF n=8
0.7	0.4
0.4	7.3
0.9	1.9
5.4	6
0.4	1.1
Mean=1.56	0.8
Median=0.7	9.9
	3.8
	Mean=1.56
	Median=2.85

The Mann Whitney U-value is 9. The p-value is .12356.

Table 4.99.1 Day 0 CRP values in patients who develop AF total n=8 and also those who do not develop AF total n=13

CRP Day 0 (mg/L) AF n=6	CRP Day 0 (mg/L) no AF n=12
14	134
16	14
13	19
11	7.5
0.8	0.8
7.3	13
	1.7
	10
	11
	32
	7.8
	49

The Mann Whitney U-value is 30. The p-value is .60306.

Table 4.99.2 Day 1 CRP values in patients who develop AF total n=8 and also those who do not develop AF total n=13

CRP Day 1 (mg/L) AF n=8	CRP Day 1 (mg/L) no AF n=12
110	80
187	339
63	111
102	106
244	86
72	95
86	85
6.3	129
	127
	106
	207

	120

The Mann Whitney U-value is 33.5. The p-value is .28014.

Table 4.99.3 Day 2 CRP values in patients who develop AF total n=8 and also those who do not develop AF total n=13

CRP Day 2 (mg/L) AF n=8	CRP Day 2 (mg/L) no AF n=12
200	132
207	287
209	219
307	288
323	287
177	198
194	216
150	340
	317
	317
	305
	306

The Mann Whitney U-value is 29. The p-value is .15272.

Table 4.99.4 Day 3 CRP values in patients who develop AF total n=8 and also those who do not develop AF total n=13

CRP Day 3 (mg/L) AF n=8	CRP Day 3 (mg/L) no AF n=13
187	76
291	239
245	281
235	275
283	332
164	201
141	240
144	187
	333
	287
	289
	344
	282

Comparing both groups the Mann Whitney U-value is 30.5. The p-value is .12852.

Table 4.99.5 Median CRP levels (mg/L) in patients that did develop AF n=8

Timing of sample	Median CRP(mg/L)	IQR	Total patients n=8
Pre-op	0.7	(0.4-3.15) 2.75	n=5
Day 0	12	(7.3-14) 6.70	n=6
Day 1	94	(67.5-148.5)81	n=8
Day 2	203.5	(185.5-258)72.50	n=8
Day 3	211	(154-264)110	n=8
Day 4	176.5	(128-281.5)153.50	n=8
Day 5	163	(121.5-279)157.50	n=8
Day 6	167	(122-350)228	n=7
Day 7	193	(149-314)165	n=7

Table 4.99.6 Median CRP levels (mg/L) in patients that did not develop AF n=13

Timing of sample	Median CRP(mg/L)	IQR	Total patients n=13
Pre-op	2.85	(0.95-6.65)5.70	n=8
Day 0	12	(7.65-25.5)17.85	n=12
Day 1	108.5	(90.5-128)37.50	n=12
Day 2	287.5	(217.5-311.5)94	n=12
Day 3	281	(220-310.5)90.50	n=13
Day 4	242	(182-258)76	n=13
Day 5	188	(139.5-247.5)108	n=13
Day 6	166	(102-252.5)50.5	n=13
Day 7	172	(97.5-276.5)179	n=13

The IL-6 results are non normally distributed and therefore Mann Whitney U tests were conducted on the data. In table 4.92 the median pre-op IL-6 value in the group that went on to develop AF was 2.1ng/ml and in the group that did not develop AF it was also 2.1ng/ml. U value: 21.5 and the p-value is .53526 (two tailed). Therefore, the difference in the pre-op IL-6 values was not statistically significant. In a similar manner to other biomarkers, I also decided to study the day 0,1 2 and 3 IL-6 values in patients who developed AF and also in those who did not to determine whether IL-6 may have been associated with the onset of AF or not. In table 4.93 the median day 0 IL-6 value in the group which developed AF was 590.35 ng/ml and in the group that did not develop AF it was 600 ng/ml. The U-value is 35.5 and the p-value is.35238. The difference in the day 0 IL-6 values between groups was not statistically significant. In table 4.94 the median day 1 IL-6 value in the group which developed AF was 458.4 ng/ml and in the group that did not develop AF it was 370.1ng/ml. The U-value is 30.5 and the p-value is .4965 which is not statistically significant. In table 4.95 the median day 2 IL-6 value in the group which developed AF was 117.35ng/ml and in the group that did not develop AF it was 303.6ng/ml. The U-value is 30.5 and the p-value is .63122. In table 4.96 the median day 3 IL-6 value in the group which developed AF was 386.65ng/ml and in the group that did not develop AF it was 128.25 ng/ml. The U-value is 21 and the p-value is .35758, which again does not reach statistical significance. Table 4.97 illustrates the median pre-operative IL-6 levels and also the daily IL-6 levels and interquartile ranges in those who develop AF post-operatively and table 4.98 demonstrates the results for those who do not. Figures 4.5 and 4.6 illustrate IL-6 levels (pg/ml) in patients pre-operatively and up to day 7 in patients that developed AF and those that did not.

The CRP results do not follow a normal distribution. In table 4.991 the median pre-op CRP value in the group that went on to develop AF was 0.7mg/L and in the group that did not

develop AF it was 2.85mg/L. The U-value is 9 and the p-value is .12356 (two tailed). I studied the day 0,1 2 and 3 CRP values in patients who developed AF and also in those who did not to determine whether CRP may have been associated with the onset of AF.

In table 4.992 the median day 0 CRP value in the group which developed AF was 12mg/L and in the group that did not develop AF it was also 12mg/L. The U-value is 30 and the p-value is .60306. The difference in the day 0 CRP values between groups was not statistically significant. In table 4.993 the median day 1 CRP value in the group which developed AF was 94 mg/L and in the group that did not develop AF it was 108.5mg/L. The U-value is 33.5 and the p-value is .28014, which is not statistically significant. In table 4.994 the median day 2 CRP value in the group which developed AF was 203.5mg/L and in the group that did not develop AF it was 287.5mg/L. The U-value is 29 and the p-value is .15272. In table 4.995 the median day 3 CRP value in the group which developed AF was 211 mg/L and in the group that did not develop AF it was 281 mg/L. The U-value is 30.5 and the p-value is .12852, which again does not reach statistical significance. Tables 4.996 and 4.997 illustrate the median pre-operative CRP levels and also the daily median CRP levels from days 0-7 and interquartile ranges in those who develop AF post-operatively and these values for those who do not.

4.14 Discussion

In the analysis for IL-6, some of the very high extrapolated values were capped to 2 x the top standard; this value was 600pg/ml. The reason for doing this is that very high sample values are above the validated range that the kit will measure and assigning a sensible value allows them to be included in the statistics. Ideally these samples should be re-assayed at a higher dilution, but that was not possible. We know that IL-6 levels are raised in certain types of cancer and following surgery and it would be useful to have a baseline level of increases following surgery to determine whether the increases are different for disparate surgeries.

The limitations were that the numbers of patients were small and some IL-6 samples were missing due to blood samples being misplaced in the labs and also IL-6 levels not being taken post-operatively due to the work pressures in ICU.

The main limitation for CRP analysis was the missing samples which mainly affected the pre-operative values as detailed on figure 4.7. This happened due to clinical commitments and personally not being able to attend every preoperative clinic to take bloods so this task was delegated to the pre assessment nursing staff. I had met with the staff and we had discussed the study and the bloods required and the location of the pre labelled request forms. However, the large pool of nursing staff meant that these forms were not used and therefore CRP was not requested as it is not a routine pre operative test.

4.15 Key messages

- Perioperative IL-6 and CRP may be helpful in clinical risk stratification for patients undergoing oesophageal surgery, however the numbers in the cohort were small and a further study with a larger sample size is required.

5 CHAPTER 5

DOES THE USE OF THE MODIFIED GLASGOW PROGNOSTIC SCORING SYSTEM (MGPS) PRE-OPERATIVELY AND POST-OPERATIVELY (POGPS) ASSIST IN DETERMINATION OF ASSOCIATIONS WITH MORBIDITY AND MORTALITY IN OESOPHAGECTOMY PATIENTS? ARE THE RESULTS OF SERIAL POST OESOPHAGECTOMY CARDIOPULMONARY (CPET) TESTS AND QUALITY OF LIFE (QOL) ASSOCIATED WITH EACH OTHER?

5.1 Setting: Evaluation of use of modified Glasgow Prognostic scoring system (mGPS) pre-operatively and post-operatively (pomGPS) to look at associations with morbidity and mortality in oesophagectomy patients

The presence of malignancy is known to be associated with systemic inflammatory response. There is evidence that markers of systemic inflammatory response such as CRP and albumin have independent prognostic value in patients with cancer[191,192].The mGPS has been shown to be more sensitive than GPS where 0 points are allocated for CRP </=10mg/L and albumin ≥ 35g/L. CRP >10mg/L allocates 1 point and CRP >10mg/L and albumin <35g/L allocates 2 points[193].The post-operative mGPS has been utilised to independently predict overall survival in patients with localised clear cell renal cell cancer[194] and in light of the fact that the mGPS has been validated in a wide range of cancer scenarios I wanted to evaluate the use of the scoring systems in this cohort.

5.2 RESULTS

Figure 5.1 Flow chart of patients undergoing oesophageal surgery and their albumin data

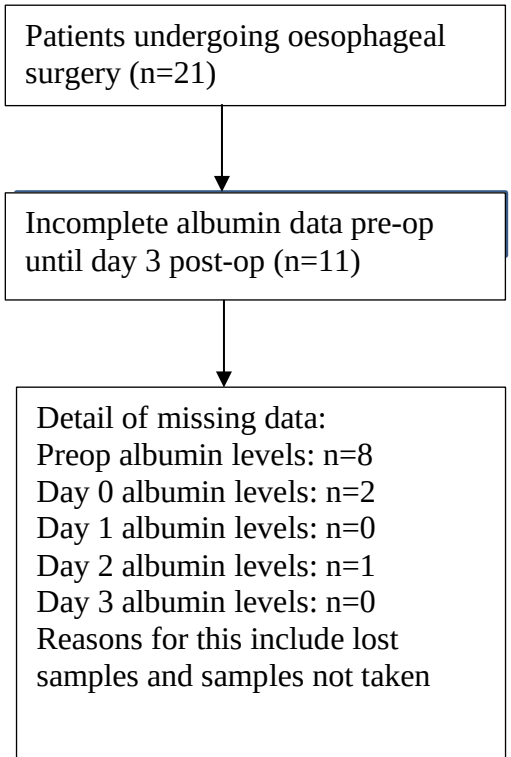


Table 5.0 Pre-op albumin (Alb) values in patients who develop AF total n=8 and also those who do not develop AF total n=13

Pre-op Alb (g/L) AF n=5	Pre-op Alb (g/L) no AF n=8
40	37
37	41
30	42
38	37
36	42
Mean: 36.2	39
Median: 37	37
	36
	Mean=38.88
	Median=38

Comparing both groups the Mann Whitney U-value is 12. The p-value is .27134.

