PROTOCOL
Title Page

Study Title: *Can left atrial strain measurements, and correlating changes in left atrium area and volume size, be used as an early predictor of atrial fibrillation?*

Acronym/Short Title: *Left Atrial Strain Values as an Early Predictor of Atrial Fibrillation*

Protocol/Study ID: IRAS 324893

REC Reference:

Version Number: 6.0, 17/05/2023

Study Summary

Study Outline	Atrial fibrillation (AF) is the most prevalent type of arrhythmia within an adult population and is characterised by disorganised, chaotic, electrical activity and thus, ineffective atrial contraction causing irregular ventricular contraction. AF can be categorised into three different groups: paroxysmal, persistent and permanent. The prevalence of AF increases with age and is associated with a range of risk factors including: hypertension, obesity and alcohol consumption. There is a plethora of both cardiac and non-cardiac causes of AF and the cause is not always clear for each patient. Furthermore, causes of AF can also present as the by-product of AF, this increases the difficulty in determining the individual patient's cause of onset. A diagnosis of AF can be incidental as not all patients are symptomatic. If AF goes undetected, this puts the patient at an increased risk of blood clot formation and stroke. Patients suspected of having AF are routinely referred for a transthoracic echocardiogram (TTE). It is common for AF patients to have a dilated left atrium (LA). This can be visually confirmed, and the LA area and volume can be measured during a TTE. LA strain measurements are not routinely measured during TTEs but may be able to detect subtle changes in longitudinal strain patterns of the myocardium. By comparing the strain patterns in patients with normal sinus rhythm (control) to those with AF, a scale can be created to suggest and predict whether a patient is likely to develop AF in the future based on these subtle changes before any changes in LA size occur. Furthermore, this can then be further developed to determine whether there are any significant differences in strain patterns between the three AF groups.
Lay Summary	Atrial fibrillation (AF) is the most common type of abnormal heart rhythm. This occurs as the chambers of the heart pump irregularly, often resulting in a fast heart rate and symptoms of irregular pounding and fluttering. There are many risk factors predisposing to AF, however, the cause cannot always be easily determined. Additionally, not all AF patients experience symptoms and consequently, this abnormal rhythm may go undetected and may be discovered accidentally. This is detrimental to patients, as untreated AF patients are at an increased risk of stroke. Patients that are suspected of having AF are referred for an ultrasound scan of the heart (echocardiogram). It is expected to see structural changes to the heart's chambers. However, structural and electrical changes of the heart may be the cause of AF but may also be a result of AF, resulting in a chicken and egg situation. It may be possible that a different type of measurement can be used during an echocardiogram to detect subtle changes in heart muscle patterns. This measurement may then serve to be an early predictor of AF. This would be determined by comparing the patterns in patients with a normal, regular rhythm to those with AF. Potential candidates are initially screened based on their echocardiogram referral. If deemed suitable for this study, the study

	process is explained to the patient and written informed consent is invited and received. The echocardiogram will be performed as normal with a focus on the area and volume measurements taken of one of the top chambers of the heart. An additional measurement will be taken to observe any subtle changes in the arrangement of heart cells within this same heart chamber. These measurements can be compared to each other to establish any relationship as well as compared to patients with and without AF.
Objectives	To determine whether there is a significant difference in atrial strain values between patients that are in normal sinus rhythm (NSR, control group) to patients that are known to have atrial fibrillation (disease group). A disease-control group of controlled hypertensive patients in NSR will also be utilised as hypertension is a known risk factor for atrial fibrillation. Providing that there is a significant difference in the values between NSR and AF patients, a further comparison will be conducted on patients in different type of AF: paroxysmal AF, persistent AF and permanent AF to determine if there is a deterioration in atrial strain values within AF patients. Left atrial area and volume size will also be measured in all patients and this can then be compared to atrial strain values.
Sample Size	234
Duration	1 year – project end date of March 2024

Statement of Compliance

The study as detailed within this protocol (Version 6, 17/05/2023) or any subsequent amendments will be conducted in accordance with the International Conference on Harmonization guidelines for Good Clinical Practice (ICH E6), the UK Policy Framework for Health and Social Care Research (2017), the World Medical Association Declaration of Helsinki (1996) and the current applicable regulatory requirements and any subsequent amendments of the appropriate regulations.

Signature Page

The signature below constitutes the approval of this protocol and the Patient Information Sheet, Informed Consent Form, Case Report Form for Research, Good Clinical Practice Certificate, Curriculum Vitae, Gantt Chart and Power Calculations attached and provides the necessary assurances that this study will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements.

