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|---|---|
| **FULL/ LONG TITLE OF THE STUDY**<br>DEVELOPMENT OF A NEW APPROACH TO MEASURE THE VOLUME OF BREAST TISSUE REMOVED DURING WIDE LOCAL EXCISION USING SPECIMEN WEIGHT AND MAMMOGRAPHIC DENSITY | |
| **SHORT TITLE/ ACRONYM**<br>Estimating breast specimen volume from weight and radiological density | |
| **Version and Date of Protocol:** | V1.0 24/Jun/2023 |
| **Sponsor:** | University Hospitals of Derby and Burton NHS Foundation Trust |
| **Chief Investigator:** | Mr Emanuele Garreffa |
| **Local Study Reference:** | UHDB/2019/088 |
| **IRAS Number:** | 325661 |
| **ISRCTN/ ClinicalTrials.gov number:** | ISRCTN15283352 |
| **Funder(s):** | Derby and Burton Hospitals Charity |
| **This protocol has regard for the HRA guidance** | |

**Confidentiality Statement**

This document contains confidential information that must not be disclosed to anyone other than the Sponsor, the Investigator Team, host NHS Trust, regulatory authorities, and members of the Research Ethics Committee.

**SIGNATURE PAGE**

The undersigned confirm that the following protocol has been agreed and accepted and that the Chief Investigator agrees to conduct the study in compliance with the approved protocol and will adhere to the principles outlined in the Declaration of Helsinki, the Derby CTSU's SOPs, and other regulatory requirement.

I agree to ensure that the confidential information contained in this document will not be used for any other purpose other than the evaluation or conduct of the investigation without the prior written consent of the Sponsor.

I also confirm that I will make the findings of the study publically available through publication or other dissemination tools without any unnecessary delay and that an honest accurate and transparent account of the study will be given; and that any discrepancies from the study as planned in this protocol will be explained.

**Protocol version 1.0 24/Jun/2023 authorisation signatures:**

**Chief Investigator:**

Signature: ……..  Date: ……/……/……

Name (please print): ……..

**For and on behalf of the Study Sponsor (if required):**

Signature: ……..  Date: ……/……/……

Name (please print): ……..

Position: ……..

**KEY STUDY CONTACTS**

| | |
|---|---|
| Chief Investigator: | Emanuele Garreffa<br>Consultant Oncoplastic Breast Surgeon<br>Royal Derby Hospital, Derby, DE22 3NE<br>Tel: 01332785538<br>Email: emanuele.garreffa@nhs.net |
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| | |
| Sponsor: | University Hospitals of Derby and Burton NHS Foundation Trust |
| Funder(s): | Derby and Burton Hospitals Charity |
| Clinical Trials Unit: | Derby Clinical Trials Support Unit<br>Royal Derby Hospital<br>Uttoxeter Road<br>Derby, DE22 3NE<br><br>01332 724639<br>uhdb.DerbyCTSU@nhs.net |
| Study Statistician: | Jacqueline Beckhelling, Medical Statistician, Derby Clinical Trials Support Unit, jacqueline.beckhelling@nhs.net |

**STUDY SUMMARY**

| | |
|---|---|
| **Study Title:** | DEVELOPMENT OF A NEW APPROACH TO MEASURE THE VOLUME OF BREAST TISSUE REMOVED DURING WIDE LOCAL EXCISION USING SPECIMEN WEIGHT AND MAMMOGRAPHIC DENSITY |
| **Local Study Reference:** | UHDB/2019/088 |
| **Study Design:** | Single-centre proof-of concept study |
| **Study Participants:** | Women undergoing breast conserving surgery for breast cancer as standard care. |
| **Planner Number of Sites:** | 1 |
| **Planned Sample Size:** | Phase 1: 110; Phase 2: 246 |
| **Treatment Duration:** | n/a |
| **Follow Up Duration:** | n/a |
| **Planned Start Date:** | 01/09/2023 |
| **Planned Recruitment End Date:** | 31/10/2024 |
| **Planned Study End Date:** | 30/11/2024 |
| **Research Question/ Aims:** | The objective of this research project is to evaluate a novel method of obtaining simple and accurate estimates of the volume of breast tissue excised during breast conserving surgery by putting the weight of the surgical specimen in relation with its radiological density. The primary endpoint is to establish whether the method is superior to the currently used method. |