Table 5.1 Day 0 albumin (Alb) values in patients who develop AF total n=8 and also those who do not develop AF total n=13

Day 0 Alb (g/L) AF n=7	Day 0 Alb (g/L) no AF n=12
32	18
16	25
30	24
31	24
28	35
23	42
22	31
Mean=26	30
Median=28	28
	20
	30
	24
	Mean=27.58
	Median=26.5

Mann Whitney U-value is 38. The p-value is .76418.

Table 5.2 Day 1 albumin (Alb) values in patients who develop AF total n=8 and also those who do not develop AF total n=13

Day 1 Alb (g/L) AF n=8	Day 1 Alb (g/L) no AF n=13
30	25
23	19
21	20
32	19
29	23
28	29
24	28
18	26
Mean=25.63	28
Median=26	23
	19
	29
	23
	Mean=23.92
	Median=23

The Mann Whitney U-value is 39.5. The p-value is .3843.

Table 5.3 Day 2 albumin (Alb) values in patients who develop AF total n=8 and also those who do not develop AF total n=13

Day 2 Alb (g/L) AF n=7	Day 2 Alb (g/L) no AF n=13
27	25
21	17
27	20
22	19
27	24
22	24
21	27
Mean=23.86	25
Median=22	26
	22
	21
	25
	20
	Mean=22.69
	Median=24

The Mann Whitney U-value is 33.5. The p-value is .36282.

Table 5.4 Day 3 albumin (Alb) values in patients who develop AF total n=8 and also those who do not develop AF total n=13

Day 3 Alb (g/L) AF n=8	Day 3 Alb (g/L) no AF n=13
24	24
21	18
18	19
26	18
21	22
26	21
19	24
19	24
Mean=21.75	24
Median=21	20
	18
	24
	20
	Mean=21.23
	Median=21

The Mann Whitney U-value is 46. The p-value is .68916.

Table 5.5 Post-operative Glasgow Prognostic Scores (po mGPS) from day of operation (D0) up to day 7 (D7) post-operatively in patients who develop AF (n=5)

Sex	Age	D0 po GPS	D1 po GPS	D2 po GPS	D3 po GPS	D4 po GPS	D5 po GPS	D6 po GPS	D7 po GPS
Male	62 yr	2	2	2	2	2	2	2	2
Female	65 yr		2	2	2	2	2	2	2
Male	73 yr	0	2	2		2	2	2	2
Male	53 yr	2	2						
Male	60 yr	0		2	2	2	2	2	2

Table 5.6 Post-operative Glasgow Prognostic Scores (po mGPS) from day of operation (D0) up to day 7 (D7) post-operatively in patients who do not develop AF (n=10)

Sex	Age	D0 po GPS	D1 po GPS	D2 po GPS	D3 po GPS	D4 po GPS	D5 po GPS	D6 po GPS	D7 po GPS
Male	53yr	2	2	2	2	2	2	2	2
Male	53yr	2	2	2	2	2	2	2	2
Male	54yr	2	2	2	2	2	2	2	2
Male	56yr	2	2		2	2	2	2	2
Male	60yr		2	2	2	2	2	2	2
Male	64yr	0	2	2	2	2	2	2	2
Male	64yr	2	2	2	2	2	2	2	2
Male	65yr	1	2	2	2	2	2	2	2
Male	66yr	2	2	2	2	2	2	2	2
Male	69 yr	0	2	2	2	2	2	2	2
Male	72yr	2	2	2	2	2	2	2	2
Male	73yr	0	2	2	2	2	2	2	2
Male	84yr	0	2	2	2	2	2	2	2

5.3 Discussion

The data was non normally distributed and so the Mann Whitney U test was used. In comparing the patient group that developed AF and the one that did not, from pre operatively to day 3 post-operatively, none of the p-values were statistically significant. One of the reasons for this was the small sample size.

All patients who developed AF post-operatively had a pre-operative mGPS score of 0 and one patient had a score of 0 on day 0. Six patients developed AF on day 2, the 53 year old patient developed AF on day 3 and the 73 year old patient on day 4. The 73 year old patient died on day 30. Three patients had cardiorespiratory complications. In the group that did not develop AF, one patient had an mGPS score of 1 pre-operatively and three patients had a score of 0 on day 0. Two patients died, one at 58 days and 5 patients had cardiorespiratory complications. The calculation of po mGPS is incomplete due to lack of albumin and CRP levels resulting from blood tests being unable to be completed and blood samples being lost. Regarding the usefulness of poGPS in patients undergoing oesophageal surgery, due to the small numbers in this cohort we are unable to draw any conclusions. More recently studies concerning 12,000 patients have resulted in an optimised score (termed the optimised Glasgow Prognostic Score, oGPS) composed of high sensitivity C-reactive protein (>3mg/l), albumin (<35g/l), neutrophil (>7.5 x 10^9) and platelet (>400 x 10^9) counts that had a superior predictive value when compared with the established GPS.[193] This could form the basis of future work with a larger cohort.

5.4 Setting: Pre-operative Cardiopulmonary (CPET) testing, sequential testing and evaluation of relationship with Quality of Life (QoL).

As previously mentioned, regarding sequential CPET testing, a literature search revealed no studies that have quantified post-operative fitness and quality of life in this population.

We originally wanted to assess whether there were differences in serial CPET tests at 6 months, 1 year and 2 years following oesophagectomy, compared with baseline pre-operative tests. Quality of life (QoL) is a factor that is very important to patients when considering the

possible outcomes of an operation as it is important in morbidity risk prediction and information provided for patients. Whilst QoL pre-operatively and post-operatively have been compared in this population, comparing post-operative QoL with post-operative fitness, as determined by CPET test results in patients who have undergone oesophagectomy, had not been studied before and so we wanted to determine whether a relationship existed between CPET results and patient's reported QoL.

5.5 RESULTS

Table 5.7 Pre-operative Cardiopulmonary Exercise Test (CPET) results for patients who develop AF. Anaerobic Threshold (AT) (ml/min/kg) and maximum rate of oxygen consumption (VO2 peak) (ml/kg/min) (n=5) Mean AT: 12.0 ml/min/kg. Mean VO2 peak: 19.2ml/kg/min.

AGE (yrs)	CPET:AT (ml/min/kg)	VO2 peak(ml/kg/min)
53	15.4	22.1
57	13.2	17.8
60	NO CPET	
62	NO CPET	
65	11.9	18.8
66	10.1	20.9
73	10.3	16.3
76	NO CPET	
Mean=64	Mean=12.18	Mean=19.18
Median=63.5	Median=11.9	Median=18.8

Table 5.8a Post-operative medical and surgical complications in all patients who develop AF (n=8)

Age (yr)	Medical complicn	Details: Cardiorespiratory/ Non cardioresp	Surgical complicn	Details	Deceased at 30 days
53	Y	Non cardiorespiratory: raised WCC/ CRP	N		N
57	Y	Cardiorespiratory: pleural effusions	Y	Anastomotic leak	N
60	Y	Cardiorespiratory: chest sepsis	Y	Hydropneumothorax	N
62	Y	Cardiorespiratory and AKI	Y	Dehiscence of roof top incision	N
65	Y	Cardiorespiratory: respiratory failure+ delirium	Y	Pneumothorax	N
66	N		N		N
73	Y	Cardiorespiratory: sepsis	Y	Intraop splenectomy+ blood transfusion	Y
76	N		N		N

Table 5.8b Binomial logistic regression to determine relationship between pre-operative maximum rate of oxygen consumption (VO2 peak) (ml/kg/min) and development of medical complications post-operatively in patients who develop AF.

	Coefficient	Std error	z value	p value (Wald)
Intercept	13.25	14.95	0.89	0.38
VO2 peak	-0.59	0.73	-0.82	0.41

Table 5.8c Binomial logistic regression to determine relationship between pre-operative Anaerobic Threshold (AT) (ml/min/kg), age of patient and development of surgical complications post-operatively in patients who develop AF.