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L. M. Hwozdyk Date: **17/05/2023**

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Contents

Abbreviations:

AF	=	atrial fibrillation
AFI	=	automated function imaging
ANOVA	=	analysis of variance
BSE	=	British Society of Echocardiography
CI	=	Chief Investigator
EF	=	ejection fraction
GDPR	=	general data protection regulation
GLS	=	global longitudinal strain
HRA	=	Health Research Authority
ICF	=	Informed Consent Form
LA	=	left atrium/atria
LV	=	left ventricle
NHS	=	National Health Service
NSR	=	normal sinus rhythm
PACS	=	picture archiving system
PAF	=	paroxysmal atrial fibrillation
PALS	=	peak atrial longitudinal strain
PI	=	Principal Investigator
PIS	=	Participant Information Sheet
REC	=	Research Ethics Committee
R&D	=	Research & Development
STE	=	speckle tracking echocardiography
TTE	=	transthoracic echocardiogram

1. Introduction

Atrial fibrillation (AF) is the most prevalent type of arrhythmia within an adult population and is characterised by disorganised, chaotic, electrical activity and thus ineffective atrial contraction causing irregular ventricular contraction (Wyndham, 2000). AF can be categorised into three different groups: paroxysmal, persistent, and permanent. The prevalence of AF increases with age and is associated with, but not limited to, a range of risk factors including hypertension, obesity, and alcohol consumption (Staerk, *et al*., 2017). There is a plethora of both cardiac and non-cardiac causes of AF and the cause is not always clear for each patient. Furthermore, causes of AF can also present as the by-product of AF, this increases the difficulty in determining the individual patient's cause of onset. A diagnosis of AF can be incidental as not all patients are symptomatic. If AF goes undetected, this puts the patient at an increased risk of blood clot formation and stroke (Markides and Schilling, 2003).

Patients suspected of having AF are routinely referred for a transthoracic echocardiogram (TTE). It is common for AF patients to have a dilated left atrium (LA) and this can be visually confirmed in addition to the LA area and volume measurements (Van De Vegte, *et al*., 2021). However, as LA measurements are limited to area and volume size, LA strain measurements may be taken but these are not routinely performed during standard TTEs. LA strain is measured using a type of speckle-tracking echocardiography (STE) technique which focuses on the subtle longitudinal changes within the myocardium to analyse myocardial deformation (Gan, *et al*., 2018). Strain imaging of the LA produces three functional strain values (contractile, conduit and reservoir) which correspond to different stages of the cardiac cycle (Jarasunas, *et al*., 2018). As patients in AF lack normal atrial contraction and have a loss of atrial kick, it is expected that the contractile strain, associated with LA contraction, is not present. However, the conduit and reservoir functions can still be compared across all three study groups to create a scale to suggest and predict whether a patient is likely to develop AF in the future; prior to any changes in LA size. Furthermore, this can then be further compared to determine whether there are any significant differences in strain patterns between the different groups of AF.

The correlation between AF and a dilated LA are widely recognised, however, LA enlargement may be both a cause and consequence of AF. Dilation of the LA has been largely suggested to be due to atrial remodelling as a result of a change in atrial function and structure – as seen in the case of AF. However, there are other factors which affect atrial function and structure which ultimately result in atrial remodelling. This includes pathologies such as mitral valve disease (mitral stenosis, regurgitation, or a mixed disease), structural cardiac disease (such as heart failure, cardiomyopathies, and congenital disease) and hypertension (Van De Vegte, *et al*., 2021). As hypertension can also cause atrial remodelling and is a risk factor for AF, patients that are in normal sinus rhythm (NSR) that have controlled hypertension are included in this study as a disease-control group. It is expected that the atrial strain values of these patients will fall between those of the control group (NSR) and of those in the disease group (chronic AF).

Existing available literature (*see document Literature Review Table*) suggests that there is a significant difference in the strain values between patients in NSR to those in AF. However, limited studies have included hypertensive patients or investigated

the differences in strain values between AF patients. Furthermore, previous studies have been limited by method technique. This is because many studies have either used external STE software to create atrial strain values or have adopted global longitudinal strain (GLS) methods to track the LA. This is a significant limiting factor as GLS methods are intended to assess longitudinal function of the left ventricle.

1.1 Background

From the current literature available (*see document Literature Review Table*), it has been suggested that there is a correlation between AF and LA strain values. Existing literature suggests that patients in AF have a reduced LA strain value compared to patients in NSR. However, there are limited studies which also include controlled hypertensive patients despite collecting data regarding blood pressure. Furthermore, the majority of literature reviewed appears to focus on patients that have other co-morbidities, and it could be argued that these can affect the parameters measured and analysed. This includes studies involving patients with valvular pathologies such as aortic stenosis and aortic valve replacements as well as studies involving patients with potential structural heart deformations such as those seen with heart failure or following a myocardial infarction. These factors may alter strain patterns and LA size as a result. By listing these factors as exclusion criteria, this study should minimise any external and uncontrollable factors which may affect LA strain value and LA size.