**FUNDING AND SUPPORT IN KIND**

| **Funder(s)** | **Financial and Non-Financial Support Given** |
|---|---|
| Derby and Burton Hospitals Charity | Financial |
| Consultant Radiologist | Radiologists participating in the study will undertake research activities at no cost to the study. |
| Mr Emanuele Garreffa | The PI will undertake research activities at no cost to the study. |

**ROLES & RESPONSIBILITIES**

**Sponsor**

The Sponsor, University Hospitals of Derby and Burton NHS Foundation Trust, take on overall responsibility for appropriate arrangements being in place to set up, run and report the research project. The sponsor is not providing funds for this study, but has taken on responsibility for ensuring finances are in place to support the research.

**Funder**

The study is funded by Derby and Burton Hospitals Charity.

**Study Management Committees**

Trial Management Group (TMG)

The trial management group will meet regularly to oversee the day-to-day management of the trial, including all aspects of the conduct of the trial. Any problems with study conduct and participating centers will be raised and addressed during TMG meetings.

Trial Steering Committee (TSC) and Data Monitoring and Ethics Committee (DMEC)

This is a small proof of concept study and therefore will not require a TSC or DMEC

**Protocol Contributors**

A number of protocol contributors have been involved in the development of this protocol, these include; the Chief Investigator, Co-Investigators (incl. Statistician) and the Trial Management team. Protocol contributors are responsible for inputting into the design of the study, ensuring that it is designed transparently and efficiently.