	Coefficient	Std error	z value	p value (Wald)
Intercept	-41.87	50.29	-0.83	0.41
AT	1.14	1.53	0.74	0.46
Age(yr)	0.46	0.52	0.87	0.38

Table 5.8d Binomial logistic regression to determine the relationship between pre-operative Anaerobic Threshold (AT) in (ml/min/kg), maximum rate of oxygen consumption (VO2 peak) (ml/kg/min) and age of patient with development of AF

	Coefficient	Std error	z value	p value (Wald)
Intercept	18.03	13.45	1.34	0.18
AT	-0.54	0.49	-1.08	0.28
VO2 peak	-0.19	0.25	-0.79	0.43
Age (yr)	-0.12	0.1	-1.16	0.25

Table 5.9a Pre-operative Cardiopulmonary exercise test (CPET) results for all patients who do not go on to develop AF. Anaerobic threshold (AT) and maximum rate of oxygen consumption (VO2 peak) (n=11) Mean AT:12.6ml/min/kg. Mean VO2 peak: 20.1ml/kg/min

AGE (yrs)	CPET:AT (ml/min/kg)	VO2 peak(ml/kg/min)
53	11.9	19.4
53	13.8	23.3
54	9.7	
56	13	23.5
60	NO CPET	
64	NO CPET	
64	14.1	22.2
65	12.7	27.7
66	13.7	17.6
69	11.9	20.4

72	11.3	15
73	13	15.3
84	13.3	16.9
Mean=64.08	Mean=12.58	Mean=20.13
Median=64	Median=13	Median=19.9

Table 5.9b Post-operative medical and surgical complications in all patients who do not develop AF

Age (yr)	Medical complicn	Details: Cardiorespiratory/ Non cardiorespiratory	Surgical complicn	Details	Deceased at 30 days
53	Y	Cardiorespiratory : pleural effusion	Y	Oesophageal tear on OGD, leading effusion	N
53	N		N		N
54	N		Y	Discharging wound	N: 58days ? PE
56	N		N		N
60	N		N		N
64	N		Y	rosion of chest drain into gastric staple line and into oesophagu	N
64	N		N		N
65	N		Y	Post op apical pneumothorax	N
66	Y	Cardiorespiratory : pleural effusion/type 1 resp failure/ cardiac failure/ C diff	N		N
69	N		Y	Required dilatation of anastomotic stricture	N
72	N		Y	Wound infection and collection	N
73	Y	Cardiorespiratory:Type 1 resp failure	N		N
84	Y	Cardiorespiratory: Aspiration pneumonia + delirium	Y	Recurrent laryngeal nerve damage: left vocal cord palsy	Y

Table 5.9c Binomial logistic regression to determine the relationship between pre-operative Anaerobic Threshold (AT) in (ml/min/kg), maximum rate of oxygen consumption (VO2 peak) (ml/kg/min) and development of medical complications post-operatively in patients who do not develop AF

	Coefficient	Std error	z value	p value (Wald)
Intercept	-2.85	11.2	-0.25	0.79
AT	1.14	1.06	1.07	0.28
VO2 peak	-0.63	0.36	-1.74	0.08

Table 5.9d Binomial logistic regression to determine the relationship between pre-operative Anaerobic Threshold (AT) in (ml/min/kg), maximum rate of oxygen consumption (VO2 peak) (ml/kg/min) age of patient and development of surgical complications post-operatively in patients who do not develop AF

	Coefficient	Std error	z value	p value (Wald)
Intercept	3.56	15.83	0.23	0.82

AT	-2.35	1.63	-1.45	0.15
Age (yr)	0.23	0.18	1.27	0.2
VO2 peak	0.63	0.56	1.131	0.26

Table 5.9e Pre-operative Cardiopulmonary exercise test (CPET) Anaerobic threshold (AT) results for all patients who do not go on to develop AF (total n=13) compared with those who do go on to develop AF (total n=8)

CPET:AT (ml/min/kg) no AF n=11	CPET:AT (ml/min/kg) AF n=5
11.9	15.4
13.8	13.2
9.7	NO CPET
13	NO CPET
NO CPET	11.9
NO CPET	10.1
14.1	10.3
12.7	
13.7	
11.9	
11.3	
13	
13.3	

Comparing the groups, the Mann Whitney U-value is 23. The p-value is .65272.

Table 5.9f Pre-operative Cardiopulmonary exercise test (CPET) VO2 peak results for all patients who do not go on to develop AF (total n=13) compared with those who do go on to develop AF (total n=8)

VO2 peak(ml/kg/min) no AF n=10	VO2 peak(ml/kg/min) AF n=5
19.4	22.1
23.3	17.8
23.5	
	18.8
	20.9
22.2	16.3
27.7	
17.6	
20.4	
15	

15.3	
16.9	

The Mann Whitney U-value is 22. The p-value is .75656.

5.6 Discussion

The CPET results could not be assessed in context of the QoL due to a colleague being unable to complete that part of the study due to ill health.

Pre-operative Cardiopulmonary Exercise Test (CPET) AT and VO2 peak results for patients who developed AF and those who did not were evaluated.

Table 5.7 illustrates the pre-operative cardiopulmonary exercise test results for patients who develop AF. Three, out of eight patients did not undergo CPET testing and 2 patients had anaerobic thresholds lower than 11ml/min/kg. Table 5.8a demonstrates that 5 patients in the group who developed AF post-operatively had cardiorespiratory complications and one patient had non cardiorespiratory complications. All of the patients who had medical complications also had surgical complications.

Out of interest, I used binomial logistic regression to explore the relationship between the maximal rate of oxygen consumption and medical complications post-operatively in patients who develop AF. It was not possible to create an additive model with AT and VO2 peak in the context of the development of medical complications and AF. A model which combined VO2 peak, AT, age of patient in the context of development of AF was created. Out of interest, bearing in mind that the sample size was 5, a multivariable regression model was created to evaluate the relationship between AT, age of patient and surgical complications in patients who developed AF post-operatively. It was not possible to extend this model to include VO2 peak. The regression model did not demonstrate statistical significance. Table 5.9a illustrates the pre-operative cardiopulmonary exercise test results for patients who did not develop AF. Two out of thirteen patients did not undergo CPET testing and one patient had an AT less than 11ml/min/kg. Table 5.9b demonstrates that four out of the eleven

patients who did not develop AF post-operatively, developed cardiorespiratory complications and of the 4, 2 developed surgical complications. Five patients had surgical complications without medical complications. To explore the relationship between the maximal rate of oxygen consumption and medical complications post-operatively in patients who do not develop AF, binomial logistic regression was used. It was possible to create an additive model with AT and VO2 peak in the context of the development of medical complications in this patient group. Again, out of interest, the relationship between AT, VO2 peak, age of patient and surgical complications was also evaluated in table 5.9d, but it did not demonstrate statistical significance. In tables 5.9e and 5.9f, I decided to compare pre-operative Cardiopulmonary exercise test (CPET) AT and VO2 peak results for all patients. This included those who do not go on to develop AF with those who do go on to develop AF, to see if there was a statistically significant difference between the two groups using Mann Whitney U tests. There was not a statistically significant difference between the AT and VO2 peak results in patients that did develop AF when compared with those who did not. The limitations include small numbers and missing CPET studies and results.

5.7 Challenges regarding the sub-study concerning serial CPET testing and relationship to QoL

Serial CPET testing and evaluation of QoL was determined to be a sub-study. The patient information leaflet, consent form, study protocol and GP information were duly changed in May 2013 and an application was made to the REC committee for a substantial amendment. The REC committee advised that a letter of invitation was developed to ensure that patients did not feel pressurised to consent to further CPET tests. This was developed in addition to ensuring that the aforementioned information was updated.

This sub-study planned to obtain consent from patients to perform CPET tests at 6 months, 12 months and 2 years following their oesophagectomy and determine their Quality of Life. The

paperwork to facilitate this was completed but the sub-study was never commenced due to a research colleague being unable to commit to the study.

6 CHAPTER 6

CAN WE APPLY THE CHARLSON AGE COMORBIDITY INDEX (CACI) TO THE COHORT AND USE THIS IN ADDITION TO BIOMARKERS, CPET RESULTS AND DEVELOPMENT OF AF TO HELP A BETTER UNDERSTANDING OF FACTORS INFLUENCING THE DEVELOPMENT OF AF AND ASSOCIATED MORBIDITY AND MORTALITY IN PATIENTS UNDERGOING OESOPHAGECTOMY?

6.1 Setting: The Charlson Age-Comorbidity Index (CACI)

The Charlson Age-Comorbidity Index (CACI) which is a combined age-comorbidity score was validated for the prediction of long-term mortality in patients undergoing non cardiac elective surgery that had essential hypertension or diabetes mellitus[27].It accounts for the patient's age and 16 conditions and uses the International Diagnosis of Diseases (ICD) diagnosis codes. The CACI has been validated in a number of surgical cohorts and it has been found to be a moderately accurate tool[27]. In a colorectal surgery cohort, it was found to be a predictor of in hospital morbidity, duration of hospital stay and mortality[29].In light of this, I wanted to investigate whether combining biomarkers, CPET results and CACI would assist in understanding the mechanisms leading to the development of AF and increased mortality in patients undergoing oesophagectomy.

6.2 RESULTS

Table 6.1 Charlson Age Co-morbidity Index (CACI), Anaerobic threshold (AT) and cardiovascular biomarkers in the context of complications (table 8.2) in patients who did not develop AF (n=13)

Age yrs	CACI score	Estim RR death	CPET:AT (ml/min/kg)	MPO (ng/ml) (median d0-2)	BNP(pg/ml)(preop)	IL-6 (pg/ml)(median d0-2)	CRP (mg/L) (median d0-2)
53	3	3.04	11.9	479.9	124.2	379.8	108.5
53	7	13.37	13.8	796.4	28.5	296.9	287
54	3	3.04	9.7	800.4	26.9	461.2	69
56	5	6.38	13	533.7	<10	430.8	27
60	8	19.37	NO CPET				80
64	4	4.4	NO CPET	1000	<10	144.7	69.5
64	6	9.23	14.1	1000	15.7	486.2	84.5
65	8	19.37	12.7	1000	<10	94.7	95
66	4	4.4	13.7	287.5	35.5	394.4	62.5
69	8	19.37	11.9	657.9	42.5	394.4	46.8
72	10	19.37	11.3	440.3	73.3	451.8	106
73	11	19.37	13	1000	84.4	473.6	106
84	8	19.37	13.3	1000	87.5	325.5	6

Table 6.2 Medical and Surgical complications in patients who did not develop AF (n=13) (reference table 5.9b)

Age (yr)	Medical complicn	Details: Cardiorespiratory/ Non cardiorespiratory	Surgical complicn	Details	Deceased at 30 days
53	Y	Cardiorespiratory : pleural effusion	Y	Oesophageal tear on OGD, leading effusion	N
53	N		N		N
54	N		Y	Discharging wound	N: 58days ? PE
56	N		N		N
60	N		N		N
64	N		Y	rosion of chest drain into gastric staple line and into oesophagu	N
64	N		N		N
65	N		Y	Post op apical pneumothorax	N
66	Y	Cardiorespiratory : pleural effusion/type 1 resp failure/ cardiac failure/ C diff	N		N
69	N		Y	Required dilatation of anastomotic stricture	N
72	N		Y	Wound infection and collection	N
73	Y	Cardiorespiratory:Type 1 resp failure	N		N
84	Y	Cardiorespiratory: Aspiration pneumonia + delirium	Y	Recurrent laryngeal nerve damage: left vocal cord palsy	Y