Furthermore, some studies do not have a standardised zero-reference point as some studies used the P wave for NSR and the R wave for AF patients which may cause a discrepancy in results. In this study, it has been agreed that the zero-reference point will be the R wave to create R-R cycles. This is because the P wave is not present in AF and thus cannot be used as a definitive reference point within the AF group. Both groups will have R waves present and therefore, the reference-point can be standardised across all three groups. Additionally, many of the studies reviewed appear to focus on only one LA strain and phase within the cardiac cycle. However, some do include all three. This study will include all three strain values as well as peak atrial longitudinal strain (PALS) values. However, statistical analysis may not be doable for the contractile phase as this is not present in patients with AF. The biggest pitfall that is common to most, if not all, studies reviewed is that the methods used to calculate LA strain were originally designed to measure global longitudinal strain (GLS) of the left ventricle (LV). This study utilises the automated function imaging (AFI) LA atrial strain tool provided by GE Healthcare which is specific to the LA as opposed to GLS methods to track the LV.

The key outcomes from this study will be whether there is a significant difference in atrial strain values between NSR patients, controlled hypertensive patients and patients with either persistent or permanent AF. These electrical changes should correlate with structural changes within the LA as the LA becomes more dilated in AF patients. Furthermore, subtle differences in LA strain values may be observed between patients with paroxysmal atrial fibrillation (PAF), persistent and permanent AF to suggest a decline in atrial strain patterns with a worsening and prolongation of AF.

This will have a significant impact to the general population as AF is the most common arrhythmia. Left atrial enlargement and dilation can occur, either as a cause or complication of AF. However, the detection of this structural change and cardiac remodelling by measuring LA area and volume is often only detected when the patient has been in AF for a prolonged period of time. The utilisation of speckle-tracking echocardiography (STE) and atrial strain measurements may be able to detect subtle changes in LA strain pattern which may occur before any significant cardiac remodelling and LA dilation, and thus serve as an early predictor of AF. This will allow for closer monitoring and the correct administration of anticoagulants. Furthermore, the results from this study will also be generalisable and transferrable and may suggest that LA strain measurements should be more routinely performed during TTEs.

1.2 Research Question

Can measurements of left atrial strain, along with correlating changes in left atrium area and volume size, be used as an early predictor of atrial fibrillation?

2. Study Outline

2.1 Aims and Objectives

The aims of this research project are split into two sets of comparisons. The first is to determine whether there is a statistically significant difference between atrial strain values in normal sinus rhythm (NSR) patients (control group), to controlled hypertensive patients (disease-control group) and patients with established, chronic AF (disease group). Atrial strain values from these groups will also be compared to left atrium (LA) area and volume to determine correlation. Furthermore, providing there is a significant difference, atrial strain values between AF groups PAF, persistent, and permanent can also be compared to potentiate deterioration of atrial strain.

Null hypothesis: There is no significant difference between the atrial strain measurements in patients with normal sinus rhythm to patients with chronic atrial fibrillation and no correlation between atrial strain measurements and left atrium are and volume size.

Research / alternative hypothesis: There is a statistically significant difference between atrial strain values between patients with normal sinus rhythm and patients with atrial fibrillation and this correlates with changes seen in left atrium area and volume size.

2.2 Method

Patient selection:
Potential candidates for this study will have been referred for a TTE by a healthcare professional. The referrals will be reviewed and if the patient meets the initial eligibility criteria, they will be considered as part of this study. The initial eligibility criteria consist of being over the age of 50 (although patients will be age-matched across the three groups) and falling within the control (normal sinus rhythm), disease-control (controlled hypertension) or disease groups (chronic AF).

Data collection:
The images obtained from the TTE scan will be stored on the ultrasound machine as well as the picture archiving systems (PACS) on the computer. The measurements to be compared are:

- Left atrium area
- Left atrium volume
- Left atrium strain measurement and analysis using the R wave

The first two measurements are routinely performed during TTEs, but atrial strain measurements are not routinely performed. This additional measurement will not affect or delay the TTE results and report from being sent back to the referrer or delay any further correspondences with other healthcare professionals. Atrial strain measurements will be conducted using the AFI LA atrial strain tool provided by GE Healthcare. These three measurements will be compared across the three groups and tested for a statistically significant difference. Providing that there is a significant

difference, particularly between the control and disease groups, a subsequent comparison will be conducted between the three groups of AF using the existing data.

2.2.1 Study Type

Single centre case-control study, quantitative and a prospective study.