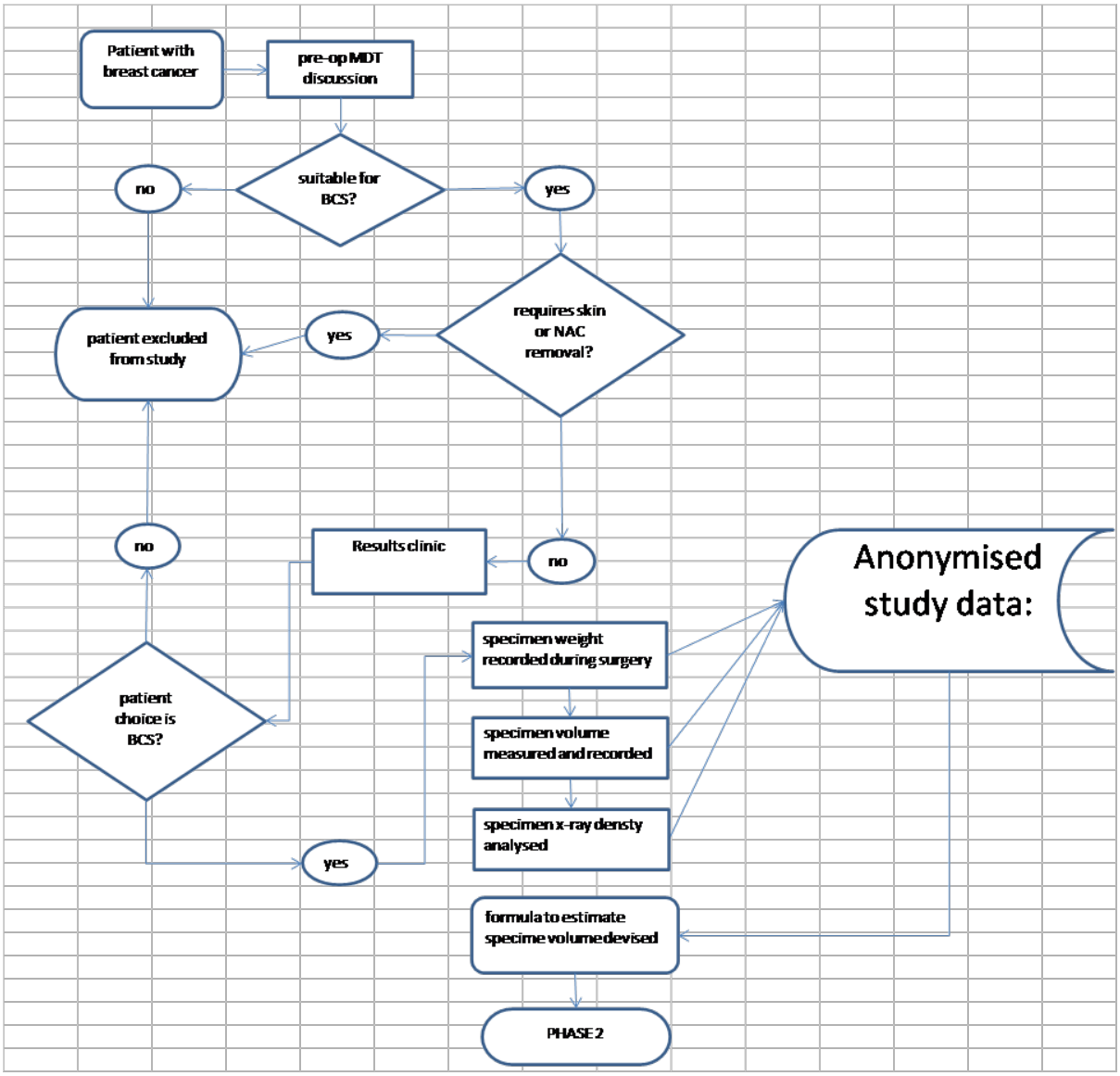
## Contents

**LIST OF ABBREVIATIONS**

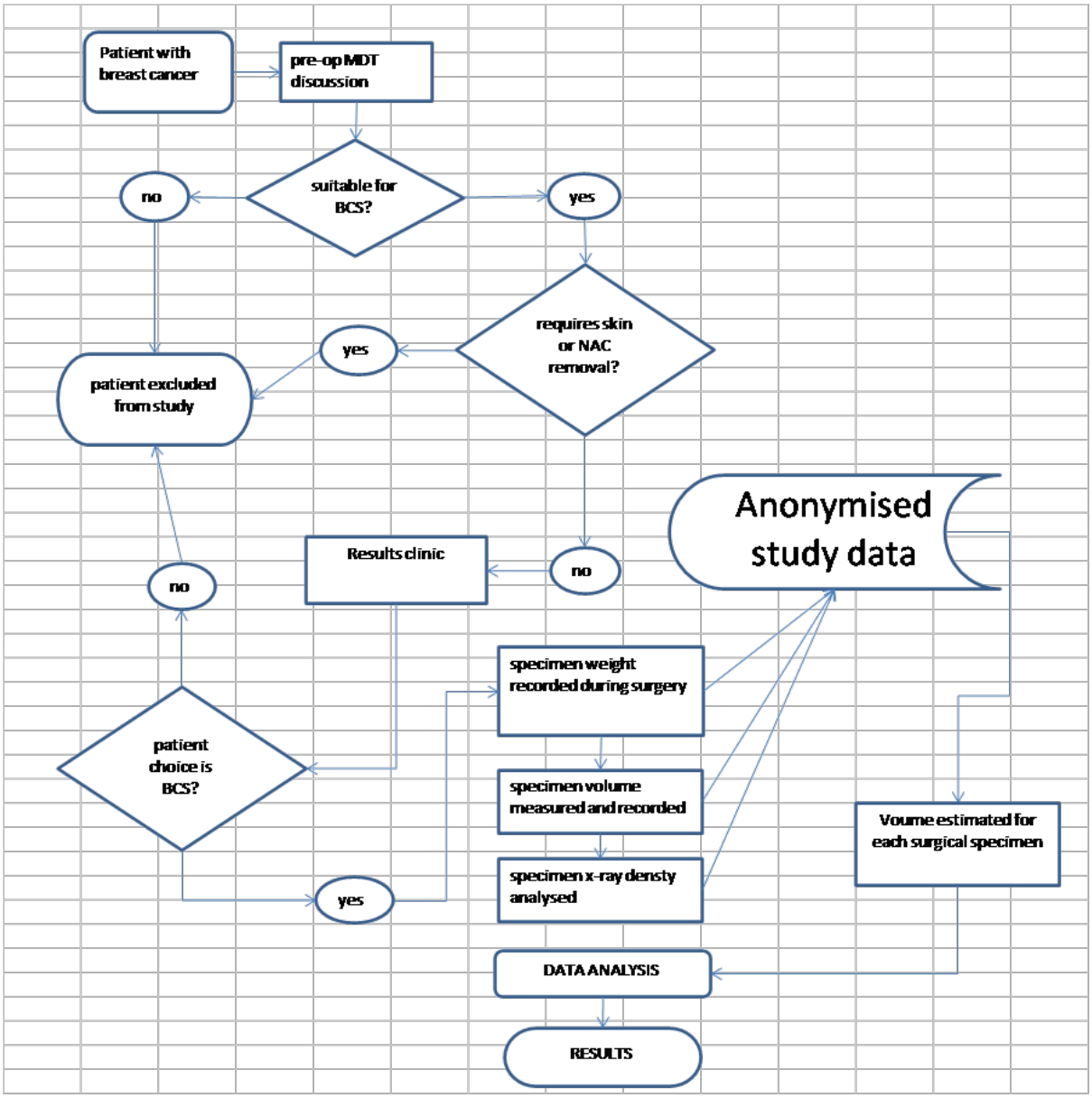
| | |
|---|---|
| AE | Adverse Event |
| BCS | Breast Conserving Surgery |
| BMI | Body Mass Index |
| CI | Chief Investigator |
| CRF | Case Report Form |
| CT | Computed Tomography |
| CWPF | Chest Wall Perforator Flap |
| DMEC | Data Monitoring and Ethics Committee |
| GCP | Good Clinical Practice |
| GMP | Good Manufacturing Practice |
| ICF | Informed Consent Form |
| ICH | International Conference on Harmonisation of technical requirements for registration of pharmaceuticals for human use. |
| ISF | Investigator Site File |
| ISRCTN | International Standard Randomised Controlled Trials |
| MDT | Multi-Disciplinary Team |
| NHS R&D | National Health Service Research & Development |
| PI | Principal Investigator |
| PIC | Participant Identification Centre |
| PIS | Participant Information Sheet |
| QA | Quality Assurance |
| QC | Quality Control |
| RCT | Randomised Control Trial |
| REC | Research Ethics Committee |
| RT | Radiotherapy |
| SAE | Serious Adverse Event |
| SDV | Source Data Verification |
| SOP | Standard Operating Procedure |
| TMG | Trial Management Group |
| TSC | Trial Steering Committee |
| TMF | Trial Master File |
| VAS | Visual Analogue Scale |
| WLE | Wide Local Excision |