Table 6.3 Charlson Age co-morbidity index (CACI), Anaerobic threshold (AT) and cardiovascular biomarkers in the context of complications in patients who did develop AF (n=8)

Age (yrs)	AF onset (day)	CACI score	Est RR death	CPET:AT (ml/min/kg)	MPO (ng/ml) (median d0-2)	BNP(pg/ml)(preop)	IL6 (pg/ml)(median d0-2)	CRP (mg/L) (median d0-2)
53	3	3	3	15.4	994.3		180.8	127.5
57	2	3	3	13.2	872.3	35	92.9	187
60	2	4	4.4	NO CPET	763.3		304.1	86
62	2	5	6.4	NO CPET	660.6	68.5	468.4	38
65	2	8	19.4	11.9	956.5	71.2	600	102
66	2	8	19.4	10.1	794	19.6	600	7.3
73	4	9	19.4	10.3	510.2	33.9	600	62
76	2	5	6.4	NO CPET	145.9		112.4	72

Table 6.4 Medical and Surgical complications in patients who did develop AF (n=8)

Age (yr)	Medical complicn	Details: Cardiorespiratory/ Non cardioresp	Surgical complicn	Details	Deceased at 30 days
53	Y	Non cardiorespiratory: raised WCC/ CRP	N		N
57	Y	Cardiorespiratory: pleural effusions	Y	Anastomotic leak	N
60	Y	Cardiorespiratory: chest sepsis	Y	Hydropneumothorax	N
62	Y	Cardiorespiratory and AKI	Y	Dehiscence of roof top incision	N
65	Y	Cardiorespiratory: respiratory failure+ delirium	Y	Pneumothorax	N
66	N		N		N
73	Y	Cardiorespiratory: sepsis	Y	Intraop splenectomy+ blood transfusion	Y
76	N		N		N

6.3 Discussion

Table 9.1 combines the results of Charlson Age Co-morbidity Index and the estimated relative risk of death with pre-operative AT values and pre-operative median values for BNP

and day 0-2 median values for MPO, IL-6 and CRP in patients who did not develop AF. Table 9.3 combines the results of Charlson Age Co-morbidity Index and the estimated relative risk of death with pre-operative AT values and pre-operative median values for BNP and day 0-2 median values for MPO, IL-6 and CRP in patients who did develop AF. A mixed effects model was attempted to analyse the data and create a risk stratification score based on the Charlson Age-Comorbidity Index, in addition to biomarkers, CPET results and development of AF, however this was not possible due to the small numbers.

7 CHAPTER 7

THE CHALLENGES WITH CONDUCTING THE STUDY

7.1 Background

This study began due to my interest in patients who developed atrial fibrillation post-operatively as I had evaluated a cohort of patients on ICU who had AF. Knowing that AF was associated with significant morbidity and mortality I hypothesized whether it would be possible to predict its development in patients post-operatively and therefore potentially prevent morbidity and mortality.

I selected oesophageal surgery as I knew that oesophagectomy operations were carried out at the GRI and on the basis of the number of operations that were undertaken, that it might be possible to recruit at least 40 patients over a period of 9 months. It was clear that recruiting 40 patients would be a suitable number for a feasibility study. On initial assessment, it appeared that the patients would have a distinct pathway leading to their surgery and therefore this could be helpful in deciding when to do investigations and when to consent patients. I had also read about the use of different biomarkers and their potential to predict AF and wanted to evaluate what role they might play in its development. I discussed my concept with my supervisors and I wrote the protocol for my study.

7.2 Ethics applications and amendment

I made an application to the Research Ethics Committee and attended the meeting which was held on the 27th September 2012. The members of the committee gave a favourable ethical opinion of the study. Research and Development approval was sought from and granted by the NHS host organisation prior to commencement of the study. Following further discussions with colleagues, I submitted an application to the Research Ethics Committee for an amendment to the study to evaluate the impact that oesophagectomy has on the perceived quality of life of the patient and also the effect it might have on cardiopulmonary exercise tests which I planned to carry out at 6, 12, 18 and 24 months following the operation. The

Committee agreed to this piece of work becoming a sub-study instead of an amendment and as such, it was approved. Unfortunately, this piece of work never came to fruition due to illness of a colleague, therefore meaning that they were unable to assist with it. I had also intended to study urinary ADMA and SDMA. Due to sample losses and therefore missing data, this was not feasible. Additionally, I collected blood results, including liver function tests and kidney function for patients and had intended to analyse these in relation to development of AF.

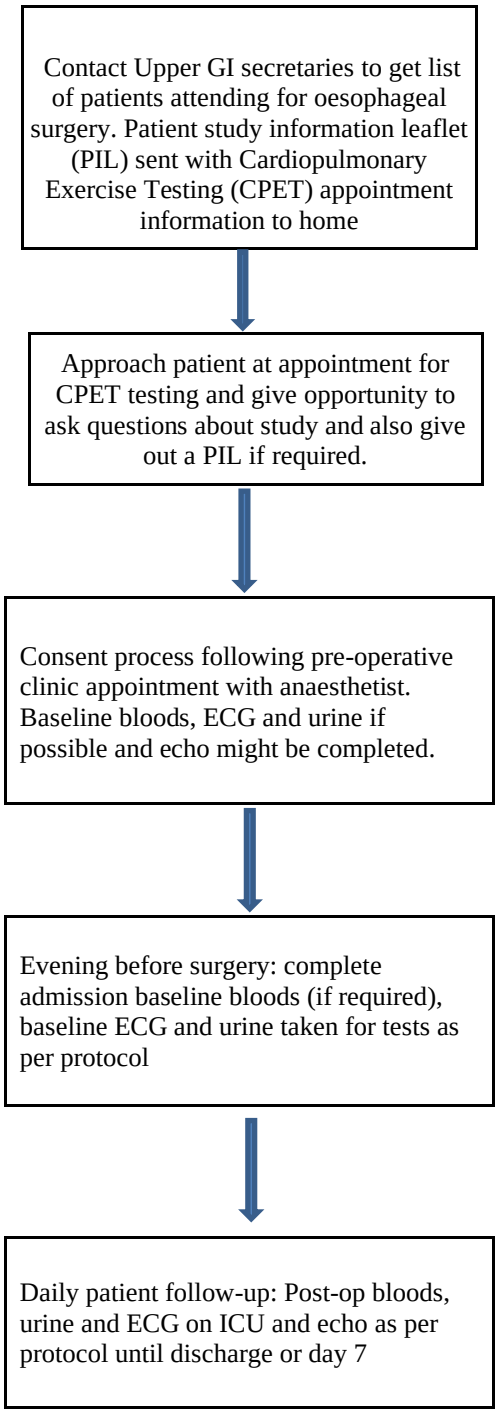
I commenced the study with a protocol and associated paperwork which included a patient pathway. This initially appeared to be straightforward, however this turned out not to be the case.

Monitoring and evaluation of a study's conduct is vital. As a result of monitoring the study as it progressed, I was able to evaluate its quality and move towards a vision of how the study could be optimised. There were several important points in the study detailed below that provided the opportunity for improvement if the study were to be repeated in future.

7.3 Study interventions based on perceived patient pathway from identification to operation

In Scotland, there is a commitment to delivering the National Cancer Quality programme, 'Beating Cancer: Ambition and Action' across NHS Scotland,[198]with a recognised need for National Cancer Quality Performance Indicators (QPI)s to support a culture of continuous quality improvement. Patients who require oesophageal surgery are identified at the multidisciplinary team (MDT) meetings which are attended by surgeons, oncologists and radiologists, in addition to other team members. Patients will often have 'tumour, node, metastasis' (TNM) stage and treatment intent stated at the multidisciplinary team meeting and those undergoing surgical resection should be offered neoadjuvant chemotherapy. Neoadjuvant chemotherapy or chemoradiotherapy prior to surgery provides a survival benefit for patients with oesophageal or gastric cancer.

Figure 7.1 Study interventions based on perceived peri-operative pathway for oesophagectomy patients



Identification of patients who are deemed to require oesophageal surgery required frequent liaison with the secretaries working with the Upper Gastrointestinal (GI) consultants, as it was not possible for me to attend MDT meetings where the decisions were originally made, due to my clinical commitments.

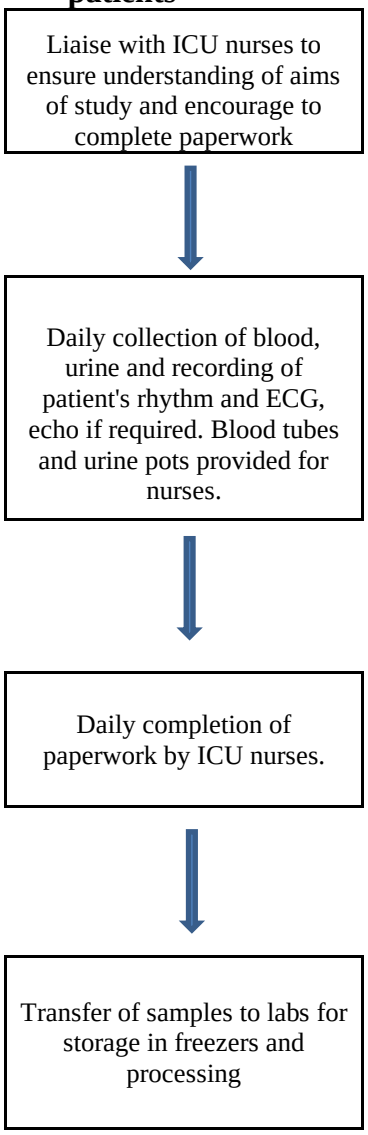
7.4 Patient information and investigations

It was challenging to discover that there was no standardised timeline and pathway for patients' visits to hospital regarding pre-operative investigations such as CPET following their neoadjuvant chemotherapy. The next step in the pathway was to give patients information and also to approach them prior to their admission and ensure that they had some bloods taken. Due to this lack of standardisation, the number of opportunities to talk to patients about the study and also to give them a patient information sheet was affected. This also led to inconsistencies regarding the requesting and completion of pre-operative echocardiograms and blood tests.

7.5 Admission to hospital

Patients awaiting oesophageal surgery were admitted on the evening of the day prior to surgery. These admissions happened at inconsistent times and so it was a labour intensive exercise for me to attend hospital at the correct time to review the patient and to ensure that pre-operative bloods and urine were taken, in addition to an ECG and echo if required.