2.2.2 Study Overview

- Study design – quantitative, observational, case-control study.
- Patients will be assigned to one of three groups: control group (normal sinus rhythm), disease-control group (controlled hypertension) and disease group (chronic atrial fibrillation).
- Patients will be required to visit the department on one occasion. This will be as part of either a routine or urgent referral for a transthoracic echocardiogram (TTE).
- TTEs are stored on both the ultrasound machines and computer picture archiving systems (PACS). Measurements can be taken on either the ultrasound machine or on the PACS following the patient's scan. The data obtained from these scans that will be utilised include patient demographics, heart rate and rhythm, LA area and volume measurements as well as LA strain measurements. This will be pseudo-anonymised using two Excel spreadsheets and the document will be password encrypted on a NHS OneDrive account
- Study site: within the Cardio-Respiratory department at Withybush General Hospital, there are five sonographers. This creates a throughput of approximately 60 TTE scans per week. With an expected data collection period of five months, this would equate to approximately 1200 outpatient scans – factoring in annual leave and sickness. Additionally, inpatient scans can boost this number, but is dependent on current demand.

2.2.3 Study Populations, Subject Selection, Recruitment and Study Schedule

Sampling design – only patients that have been referred for a transthoracic echocardiogram will be included as part of this study. Patient referrals will be screened to assess whether the patients fit into either of the three study groups. On patient arrival, patients that are deemed to be eligible thus far will be provided with an explanation of the study, patient information documents, and written informed consent is to be invited and received. Departmental sonographers may initiate Research discussion with patient either pre- during or post- scan, prior to the CI explaining the study in full and obtaining written informed consent. Providing that the transthoracic scan images are of a good enough quality to obtain strain measurements, the patient will be included as part of the study. Recruitment options will be tailored to the participant and may be either remotely or at time of clinic visit. Once potential candidates have been screened, it is envisaged that the invitation letter, Participant Information Sheet (PIS), Informed Consent Form (ICF) and a template ICF will be posted to the participant along with their appointment letter for an echocardiogram. This will allow plenty of time for potential participants to read over the PIS. The patient then may be contacted prior to their appointment via telephone to discuss the study and participant involvement and give verbal consent. If this is not feasible, the study should be discussed with patient during their appointment visit and written consent can be obtained. If the CI is not able to discuss the study with the patient at either of

these times, the patient may be telephoned following their scan to discuss and provide verbal consent.

2.2.4 Eligibility Criteria

Patients must be in one of the following categories to be included: normal sinus rhythm, normal sinus rhythm with controlled hypertension and chronic atrial fibrillation (persistent or permanent). Patients with paroxysmal AF may also be included but only as part of subsequent analysis when comparing strain measurements between AF groups. Additionally, eligible patients must conform to the following inclusion and exclusion criteria.

Inclusion criteria:

- Criterion 1: subjects will be included in the study if they are within one of the following groups:
    - Known to be in normal sinus rhythm, are normotensive and have no underlying health problems.
    - Known to be in normal sinus rhythm and have controlled hypertension. This must have been diagnosed by a healthcare professional and the patient should be taking appropriate antihypertensive medication.
    - Known to have chronic atrial fibrillation (persistent or permanent AF). This must have been diagnosed by a healthcare professional and the patient should be appropriately anticoagulated.
- Criterion 2: subjects should ideally be ≥ 50 years, but subjects will be age matched across all three groups.
- Criterion 3: patients should have an EF ≥ 50%.
- Criterion 4: good quality TTE images.
- Criterion 5: No valvular pathologies.
    - Patients with AF that have < moderate valvular pathologies may be considered (as per the British Society of Echocardiography Guidelines).

Exclusion criteria:

- Criterion 1: subjects with any valvular pathologies in the control and disease-control groups.
- Criterion 2: subjects with AF that have ≥ moderate valvular pathologies (as per the British Society of Echocardiography Guidelines).
- Criterion 3: subjects with an EF < 50%.
- Criterion 4: subjects < 50 years old.
- Criterion 5: poor quality TTE images.
- Criterion 6: inability to provide informed consent.
- Criterion 7: permanent atrial / ventricular pacing.
- Criterion 8: previous cardiac surgery.
- Criterion 9: unsatisfactory tracking of the LA endocardial border.
- Criterion 10: patients unwilling to have their results potentially published

2.2.5 Power Calculation

The study aims to investigate significant differences in atrial strain values between subjects with atrial fibrillation (AF), hypertension (no AF), and healthy volunteers. To

achieve statistical power for this study, a large enough sample size must be obtained. This will be achieved via the use of power calculations utilising G*Power 3.1 Statistical support has been provided and a sample size calculation was based on data found within the literature and involved detecting the smaller difference of left atrium contractile % between subjects with atrial fibrillation and healthy volunteers. The following criteria will be utilised: alpha level of 0.05, beta level of 15% and statistical power of 85%. Hence using this minimum value to determine our sample size (expected difference of 4.0, power = 0.85 and SD ± 7.5) and one-way ANOVA at three levels, a minimum sample size of at least 78 subjects in each group will be required for this study, total of 234 participants required. See Appendix Figures 1 & 2 for power calculation plots.