**STUDY FLOW CHART**

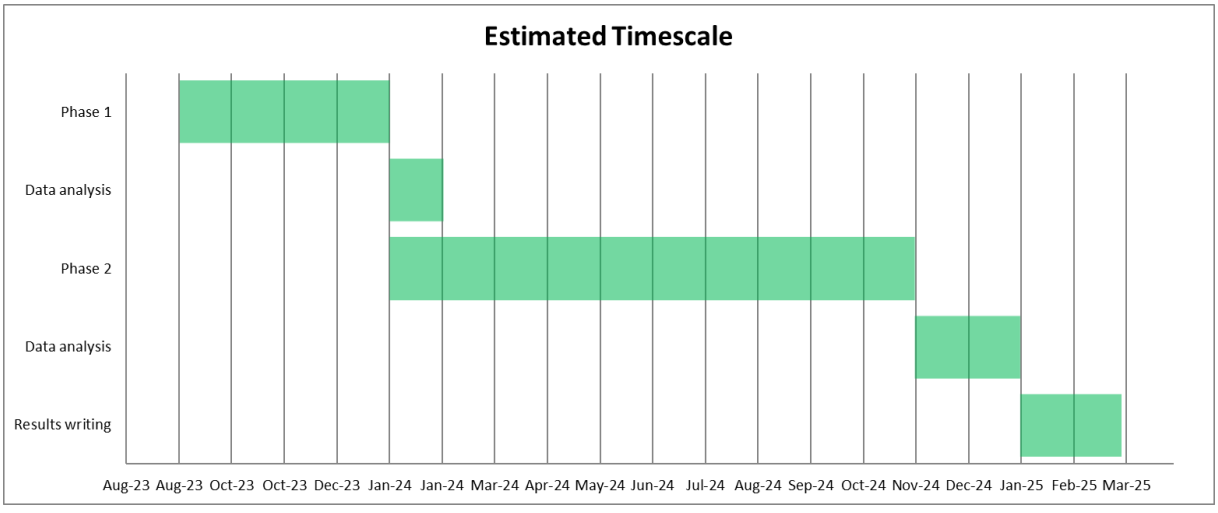
**Phase 1:**



**Phase 2:**




**Estimated timescale:**



## 1. BACKGROUND

There are several published methods to estimate the breast volume using mammogram measurements as reference [1,2]. All these methods are based on the assumption that breasts are equally composed of fibro-glandular tissue, which has been attributed the same density of water (1g/ml), and fatty tissue (with a density of 0.92g/ml). The resulting breast density based on this assumption is 0.958g/ml.

A similar approach has been used in attempt to estimate a wide local excision (WLE) specimen volume from its weight [3,4]. All these methods are limited by the fact that the percentage of breast fibro-glandular tissue is not the same for every woman and every surgical specimen. It does also not take into account the proportion of the specimen occupied by the breast tumour itself, which will be denser.

Breast cancer patients treated with WLE subsequently receive adjuvant breast radiotherapy (RT) to reduce the risks of local recurrence. This can be accompanied by a further radiation dose to the breast tissue adjacent to the surgical excision (tumour bed boost), which is the area at higher risk for recurrence. During surgery, metal clips are left in the breast to help the radiation oncologist to identify the target area for the boost.

The delivery of tumour bed boost in an accurate way is of paramount importance. The recently published up to 20-year follow-up data of the European Organization for Research and Treatment of Cancer (EORTC) “boost no boost” trial show that the ipsilateral recurrence rate is lower in patients receiving boost (12%) vs. no boost (16.4%) [5]. However, boost RT may result in varying degrees of focal retraction or indentation and asymmetry due to either local fibrosis or major fat necrosis resulting in breast volume loss or change of shape [6].

## 2. RATIONALE

The aim of this study is to evaluate a novel method of obtaining simple and accurate estimates of the volume of a WLE specimen by putting the specimen weight in relation with its radiological density.

In women with smaller breasts and larger tumours oncoplastic breast surgery techniques such as volume replacement with chest wall perforator flaps (CWPF) can be used to enable breast conservation and avoid deformity. In CWPF, the surgical cavity resulting from WLE is filled volume-for-volume with non-breast tissue (skin and fat) harvested from the surrounding chest wall.

A previous research study demonstrated how, in the context of CWPF volume replacement, the standard approach for tumour bed contouring (based solely on the metal clips placed by the surgeon) is not accurate (contoured volume significantly smaller than specimen volume). A new method of contouring has been proposed including the clips and the typical appearance of the flap in the planning CT scan [7,8].

On average, the tumour bed volume obtained with this method closely matched the estimated surgical specimen volume obtained from its weight (only 1.02 ml smaller on average). However, the variability between the individual cases was high (SD 43.6). This is thought to be related with the difference in breast density between the patients leading to a non-accurate estimate of the specimen volume based on its weight [8].

In the context of volume replacement surgery, having an accurate estimate of the volume of breast tissue removed with surgery could help to increase the accuracy of tumour bed contouring. If the boost volume is underestimated, then the target penumbral breast tissue will be missed (only the central portion of non-breast flap tissue would be irradiated) potentially leading to higher rates of local recurrence. If the boost volume is overestimated, then an excess dose of radiation would be administered with negative impact on the final cosmetic outcome (increased fibrosis and loss of volume).

During a meeting with a group of breast cancer patients held during the design phase of this study, the importance of accurate radiotherapy planning has been unanimously acknowledged by all participants. In fact, both under- and overestimation of the boost target volume (and subsequent increased risk of local recurrence or impaired cosmetic outcome respectively) could have negative impact on patients' quality of life and require further treatment including surgery.

To determine the long-term effects of an increased accuracy of the RT boost (impact on local recurrence rate and cosmetic sequelae) is beyond the scope of this initial project due to the timelines of these events. Nevertheless, these aspects could be potentially explored with a long-term study and the findings of the proposed research project will be of key importance in the design of that.