Figure 7.2 Perceived peri-operative investigation pathway for oesophagectomy patients



For information sheet to assist nursing staff understand the practicalities of study see appendix (pg 173-176)

7.6 Recording of cardiac rhythm

Post-operatively it was challenging to record the onset of atrial fibrillation as due to my on call and work rota, I mainly had to rely on the nursing staff on duty on ICU. I held sessions

on ICU regarding the study and how to record different parameters as well as sharing a guidance sheet for the study, however, frequently no specific time of onset or duration of AF was recorded. The ECGs were often not done due to patient agitation or nurses having insufficient time. Time pressures also led to the daily document, which was created to monitor these investigations being inconsistently completed by nursing staff on ICU. The protocol also detailed that patients who developed AF required an echocardiogram from a dedicated consultant whose contact details were provided. Again, due to time pressure and availability of the staff on ICU, inconsistent contact was made with the consultant who carried out echos when patients went into AF. This led to several opportunities to complete this investigation being missed.

7.7 Laboratory sample collection and transport

The collection of blood and urine samples and their transportation to the labs was not a standardised process and depending on the time of day that the blood and urine samples were taken, a porter, a nurse or sometimes the chief investigator delivered them to the labs. This process varied depending on whether the samples were taken in, or out of hours. As part of the study protocol, within the labs there was a box for samples which was specifically for the study. I had negotiated with the biochemistry lab and there was a verbal agreement that the box should be emptied regularly. However, there was inconsistent transfer of the samples into the study box and therefore some samples did not arrive in the labs and did not get processed.

7.8 Storage of samples

The study samples were stored in one laboratory freezer, which was set at -60^{o}C. This freezer was situated in a hospital corridor remote from the laboratories. Prior to the study commencing I had not completed a risk assessment regarding the sample storage and I had made assumptions about how laboratories would manage their storage of study samples. The freezer which held some of my study samples broke down over a weekend and this led to

them defrosting as the breakdown was not picked up until Monday morning. This resulted in 14 serum samples, in addition to multiple urine samples being unable to be salvaged.

Figure 7.3 Photographs of remote laboratory freezer at the GRI

7.9 Freezer breakdown

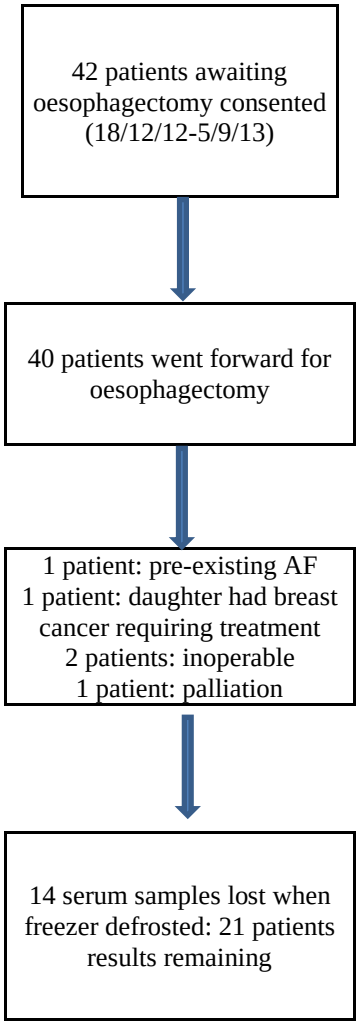
After the event, I enquired about the cause of the freezer breakdown and I was informed that it was due to the compressor within it breaking. I further enquired as to the governance of the laboratory freezers and was told that it was the responsibility of the Clinical Pathology Accreditation (CPA). I was told that the freezer in question was 3 years old and that this freezer had not previously broken down. Due to the freezer being located at a distance from the laboratory this resulted in the breakdown being missed, as the alarm system for the laboratories was not connected to the remote freezer and over weekends and out of hours there was no consistent checking of the freezers.

7.10 Governance of freezers in laboratories

After the incident regarding the freezer breakdown, I discussed the laboratory governance process and whether there had been a critical incident lodged and a root cause analysis completed. I also enquired if there had been a contingency plan for freezer failure prior to this event. I was informed that a critical incident form was not lodged as there was no requirement in the laboratory for this, due to the lost samples not being part of a clinical trial. I also enquired as to whether governance guidelines had been changed or created since the freezer breakdown. At the time that the incident occurred there was no governance regarding how samples were sorted and placed in a freezer and no consideration regarding minimisation of the potential risk of sample loss if the freezer broke down, in terms of separating samples from the same study between freezers. There exists very clear mandatory guidance about what should happen to samples from clinical trials and how samples are stored. There was no clear guidance for the samples that are not involved in clinical trials, such as research samples and in this case, the onus is on the lab.

Of note, there is no British regulation for laboratory freezers. I was informed that since 2017 there has been a change in accreditation in the laboratory where the freezer was based and it is now United Kingdom Accreditation Service (UKAS) accredited. UKAS provides accreditation to the internationally recognised standard ISO 15189 which details Medical Laboratories requirements for quality and competence. This has resulted in clear guidelines detailing how the freezers are now alarmed. The alarm system is connected to the main laboratory and as a result, remote freezers are now monitored 24 hours a day and 7 days a week. The labs are subject to annual review by UKAS and they are inspected annually as part of UKAS accreditation. Since 2017 there have been no similar events or freezer breakdowns.

Figure 7.4 Study consort diagram



7.11 Discussion

Patient pathway

In the event that a similar study might be repeated in the future, the lead researcher reflected on which aspects of it they might be able to improve. The ability to standardise the patient pathway from selection for surgery until the operation would enable a more streamlined consent process for the patient and also for optimising investigations. Following this study, the centralisation of oesophageal surgery on one site has been a key factor, resulting in endeavours to streamline the pathway.

Rhythm recognition

The technology exists for the ability to record the onset of AF and measure its duration. This could be programmed and preset on the monitors on ICU and when patients developed this rhythm, an automatic rhythm print out could be produced. This would minimise work for the nursing staff. Frequently, dysrhythmias are empirically treated on ICU with electrolytes, fluids and rate controlling medication. An automated identification of AF would be helpful in providing information about the precise onset and duration of AF and therefore, when combined with analysis of biomarkers, could better inform knowledge about whether there is a significant relationship between them.

Investigation pathway process

When the lead researcher reflected upon what happened with regards to loss of serum and urine samples, they acknowledged that they had not given the safe storage of samples enough consideration, as they had incorrectly assumed that a laboratory dealing with thousands of daily samples would have safety mechanisms and contingency plans in the event of a freezer breaking down. In retrospect, they should have completed a risk assessment at the beginning of the study to identify the potentially critical 'pinch' points in the process and they should have made a contingency plan.

In future, they would ensure that samples were separated and stored in different freezers to ensure that even if a freezer did break down or if the alarm malfunctioned and samples were lost, the number of lost samples relative to the total number of samples in the study would be small.

7.12 Limitations

The lead researcher reflected on the validity of their pilot study and how well the outcomes for the study participants would be reflected in individuals beyond the study.

Earlier in the thesis factors affecting the internal validity of the study were mentioned and therefore factors affecting the relationship between oesophageal surgery and developing AF.

These factors included eg. whether all episodes of AF in the cohort studied were able to be detected.

The external validity of the results was evidently dependent on the internal validity of the study which was limited due to patient recruitment being restricted to patients that were due to undergo oesophageal surgery at Glasgow Royal Infirmary. As mentioned previously this was due to time limitations and also the practicalities of recruiting patients when the lead researcher also had to fulfil a clinical commitment in a hospital several miles away. Another factor influencing internal validity was the loss of patient samples due to freezer breakdown. This left the lead researcher with a small number of samples to analyse and resulted in logistic regression being completed out of interest rather than having enough samples to be able to do more meaningful analysis. Limitations of this pilot observational study included the variation in the cohort studied and potential confounding factors that had not been fully accounted for such as age, sex, co-morbidities and medications that patients were taking could have an effect on whether patients in the study develop AF or not. Data concerning the patients including electrolyte levels such as magnesium and potassium and also fluid balance and electrolyte requirements could also be potential confounding factors when looking at the onset of AF.

The lead researcher knew that as a pilot study it would be too small to match patients to ensure equal distribution of confounders. Confounding can have a significant impact on internal validity and so, they decided to use logistic regression as a way of addressing potential confounders. In the results, due to the small sample size following sample losses they were limited in doing logistic regression. They also did not restrict the entry of patients with potential confounding factors into the study as restricting entry would have risked bias. Selection bias originated when patients were enrolled in the study. In the study the selection criteria were that patients had to be awaiting trans-hiatal, trans-thoracic oesophagectomy or

oesophagogastrectomy, be males or non-pregnant females > 18 years old, not have AF and have capacity to give their written consent. Ideally, studying a group that was matched by age and gender to the group undergoing oesophageal surgery, would have helped to address bias, however, this was not possible in my study.

Information bias occurs as a result of having incomplete data. To deal with missing data you can use linear regression and if you are using logistic regression, imputation of missing data can be done.

The way that was chosen to deal with missing data was to omit the cases with the missing data and to analyse the remaining cases.

Problems with missing data include bias in the estimation of parameters and it can also reduce how representative a sample is.

Linear regression could also have been trialled to estimate missing values, by using the best predictors of the variable as independent variables and the variable with the missing data as the dependent variable in a regression equation. When replaced values are predicted from other variables the assumption is that there is a linear relationship between the variables used in the regression equation when there may not be one. Often they fit together too well and standard error is deflated.

7.13 Conclusions

In conclusion, due to these factors, this pilot study has limited internal validity and therefore external validity. In future, a larger study would need to be done with collection of more data, including nutritional status, baseline medications, co-morbidities and also consideration of matching patients undergoing oesophageal surgery to those with similar characteristics who are not undergoing oesophageal surgery. Along with robust data collection, the statistical analysis would be more meaningful, ensuring greater internal and external validity and therefore the results might be more generalizable.

In general terms, this study reinforced the need for further work regarding investigation into why patients were developing AF and how this arrhythmia might be predicted and prevented.

Positive aspects of the study

R&D and Ethics approval resulting in the recruitment and consenting of 40 patients over a 9 month period. This was a notable achievement.