2.2.6 Statistical Analysis Plan

Continuous data normality will be assessed utilising the Shapiro-Wilk test. Non-parametric data will be analysed via Mann-Whitney U tests and Kruskal-Wallis one-way ANOVA tests. If data shows a normal distribution, to compare the LA strain values between the control (NSR) and disease (AF) groups an independent T-test can be performed; this will establish whether there is an initial statistically significant difference between the two. Following this, to compare the mean strain values for each phase between the three groups, a one-way ANOVA must be performed. A one-way ANOVA can also be performed to determine any significant differences between the strain values between AF groups. These data analysis methods will also be repeated to analyse LA area and volume. To assess whether LA area and volume size correlate with LA strain, a Pearson correlation coefficient must also be performed. Furthermore, to determine whether LA strain may be used as an early predictor of AF, linear regression models must be adapted. For categorical data, such as gender, chi-squared tests will be performed to determine any significant difference between groups. All statistical tests will be performed using the Prism GraphPad software.

It is expected that there will be a significant difference in strain values between NSR and AF participants. It is also expected that an increase in LA area and volume size will correlate with a decrease in LA strain values.

2.3 Study Outcomes

The key outcomes from this study will be whether there is a significant difference in atrial strain values between NSR patients, controlled hypertensive patients and patients with either persistent or permanent AF. These electrical changes should correlate with structural changes within the LA as the LA becomes more dilated in AF patients. Furthermore, subtle differences in LA strain values may be observed between patients with PAF, persistent and permanent AF to suggest a decline in atrial strain patterns with a worsening and prolongation of AF.

This will have a significant impact to the general population as AF is the most common arrhythmia. Left atrial enlargement and dilation can occur, either as a cause or complication of AF. However, the detection of this structural change and cardiac remodelling by measuring LA area and volume is often only detected when the patient has been in AF for a prolonged period. The utilisation of speckle-tracking echocardiography (STE) and atrial strain measurements may be able to detect subtle changes in LA strain pattern which may occur before any significant cardiac

remodelling and LA dilation, and thus serve as an early predictor of AF. This will allow for closer monitoring and the correct administration of anticoagulants.

2.4 Timescale

The project completion date is estimated to be March 2024 with the possibility of publishing following this. An overview of the entire project timescale can be visualised in the Gantt Chart – *see Appendix Figure 3*. The project has been split into three phases: project planning, data collection and analysis and dissertation write up and submission. The planning stage includes sponsorship and ethics approval and has an estimated completion date of May 2023. Following this, data collection and analysis may commence and in order to allow for sufficient data collection, a deadline of the end of November 2023 has been set. Furthermore, the end of January 2024 has been set to complete data analysis. The final stage consists of the write up of results, discussion and conclusion. Some of which may commence alongside data analysis and should be complete at the end of February 2024, ready for March submission. The date of the last visit for the patient will denote the end of the study. There are no follow-ups post echocardiogram required for this study, only data collection and analysis after this point.

2.5 Publication Policy

As findings from this study may be applicable in a much wider setting, it is important that any significant findings and benefits to patients are widely disseminated throughout research. Patients will be informed as part of the participant information document as well as verbally during their appointment regarding the intentions to possibly publish study findings. The participants are welcome to access and receive a copy of the findings and publication if they wish to do so post-study. It will be reiterated, and patients should be assured that any publishable findings will be completely anonymised and that no identifiers will be present in the publication which can link back the results to individual participants. Subjects who are not happy to have their results potentially published will be excluded from the study.

3. Screening, Consent and Withdrawal

3.1 Screening Duties

The Chief Investigator will be responsible for screening potential participants. This will be done by reviewing both in-patient and out-patient referrals to look for patients that are in one of the following categories: normal sinus rhythm, normal sinus rhythm with controlled hypertension and chronic atrial fibrillation. Subsequently, this can be further refined by excluding patients based on age, valvular pathologies and ventricular dysfunction.

3.2 Informed Consent Procedures

The CI will be responsible for inviting and receiving informed consent. Once potential candidates have been screened, it is envisaged that the invitation letter, PIS, ICF and a template ICF will be posted to the participant along with their appointment letter for an echocardiogram. The study team will telephone participants after they have received this information and prior to their TTE to explain the study details and to confirm whether they are interested in participating. Verbal, telephone consent can be given and recorded at this time. Written consent can also be given if the patient wishes to complete the received ICF, following the template ICF. Additionally, written consent may be given if the research team are unable to contact the patient prior to their appointment. This can be completed during the echocardiogram appointment via written informed consent. If patients are not contactable prior to their appointment, or patients contacted during their appointment wish to have more time to think about their decision, the patient may be contacted following their scan to obtain verbal, telephone consent. Similarly, if the patient is not contactable prior to their scan, and the CI is unable to discuss the study with the patient during their appointment, then again, the patient may be contacted via telephone following the TTE to obtain verbal, telephone consent. Furthermore, if a potential candidate for the study is an in-patient, after the referral has been screened, the CI will go to the appropriate ward and provide the patient with the PIS, ICF and template ICF. The study may be explained to the patient at this time or at the time of the scan and only written informed consent can be given in this instance. Participants will be enrolled into the study once verbal or written consent have been given. If telephone consent is obtained, a copy of the signed consent form will be provided to the participant – either during their appointment visit or via post following the scan.

It will be explained to patients that they have been identified as potential candidates for this study and a lay summary will be provided to outline the research project. The patient should be reassured that the scan will not differ because of their involvement in the study and there will be no delay in the results and TTE report as a result. The patient should also be informed that if they choose not to participate in the research study, this will not affect their standard of care in any way. Participants will be encouraged to ask questions and they will have the right to decline to take part in the study without providing any reasons. It should be explained to the patient that it is highly unlikely that there will be any incidental findings. However, any incidental findings would be dealt with accordingly, as they usually would as per standard of care, and would be reported back to the referring clinician. Unless GPs are the initial echocardiogram refer, they will not be directly contacted regarding incidental findings

but should have access to patient information and testing using the Welsh Patient Administration System and Welsh Clinic Portal system. The cardio-respiratory department does not have any direct contact or involvement with life insurance companies, however, if a patient has already been referred for an echocardiogram, it could be argued that they are currently awaiting investigations regardless of their involvement in the study.

3.3 Study Withdrawal

Not all patients that provide written informed consent to be involved in this study will be deemed to be suitable and thus will not be included. If it is deemed that the quality of the TTE images obtained are not of a high enough quality (LA and LA walls must be fully visible and clear), then the subject will not be included in the study. This is because unclear images make it very difficult to accurately measure the LA area and volume. Clear LA wall borders are required to obtain atrial strain measurements. Including unclear images in the study will not provide an accurate measurement of LA strain for comparison. This will be explained to the patient prior to receiving consent and in the participant information sheet. The patient should be reassured that if their images are deemed to be of an inadequate standard for LA strain analysis, this does not mean that they do not have a diagnostic use as per referral request.

Participants have a right to withdraw their consent at any point in the study. As the data will have been pseudo-anonymised, if consent withdrawal is following data collection, the data obtained thus far must be uncovered to match the patient identifiers which are to be kept separately from patient data. Once the patient's data has been correctly identified, this will be removed from records and not included in any data analysis. However, if the participant chooses to withdraw following any processing of data, it may not be possible to remove this data from the analysis.

4. Risk, Ethical Considerations and Confidentiality

4.1 Risks and Benefits

This research study is very low risk. TTEs have no known associated risks. Some pressure is applied during the scan to obtain images, and this may be sensitive for some patients, however, this is the case regardless of the study. The potential benefit of this study is that participants may be identified as having atrial strain patterns which are similar to those seen in patients with AF or may be an early predictor of AF. If this is the case, the patient can be monitored more closely and appropriately medicated if required.

4.2 Ethical Considerations

Joint Health Research Authority (HRA) and NHS Research Ethics Committee (REC) approvals are required for this project to uphold standards surrounding confidentiality, anonymity and informed consent. This is primarily due to the transferable and generalisable nature of the study outcomes and the identification of potential candidates as a result of previous NHS service use.

4.3 Patient and Public Involvement

Patients will not be involved in the development of the study and will only be involved as part of the conduction of data collection from TTEs. Patients will be informed prior to providing informed consent that there may be a possibility of publication and dissemination and will have been reassured that there will be no identifiable information present within any potential publications.

4.4 Confidentiality

For patients that wish to participate in this study, all signed Informed Consent Forms will be securely stored in the Investigator Site File within the Cardio-Respiratory Department and must not leave the department under any circumstances. The participant data will undergo pseudo-anonymisation via the use of two password-protected excel spreadsheets. One spreadsheet will contain participant numbers and corresponding hospital ID numbers. The second spreadsheet will include participant numbers along with patient demographics (i.e., sex, age, participant group, reason for patient referral, etc.). This data will be encrypted and the folder it resides in will also be encrypted and password protected. This will be stored on an NHS OneDrive account on a departmental computer. General data protection regulation (GDPR) will be maintained and followed throughout.