Additionally, regarding the investigations and samples collected on ICU, the lead researcher had some excellent feedback regarding communication with nursing staff about study conduct and clarity about what needed to be done on a daily basis for the study.

7.14 Key message

- Despite the challenges the lead researcher encountered whilst undertaking this study, they have been able to turn challenges into opportunities for learning that they have been able to take forward to inform their future practice.

8 CHAPTER 8

8.1 Conclusions

In this thesis I planned to utilise peri-operative biomarker levels, combined with results from cardiopulmonary exercise testing, in addition to the development of AF, to explore whether it was possible to determine effects on morbidity and mortality in patients undergoing oesophageal surgery. I also included the Charlson Age Co-morbidity Index risk stratification tool and the modified Glasgow prognostic scoring system pre and post-operatively to explore the feasibility of improving risk stratification in this patient group. The concept was that if there was a greater understanding of the mechanisms leading to increased morbidity and mortality in patients undergoing surgery for oesophageal cancer then this could assist in moving us closer to the personalisation of care, where risk for each patient could be individually stratified and decision making could be better informed and truly shared.

I conducted the study and have shown that ADMA levels were lower and homoarginine levels were lower in patients who had undergone oesophageal surgery and develop AF post-operatively. Both were statistically significant. The levels of BNP, myeloperoxidase, IL-6 and CRP were not significantly related to AF development in these patients. When considering the modified Glasgow prognostic scoring system pre and post-operatively, it was not possible to determine evidence of any difference between values in the patient group that did develop AF compared with the group that did not. There was no statistically significant relationship between VO2 peak and medical complications and no significant relationship between anaerobic threshold and surgical complication in patients who developed AF post-operatively. There was also no statistically significant relationship between anaerobic threshold, VO2 peak and medical and surgical complications in patients who do not develop AF. When determining the overall connection between biomarkers, cardiopulmonary exercise testing results, the Charlson Age Co-morbidity Index risk stratification tool and the modified

Glasgow prognostic scoring system pre and post-operatively in the context of developing AF and morbidity and mortality, it was not possible to complete a mixed effects model. Being honest about my study and the work detailed in my thesis, a source of disappointment for me was that as a result of the unique challenges which I encountered, it was not possible to draw any robust conclusions from the study due to sample loss and small numbers. This study was effectively one of feasibility. The next logical step would be to implement changes in the planning and processes which were identified as areas of improvement in the work described above and to take this forward to a larger study.

Confounders that were undetected in my dataset or have not been identified in previous studies were not adjusted in my statistical analyses. In contrast, there might be mediators in a causal pathway that were adjusted in the statistical analyses; it is difficult to discriminate mediators from confounders among the variables in my dataset. In addition, I could adjust only the data regarding the postoperative complications that were available in my dataset. Moreover, unfortunately there was no information regarding the accurate sequence of the postoperative complications and the causality between the postoperative AF and other complications. Therefore, caution has to be taken when interpreting the results. Despite the limitations of the study design, I think that my study is clinically significant as an exploratory research that suggests a perspective that postoperative AF might be an independent risk factor in oesophageal cancer patients who underwent oesophageal surgery.

The learning which I have benefitted from, as a result of the challenges I encountered whilst undertaking the study will be applied to any academic work which I undertake in the future and could also be extrapolated to clinical practice and beyond. I also plan to apply the knowledge which I developed as a result of what went well in the study. A point worth recognising is that some of the Glasgow-based population has reduced life expectancy when

compared to the rest of Europe and it is noteworthy that the study cohort may have had high levels of concurrent morbidity causing derangement of the variables studied.

Further to the work that has been done in the area of oesophagectomy and AF, a study published in May 2016 evaluating 583 patients found that in oesophageal cancer patients who underwent oesophagectomy, the development of new onset AF in 63 patients during the post-operative period might be independently associated with mortality. Risk factors for an increased incidence of AF included patients that were older and those taking pre-operative calcium channel blocker medication. [78] Only a few studies have focused on the association between mortality and AF after oesophagectomy. A study by McCormack et al in 2014 found no significant difference in mortality after oesophagectomy with the median survival was 40 months versus 53 months in the cohort that developed AF and the cohort that did not respectively ($p=0.353$)[195]. In 2015 a systematic review and meta-analysis evaluating the relationship between biomarkers and AF looked at 472 studies from which 16 were selected. These 16 studies comprised 2915 patients. The results suggested that biomarkers such as IL-6 and CRP were significantly associated with onset of AF and states that this should be further investigated[196].

The RE-LY (Randomised Evaluation of Long-Term Anticoagulation Therapy) study compared dabigatran to warfarin in patients with AF and found that high-dose dabigatran reduced stroke risk without increasing the risk of major bleeding among AF patients. In a subset of the RE-LY study, blood samples were taken in 2514 patients for NT-pro BNP and cardiac troponin-I (cTnI). Persistent elevation of either or both cardiac biomarkers at baseline and 3 months was associated with a higher risk for cardiovascular events such as stroke and thromboembolic events and mortality ($p<0.0001$)[197].

In terms of adding to the literature, I have evaluated the levels of several cardiovascular biomarkers in the context of development of atrial fibrillation, morbidity and mortality and I have also evaluated the conduct of the study in terms of potential areas in which there would be room for improvement such as streamlining identification of appropriate patients in a timely manner and being able to approach them at appropriate points of the patient journey. As noted previously, there were also challenges with requesting investigations and also with laboratory processes and storage of samples was a particular area that was extremely challenging. However, the lessons have been acknowledged and the application of related learning will be used in the future.

8.2 Future plans

Future work in this area would include running a larger study based on the feasibility study which I have described above. This format and planning of this study would be informed by the lessons learned, which are detailed in chapter 3. It would consider each stage of the patient pathway with respect to their oesophageal cancer and also with respect to their preferences and what is important to them, so that their care could truly be personalised. This would include commencing with taking a comprehensive history from the patient, including details of their co-morbidities, nutritional status and regular medications, considering the effect they might have on the development of AF and longer-term outcomes. I would seek to determine the patient's quality of life at the outset of the pathway and ensure that they have honest conversations with the surgical team and also allied health professionals about what matters to them and to help them understand the journey that they are embarking upon and also to ensure that there is shared decision making based on appreciation of what is known about the risks of undertaking chemotherapy and also an operation. Using the baseline knowledge about the patients and their preferences, I would also consider the effects of neoadjuvant chemotherapy, as unfortunately during this study I did not have the capacity to

inquire about chemotherapy complications and consider whether patients who had problems after chemotherapy had increased morbidity and mortality following surgery. The side effects of chemotherapy include predisposition to infection and inflammation. It is unclear what effect chemotherapy would have on levels of inflammatory biomarker baseline levels when completed more than 6 weeks prior to surgery, and subsequently predisposition to development of AF. Ideally a separate trial looking at biomarker levels in a cohort 6 weeks after undergoing chemotherapy compared with a cohort matched with regard to baseline characteristics, who did not, might be able to illicit the effect it has on biomarker levels and subsequent AF development. Consideration of capturing every episode of AF, no matter how brief, would play a part in a future study. Due to the particular limitations of the resources available, including monitors and nursing staff in the study described above, it was not possible to record and make a note of short runs of AF, which in turn may well have affected the biomarker results. In terms of the management of AF on the ICU, I would plan to stratify the treatments used, ranging from simpler interventions such giving fluids, to giving electrolytes and medications such as amiodarone. Additionally, recording whether any patients had to undergo cardioversion due to cardiovascular instability secondary to AF would be important. Having comprehensively evaluated patients' nutritional status at the beginning of the study, I would evaluate their feeding regimes post-operatively and place this in the context of blood results and nutritional indicators such as weight. A risk assessment should be completed prior to the study, to make certain that there is a streamlined pathway to enable the collection of plasma and urine samples and to safeguard their delivery to the laboratory and ensure their subsequent processing. The follow up period would also be extended to five years to determine long term outcomes in these patients. Comparison of CPET tests at 6, 12, 18 and 24 months following oesophagectomy, in the context of quality of life would be helpful. This could be measured by patients with a quality of life questionnaire

with a Likert scale. It could also include evaluation of what activities people are able to return to following their operation and the impact that this has on their perceived quality of life. Depending on the age of patients undergoing the operation this may differ in terms of either going back to work or being able to return to pursuing a hobby that they enjoy. It would be informative to discover whether there is deterioration in CPET test results following oesophagectomy initially at 6 months following the operation and then whether there is a gradual return to pre-operative levels or whether this is never achieved. It would also be helpful to see whether patients experience an improved quality of life after their operation, whether this achieved after a certain period of time or whether this is not the case at all. Answering the question regarding what type of relationship there is between post-operative CPET test results and self-assessment of Quality of Life would be informative for future planning in terms of rehabilitation of this patient group. Additionally, the impact of socioeconomic status and the access to care on this patient cohort would be a valuable area of study to reflect on in the future. The considerations above regarding a future study would help to manage risk better by engaging in decision-making that is shared, therefore leading to reduced harm and reduce unwanted variation in practice. A truly personalised approach applied to the pathway for patients undergoing treatment for oesophageal cancer would be the ultimate goal. These concepts could then be extrapolated to other areas of health and care to help ensure we remain realistic.

APPENDIX 1: ETHICAL APPROVAL FOR STUDY AND AMENDMENT TO PROTOCOL

WoSRES
West of Scotland Research Ethics Service

NHS
Greater Glasgow and Clyde

West of Scotland REC 3
Ground Floor – The Tennent Institute
Western Infirmary
38 Church Street
Glasgow G11 6NT
www.nhsggc.org.uk

Professor John Kinsella
Professor and Head of Section
University Department of Anaesthesia, Pain & Critical Care Medicine
University of Glasgow
Academic Department of Anaesthesia Pain and Critical Care Medicine
Level 4 Walton Building
Glasgow Royal Infirmary
Castle Street
Glasgow G4 0SF

Date 5th October 2012
Your Ref
Our Ref
Direct line 0141 211 2123
Fax 0141 211 1847
E-mail Liz.Jamieson@ggc.scot.nhs.uk

Dear Professor Kinsella

Study title: **The association of asymmetric dimethylarginine with new onset Atrial Fibrillation in patients following Oesophagectomy**
REC reference: **12/WS/0232**
IRAS Project reference: **109261**

The Research Ethics Committee reviewed the above application at the meeting held on 27 September 2012. Thank you for attending to discuss the study.