4.5 Premature Termination or Suspension of Study

In the unlikely event that the study was to be prematurely terminated or suspended (potentially due to insufficient adherence to protocol requirements or perhaps insufficient data availability), written notification will be provided to all parties involved in the study (REC, sponsor, participants, clinical supervisors and academic

supervisor). This notice will document the reasons for premature termination or suspension and outline the steps to be taken regarding collected data.

4.6 Future Use of Stored Specimens and Other Identifiable Data

There are no current plans to use the data collected post study. However, the data will be securely stored and encrypted as it may be deemed to be necessary that the pseudo-anonymisation is uncovered in the patients' best interests.

4.7 Record Retention, Storing and Archiving

TTE scans are stored on both the ultrasound machine and on the computer through PACS. Scans stored on the machine are routinely archived after 3 months; clinical guidelines suggest than scans should be accessible on PACS server for 7 years post-investigation. These are to be accessible to healthcare professionals throughout following the patient scan for clinical progression external to the research project. Data which has been obtained from these scans will be stored in two locations. The first of which is across two excel spreadsheets to maintain pseudo-anonymisation. Both spreadsheets will be password protected and folders encrypted on an NHS OneDrive account. These will be located on a single departmental computer.

4.8 Study Oversight

The CI will be responsible for study oversight, including monitoring safety, ensuring that the study is conducted according to the protocol and ensuring data integrity. The CI will review the data obtained and will also perform a reproducibility study. Any issues that arise (unanticipated problems, protocol deviation, and significant events) will be promptly reported to the REC, the sponsor, clinical supervisors and academic supervisor.

4.9 Study Reporting

Regular updates will be provided to the clinical supervisors and academic supervisor involved in this project as well as to the Sponsor. Regular meetings will be held between all parties to ensure the project is on track and progressing as planned. This may be in person, via Teams or through email updates.

4.10 Safety Reporting

No adverse events are anticipated during this study. Subjects included in this study will have either been urgently or routinely referred for a TTE. The extra measurements taken from the patient’s scan occur after the patient has left, thus there is no alteration to the scan itself for patient experience.

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Appendix

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[1] -- Friday, January 20, 2023 -- 20:14:29
F tests - ANOVA: Fixed effects, omnibus, one-way

Analysis:    A priori: Compute required sample size
Input:       Effect size f                  =   0.2177324
             α err prob                     =   0.05
             Power (1-β err prob)           =   0.85
             Number of groups               =   3
Output:      Noncentrality parameter λ      =   11.0933311
             Critical F                     =   3.0349206
             Numerator df                   =   2
             Denominator df                 =   231
             Total sample size              =   234
             Actual power                   =   0.8509156
```

Figure 1: Computed sample size of 234 utilising G*Power 3.1. *The following criteria was used: effect size of 0.25, alpha level of 0.05, beta level of 0.15 (15%), statistical power of 0.85 (85%) and a total of 3 groups – control, disease-control, and control.*

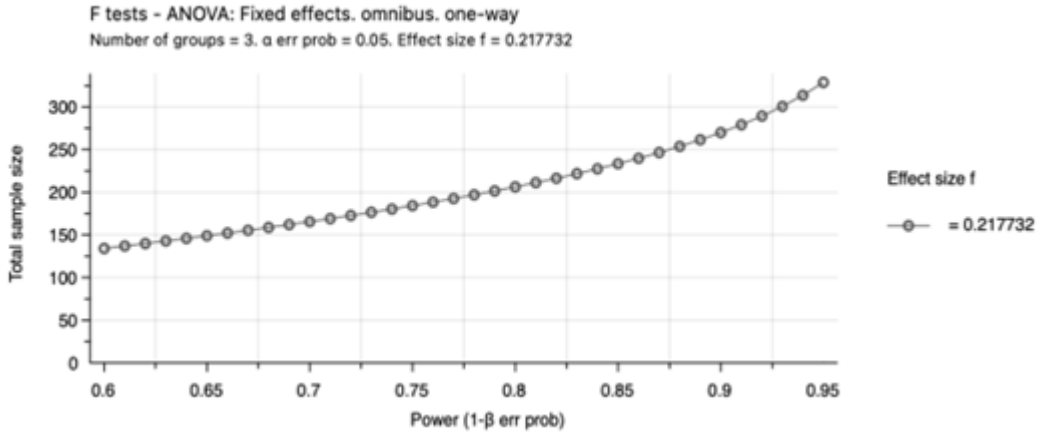
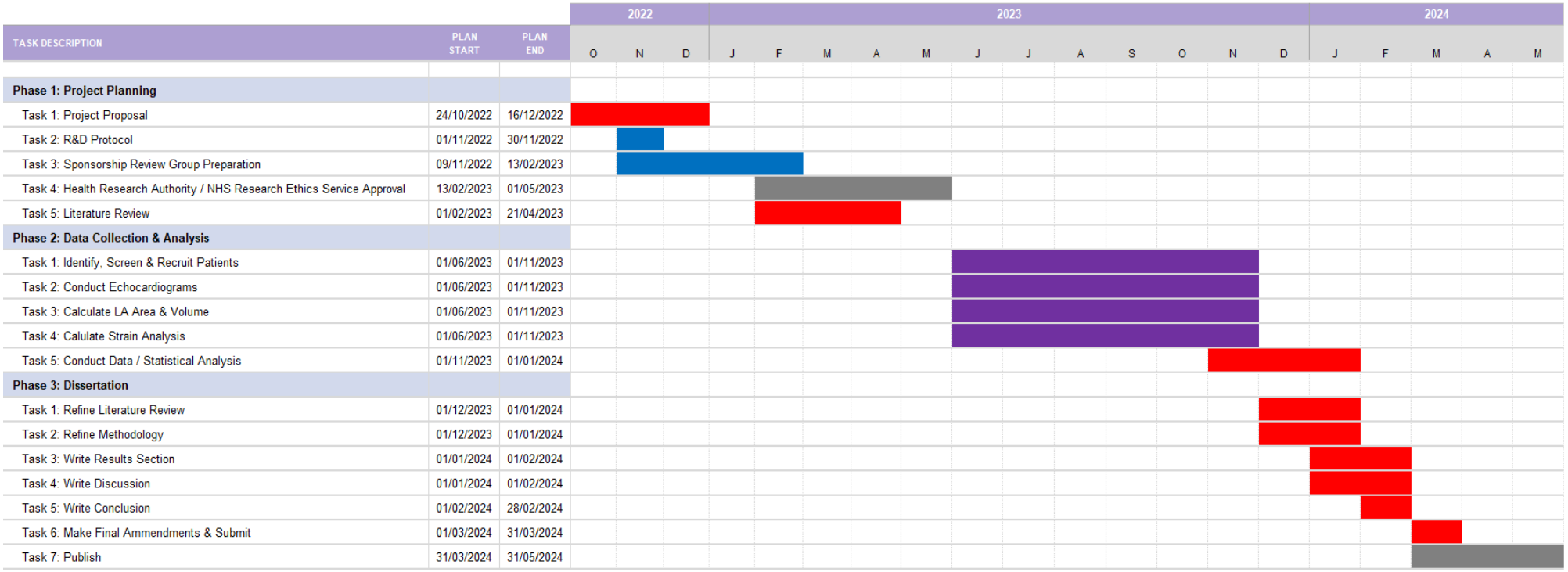


Figure 2: Plotted total sample size over power utilising G*Power 3.1, producing a sample size of 234 to achieve 85% significance. *The following criteria was used: effect size of 0.25, alpha level of 0.05, beta level of 0.15 (15%), statistical power of 0.85 (85%) and a total of 3 groups – control, disease-control, and control.*

Research Project: *Can left atrial strain measurements, and correlating changes in left atrium area and volume size, be used as an early predictor of atrial fibrillation?*
Lucy Hwozdyk



TASK DESCRIPTION	PLAN START	PLAN END
Phase 1: Project Planning		
Task 1: Project Proposal	24/10/2022	16/12/2022
Task 2: R&D Protocol	01/11/2022	30/11/2022
Task 3: Sponsorship Review Group Preparation	09/11/2022	13/02/2023
Task 4: Health Research Authority / NHS Research Ethics Service Approval	13/02/2023	01/05/2023
Task 5: Literature Review	01/02/2023	21/04/2023
Phase 2: Data Collection & Analysis		
Task 1: Identify, Screen & Recruit Patients	01/06/2023	01/11/2023
Task 2: Conduct Echocardiograms	01/06/2023	01/11/2023
Task 3: Calculate LA Area & Volume	01/06/2023	01/11/2023
Task 4: Calulate Strain Analysis	01/06/2023	01/11/2023
Task 5: Conduct Data / Statistical Analysis	01/11/2023	01/01/2024
Phase 3: Dissertation		
Task 1: Refine Literature Review	01/12/2023	01/01/2024
Task 2: Refine Methodology	01/12/2023	01/01/2024
Task 3: Write Results Section	01/01/2024	01/02/2024
Task 4: Write Discussion	01/01/2024	01/02/2024
Task 5: Write Conclusion	01/02/2024	28/02/2024
Task 6: Make Final Ammendments & Submit	01/03/2024	31/03/2024
Task 7: Publish	31/03/2024	31/05/2024

Figure 3: A Gantt chart demonstrating the entire research project outline and timeline.

Key:
- *Red: university-based steps and deadlines*
- *Blue: research and development*
- *Grey: external (i.e., ethics approval and publication)*
- *Purple: data collection and processing*