Ethical opinion

Discussion

The Committee asked you to summarise the study and explain how participants would be recruited and consented. You advised that suitable participants would be identified by the Multi Disciplinary Team and sent an Information Sheet with their appointment for their standard pre-operative cardiopulmonary exercise test (CPX). When the patients come for this test they would be able to ask the nurse and the researcher any questions they may have. They would then be seen one week before their operation by the clinician and at that time if they decide to take part in the study consent would be taken by someone from the Research Team.

After you left the meeting the Committee discussed the issue of the direct care team identifying possible suitable participants for research. In light of the type of patients involved and the benefits to be gained from the study the Committee agreed to allow this recruitment process on this occasion.

The members of the Committee present gave a favourable ethical opinion of the above research on the basis described in the application form, protocol and supporting documentation, subject to the conditions specified below.

Ethical review of research sites

NHS Sites

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

Conditions of the favourable opinion

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

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

Management permission ("R&D approval") should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements.

Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of approvals from host organisations

Other Conditions specified by the REC

1) The Participant Information Sheet requires to be changed as follows:

- At 'Do I have to take part' 'No' should be inserted at the beginning of the paragraph.

2) The Consent Form requires to be changed as follows:

- The words 'please initial' should be above the boxes.
- In the first statement the version number and date of the Participant Information Sheet is incorrect.

It is responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

You should notify the REC in writing once all conditions have been met (except for site approvals from host organisations) and provide copies of any revised documentation with updated version numbers. Confirmation should also be provided to host organisations together with relevant documentation

Approved documents

The documents reviewed and approved at the meeting were:

Document	Version	Date
Covering Letter		27 August 2012
Evidence of insurance or indemnity		08 August 2012
GP/Consultant Information Sheets	1.1	24 July 2012
Investigator CV		
Letter from Statistician	1.0	
Other: Educational Supervisor Report		24 July 2012
Other: Data collection sheet	1.1	22 July 2011
Other: CV - K Tober (student)		
Participant Consent Form	1.0	22 July 2012
Participant Information Sheet	1.3	10 August 2010
Protocol	1.3	22 July 2012
REC application		11 September 2012

Membership of the Committee

The members of the Ethics Committee who were present at the meeting are listed on the attached sheet.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

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

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

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

Feedback

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

Further information is available at National Research Ethics Service website > After Review

12/WS/0232	Please quote this number on all correspondence

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

Yours sincerely

Liz Jamieson
Committee Co-ordinator
On behalf of Dr Adam Burnet, Chair

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

Copy to: Dr Maureen Travers, NHS GG& C, Central R&D Office

1. Application for amendment to protocol

NOTICE OF SUBSTANTIAL AMENDMENT

Please use this form to notify the main REC of substantial amendments to all research other than clinical trials of investigational medicinal products (CTIMPs).
The form should be completed by the Chief Investigator using language comprehensible to a lay person.

Details of Chief Investigator:

Title	Forename/Initials	Surname
Professor	John	Kinsella

Work Address	Academic department of Anaesthesia,Pain and Critical Care Medicine Level 4 Walton Building Glasgow Royal Infirmary, Castle Street, Glasgow
PostCode	G4 0SF
Email	
Telephone	01412114625
Fax	01412111191

Full title of study:	The association of asymmetric dimethylarginine with new onset atrial fibrillation in patients following oesophagectomy
Lead sponsor:	Central R&D Office
Name of REC:	West of Scotland Research Ethics Committee (3)
REC reference number:	12/WS/0232
Name of lead R&D office:	Central R&D Office
Date study commenced:	October 2012
Protocol reference (if applicable), current version and date:	Version 1.4 06/11/2012
Amendment number and date:	Version 1.5 12/05/13

Type of amendment

(a) Amendment to information previously given in IRAS

(●) Yes ( ) No

If yes, please refer to relevant sections of IRAS in the "summary of changes" below.

(b) Amendment to the protocol

(●) Yes ( ) No

If yes, please submit either the revised protocol with a new version number and date, highlighting changes in bold, or a document listing the changes and giving both the previous and revised text.

(c) Amendment to the information sheet(s) and consent form(s) for participants, or to any other supporting

documentation for the study

(●) Yes ( ) No

If yes, please submit all revised documents with new version numbers and dates, highlighting new text in bold.

Is this a modified version of an amendment previously notified and not approved?

( ) Yes (●) No

Summary of changes

Briefly summarise the main changes proposed in this amendment. Explain the purpose of the changes and their significance for the study.
If this is a modified amendment, please explain how the modifications address the concerns raised previously by the ethics committee.
If the amendment significantly alters the research design or methodology, or could otherwise affect the scientific value of the study, supporting scientific information should be given (or enclosed separately). Indicate whether or not additional scientific critique has been obtained.

The patients that have already been recruited for the ADMA/oesophagectomy study will be re-consented for the CPEX/QoL part of the study.
Pre-operative cardiopulmonary exercise testing, is routinely carried out for patients requiring oesophagectomy. Studies have shown that preoperative VO2 max and anaerobic threshold measurements from CPET can be used to predict risk of post-operative morbidity in oesophagectomy patients. There are however no studies that have quantified postoperative fitness in this population. We want to assess how different serial CPEX tests at 6 months, 1 year and 2 years following oesophagectomy are compared with baseline pre-operative tests. This has not been studied before in this population.
Quality of life is a factor that is very important to patients when considering the possible outcomes of an operation34 and it has been named the 'missing axis'35 in morbidity risk prediction and information provided for patients. Whilst QoL preoperatively and postoperatively have been compared in this population36, comparing postoperative QoL with postoperative fitness in patients undergoing oesophagectomy has not been studied before.

Any other relevant information

Applicants may indicate any specific issues relating to the amendment, on which the opinion of a reviewing body is sought.

For return CPEX testing at 1 year and 2 years funding for transport will be provided from the University of Glasgow Academic Department of Anaesthesia.

List of enclosed documents

Document	*Version*	*Date*
Information sheet	1.5	12/05/2013
Protocol	1.5	12/05/2013
Consent	1.2	12/05/2013
GP information	1.2	12/05/2013

Declaration by Chief Investigator

1. *I confirm that the information in this form is accurate to the best of my knowledge and I take full responsibility for it.*
2. *I consider that it would be reasonable for the proposed amendment to be implemented.*

This section was signed electronically by professor john kinsella on 30/05/2013 10:45.

Job Title/Post:	professor
Organisation:	university of glasgow
Email:	john.kinsella@glasgow.ac.uk

Declaration by the sponsor's representative

I confirm the sponsor's support for this substantial amendment.

This section was signed electronically by Dr Maureen Travers on 28/05/2013 11:10.

Job Title/Post:	Research Coordinator
Organisation:	NHS Greater Glasgow and Clyde
Email:	Maureen.Travers@ggc.scot.nhs.uk

2. Ethical approval for sub-study

WoSRES
West of Scotland Research Ethics Service

NHS
Greater Glasgow and Clyde

West of Scotland REC 3
Ground Floor - The Tennent Institute
Western Infirmary
38 Church Street
Glasgow G11 6NT
www.nhsggc.org.uk

Professor John Kinsella
Professor and Head of Section University
Department of Anaesthesia, Pain and Critical Care Medicine
University of Glasgow / NHS Greater Glasgow and Clyde
Level 4 Walton Building
Glasgow Royal Infirmary
Castle Street
Glasgow G4 0SF

Date 18TH June 2013
Your Ref
Our Ref
Direct line 0141 211 2123
Fax 0141 211 1847
E-mail Liz.Jamieson@ggc.scot.nhs.uk

Dear Professor Kinsella

Study title: **The association of asymmetric dimethylarginine with new onset atrial fibrillation in patients following oesophagectomy**
REC reference: **12/WS/0232**
Amendment number: **AM02**
Amendment date: **30 May 2013**
IRAS project ID: **109251**

The above amendment was reviewed by the Sub Committee in correspondence.

Ethical opinion

The members of the Committee taking part in the review decided that they could not give a favourable ethical opinion of the amendment, for the following reasons:

- The Sub Committee agreed that the character of the project had changed from an in-patient biochemical study to a long-term follow up in a vulnerable high risk population who may be very seriously ill or even have passed away.

I regret to inform you that the amendment is therefore not approved. The study should continue in accordance with the documentation previously approved by the Committee.

If you would find it helpful to discuss any of the matters raised above or seek further clarification from a member of the Committee, you are welcome to contact Mrs Liz Jamieson, Committee Co-ordinator, contact details at the beginning of this letter.

The Sub Committee would however like to suggest that this could be a 'Sub Study' whereby participants would be asked if they would like to take part in the sub study. Before any contact is made the participant's current status must be checked to ensure that there is no

upset to either the participant who may be very unwell or the family. A Letter of Invitation, Participant Information Sheet and Consent Form will require to be developed. The Protocol should be updated to reflect that this is a 'Sub Study'.

Options for further ethical review

1. **Modifying the amendment**

You may modify or adapt the amendment, taking into account the Committee's concerns. Modified amendments should be submitted on the standard Notice of Amendment form. The form should indicate that it is a modification of the above amendment. Please ensure that you resubmit those documents that have been added or revised and need to be reviewed. There is no requirement to resubmit any documents that were submitted with the original amendment and are still relevant to it but have not changed. However, the standard Notice of Amendment form must *list* all documents that are still relevant to the amendment, clearly indicating those which are new or have been modified and those which remain unchanged.

The REC must receive a revised Notice of Amendment form at least 14 days before you plan to implement the amendment. The Committee will then have 14 days from the date of receiving the notice in which to notify you that the amendment is rejected, otherwise the amendment may be implemented.

2. **Appeal against the opinion**

Alternatively, you may appeal against the decision of the Committee by notifying the relevant Research Ethics Service appeals manager (see below) in writing within 90 days of the date of this letter, setting out your representations with respect to the opinion. The appeal would be based on the notice of substantial amendment and supporting documentation reviewed previously, without revision. If the appeal is allowed, the amendment will be reviewed again at the next scheduled full meeting of this Committee, taking into account your representations together with the comments of a second REC on the amendment. The second REC will be appointed by the appeals manager.

You will be notified of the arrangements for the meeting of the REC and will be able to attend and/or make further written representations if you wish to do so.

The appeals manager is:

Joan Kirkbride
Director of Operations
National Research Ethics Service

Email: joan.kirkbride@nhs.net

Documents reviewed

The documents reviewed at the meeting were:

Document	Version	Date
GP/Consultant Information Sheets	1.2	12 May 2013
Participant Consent Form	1.2	12 May 2013
Participant Information Sheet	1.5	12 May 2013
Protocol	1.5	12 May 2013
Notice of Substantial Amendment (non-CTIMPs)	AM02	30 May 2013

Membership of the Committee

The members of the Committee who took part in the review are listed on the attached sheet.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

We are pleased to welcome researchers and R & D staff at our NRES committee members' training days – see details at http://www.hra.nhs.uk/hra-training/

12/WS/0232:	Please quote this number on all correspondence

Yours sincerely

Liz Jamieson
Committee Co-ordinator
On behalf of Dr Adam Bumel, Chair

Enclosures: List of names and professions of members who took part in the review

Copy to: Dr Maureen Travers, R&D - NHS Greater Glasgow & Clyde

APPENDIX 2: PATIENT AND GP STUDY INFORMATION

Patient study information v1.3 10/8/2012

University Department of Anaesthesia,
Walton Building, Glasgow Royal Infirmary.
Castle Street, G4 OSF.

Association of ADMA and new onset AF following oesophagectomy study

Patient information sheet

We would like to invite you to take part in a research study. Before you make a decision, you need to understand why the research is being done and what it would involve for you. Please take time to read the following information carefully. Talk to others about the study if you wish. Please do not hesitate to ask if there is anything that is unclear or if you would like more information.

Who is conducting the research?
Dr. Katherina Tober and Professor John Kinsella from the University Department of Anaesthesia at Glasgow Royal Infirmary are conducting the research.

What is the purpose of the study?
Atrial fibrillation (AF) is an irregular heart rhythm with which many people are diagnosed each year. Patients in the community with AF are often managed with medication to prevent symptoms such as palpitations and may also be on drugs which thin the blood.
Following an oesophagectomy, AF occurs in approximately 11-22% of patients and often requires medication initially but this is infrequently needed for longer term management.
Our observational study hopes to clarify why some people develop AF after oesophagectomy and why others do not, by establishing whether levels of asymmetric dimethylarginine (ADMA), an amino acid which is naturally produced in our bodies, is associated with new onset atrial fibrillation (AF).
We hope to contribute to the relatively limited knowledge that exists regarding ADMA and the development of AF. If a link exists, this could mean the potential to predict which patients may develop atrial fibrillation post-operatively and therefore prevent it occurring.

Why have I been invited to participate?
You have been invited to participate as you may have to undergo an oesophagectomy procedure within the next few weeks.

Do I have to take part?
It is your decision. We will describe the study and go through the information sheet, which you will have received in the post. You will be asked to sign a consent form to show that you

have agreed to take part. You are free to withdraw from the study at any time, without giving any reason. This will not affect the standard of care that you receive or your future treatment.

What does taking part involve?
Once you have read the information sheet and have had the opportunity to ask any questions you will be asked to sign a consent form for the study. Your GP will be made aware of your involvement with the study.
The study involves a small amount of extra blood (slightly more than two teaspoonfuls) being taken at the time of routine blood tests pre-operatively. Urine samples will also be taken for analysis around this time. These blood and urine samples will be taken daily for 7 days or for the duration of your High Dependency Unit (HDU) stay, whichever comes first. If you develop AF this will be treated according to the HDU protocol and you will have a 12 lead ECG and an ultrasound scan of your heart.
Your GP will follow your progress post-operatively and the researchers will make you aware of any publications arising from the study.

What happens to the information?
Your identity and personal information will be completely confidential and known only to the researcher. The information obtained will remain confidential and stored within a locked filing cabinet. The data are held for up to 5 years in accordance with the Data Protection Act, which means that we keep it safely and are unable to reveal it to other people without your permission.

What are the possible benefits of taking part?
It is hoped that by taking part in this research you will be helping us to gain valuable insights regarding the biochemical pathways involved in the development of AF in patients following oesophagectomy. In the future we anticipate that this will help us to predict which patients will develop AF post-operatively and therefore we may be able to prevent it.

Who has reviewed the study?
The study has been reviewed by the West of Scotland Research Ethics Service (WoSRES).

Who do I contact if I have further questions about the study?
We will give you a copy of the information sheet and signed consent form to keep. If you would like more information about the study and wish to speak to someone not closely linked to the study, please contact:
Dr. Malcolm Booth, Consultant in Anaesthesia and Intensive Care Medicine, Glasgow Royal Infirmary.
Telephone: 0141 211 4225

Study Contacts

Dr. Katherina Tober
Clinical research fellow in anaesthesia,
Glasgow Royal Infirmary
Telephone: 0141 211 4620
Email: katherinatober@nhs.net

Professor John Kinsella
Academic lead, University Dept Anaesthesia
Glasgow Royal Infirmary
Telephone: 0141 211 4625

What if I have a complaint about any aspect of the study?
If you unhappy about any aspect of the study and wish to make a complaint, please contact the researcher in the first instance. The normal NHS complaint mechanism is also available to you.

Thank-you for your time and co-operation.

GP study information v 1.1 24/7/2012

University Department of Anaesthesia,
Walton Building, Glasgow Royal Infirmary.
Castle Street, G4 OSF.

Re: Association of ADMA and new onset AF following oesophagectomy study

Dear Dr,
I am writing to inform you that your patient ……
has consented to take part in the above study which concerns the investigation of asymmetric dimethylarginine (ADMA) levels and their association with new onset atrial fibrillation (AF) following oesophagectomy.
Participation in this study will involve some additional blood being taken from patients in order to analyse levels of biomarkers including ADMA, symmetric dimethylarginine (SDMA) and laevo (L) arginine. Urine will also be sampled and tested for ADMA, SDMA and dimethylarginine (DMA) levels in addition to other biomarkers.
These tests will be carried out pre-operatively and then daily post-operatively on HDU for a 7 day period or for the duration of their stay, whichever is shorter.
If a patient develops new onset atrial fibrillation then this will be managed according to our local protocols.

Risks of participation relate to venepuncture.

Please do not hesitate to contact us if you should require any additional information.

Yours sincerely,

Katherina Tober

Clinical research fellow in anaesthesia
ST5 in Anaesthesia
Telephone: 01412114625
E-mail: katherinatober@nhs.net

1. **Patient consent form and study forms for completion on ICU**

Patient study consent v 1.0 22/7/2012

University Department of Anaesthesia,
Walton Building, Glasgow Royal Infirmary.
Castle Street, G4 OSF.

Subject number:

Association of ADMA and new onset AF following oesophagectomy study

Consent Form

Please initial box

I confirm that I have read and understand the information sheet dated 10/08/2012 (version 1.3) for the above study and have had the opportunity to ask questions. ☐

I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐

I understand that sections of my medical notes may be looked at by the research team where it is relevant to my taking part in the research. I give my permission for the research team to have access to my records. ☐

I understand that if I am unable to participate in the exercise test that the information from my questionnaire will still be used for the study. ☐

I understand that my GP will be made aware of my participation in this study. ☐

I agree to take part in the above study. ☐

……. ……..
……..
Name of participant Date Signature

……. ……..
…….
Name of Researcher Date Signature

One copy to the patient, one copy to the researcher, one original for the patient's notes

University Department of Anaesthesia,Walton Building, Glasgow Royal Infirmary.Castle Street, G4 OSF.

BLOOD SAMPLES

Association of ADMA and new onset AF following oesophagectomy study

ADMA, SDMA, homoarginine, myeloperoxidase, IL-6

Fill: 1x green top vacutainer and 1x red top vacutainer

BNP: Fill: 1 x purple top vacutainer

URINE SAMPLES

Nitrate, DMA, SDMA, ADMA

Fill: 1x Universal container with 10ml urine

Send to **Biochemistry**: FAO **Dinesh Talwar** and **Laura Willox** (ADMA study)

Purple, red and green top and urine sample can be sent with one biochemistry request form

Daily checklist for ADMA study patients

Please initial box

Day 0 (following return from theatre)
Bloods: Routine bloods plus triglycerides, glucose, CRP, ADMA, SDMA, homoarginine, BNP, myeloperoxidase, IL-6 ☐
Urine: nitrate, DMA, SDMA, ADMA
ECG
Rhythm: Is patient in sinus rhythm? If in AF manage as per local guidelines
Aim to get an echo within 24 hours if AF sustained >30min (To request echo see below)

Day 1
Bloods: as per day 0 excluding BNP ☐
Urine: as per day 0
ECG and evaluate rhythm as per day 0

Day 2
Bloods: as per day 0 ☐
Urine: as per day 0
ECG and evaluate rhythm as per day 0

Day 3
Bloods: as per day 0 ☐
Urine: as per day 0
ECG and evaluate rhythm as per day 0

Day 4
Bloods: as per day 1 ☐
Urine: as per day 0
Evaluate rhythm as per day 0

Day 5
Bloods: as per day 1 ☐
Urine: as per day 0
Evaluate rhythm as per day 0

Day 6
Bloods: as per day 1 ☐
Urine: as per day 0
Evaluate rhythm as per day 0

Day 7
Bloods: as per day 0 ☐
Urine: as per day 0
Evaluate rhythm as per day 0

If you have any questions regarding this study please do not hesitate to contact one of the researchers.

Many thanks for your help.

Researchers:
Dr Katherina Tober
E-mail: katherina@doctors.org.uk
Contact: 07920099479

Professor John Kinsella
Contact: 0141 211 4625

Echo requests (in daylight hours)
Dr. Alex Puxty
ICU Consultant
Glasgow Royal Infirmary
Contact: 0141 211 4225

Dr Carol Murdoch
ICU Consultant
Glasgow Royal Infirmary
Contact: 0141 211 4225

Dr Martin Hughes
ICU Consultant
Glasgow Royal Infirmary
Contact: 0141 211 4225

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