## 3. OBJECTIVES AND OUTCOME MEASURES/ ENDPOINTS

### 3.1. Objectives

**Phase 1:** To develop a regression model to estimate tissue sample volume using a combination of explanatory variables potentially including specimen weight, radiological density, patient 's age, patient's BMI, tumour histology and grade.

**Phase 2:** To compare the estimates of volume derived from the regression model identified in phase 1 and then the currently used estimation method (dividing the sample weight by the approximate density of 0.958g/ml to determine which method produces the most accurate estimates.

### 3.2. Outcomes

**Phase 1 primary outcome:** A regression model using a combination of variables from specimen weight, radiological density, patient 's age, patient's BMI, tumour histology and grade that predicts surgical specimen volume as accurately as possible.

**Phase 1 secondary outcomes:** not applicable.

**Phase 2 primary outcome**: The 95% limits of agreement (Bland and Altman [15]) with 95% confidence limits of the novel method using the regression model and the current method (dividing the specimen weights by 0.958, regardless of their radiological density) against the gold standard measurement of specimen volume (the water displacement / Archimedes method).

**Phase 2 secondary outcome 1**: The Intraclass correlation (ICC) of the estimates using the new method and the current method. This will indicate how strongly the two methods resemble each other.

**Phase 2 secondary outcome 2**: A within-patient comparison of the new method and the current method using a paired t-test.

## 4. STUDY DESIGN

This is a proof-of-concept diagnostic study which evaluates a novel method of obtaining an estimate of the breast volume excised with a WLE by correlating specimen weight and radiological density adjusted for other variables such as age (affecting overall breast radiological density), tumour histology and grade (affecting specimen density as high-grade tumours are denser), and patient's BMI. The latter was selected as a surrogate of breast size as it is routinely measured in patients undergoing surgical procedures. It has been demonstrated that WLE surgical specimens in larger breasts are inherently larger due to the increased antero-posterior dimension of the cylindrical excision [9].

This method is then compared, together with the currently used approximate density of 0.958g/ml applied to all samples regardless of their radiological density, against volume measurement by water displacement (gold standard).

Method:
Specimen x-ray density will be assessed by a Radiologist using a Visual Analogue Scale (0-100). The presence of a tumour mass and its diameter, as well as the percentage of specimen occupied by the tumour will also be assessed. A standard procedure on how to assess specimen density with VAS and how to measure the tumour mass will be devised and training will be provided to the radiologists performing the measurements to reduce inter-observer variability.

Assessing mammographic density using a visual analogue scale (VAS) is a widely used and validated procedure and mammographic density using this methodology has been shown to correlate with a number of important factors breast cancer risk [10-12] and increased risk of interval cancers in screened populations [13]. In cancer patients it is assessed in the contra-lateral breast as the cancer itself affects breast density.

What is novel about this study is that we are assessing the density of a lumpectomy specimen using a VAS to help estimate excised volume of tissue. The density of the excised tissue may differ significantly from the overall breast density for two reasons:

- Invasive cancers contain little fat and so are a dense combination of tumour cells and fibrotic tissue even when the cancer occurs in a breast made up almost entirely of fat. The percentage of excised tissue which is cancer varies from 2% to 30%.
- Dense breast tissue is not uniformly spread throughout the breast. So, in a breast which is 50% dense tissue and 50% fat the tissue surrounding the cancer may be 100% fat or 100% dense tissue depending on the tumour location.

Following the x-ray assessment, breast specimen density will then be calculated:
Breast Specimen Density = Specimen weight / Specimen Volume.

The breast specimen volume will be modelled using regression analysis, with specimen x-ray density and other variables as explanatory variables. This will enable breast volumes to be estimated that are tailored to each specimen.

This study will require 2 phases:
**Phase 1:** obtain the equation to predict surgical specimen volumes.
**Phase 2 (proof of concept):** compare the novel method and the current method (standard density of 0.958g/ml applied to all samples regardless of their radiological density) with the gold standard (water displacement) using Limits of agreement (Bland and Altman [15]).

## 5. STUDY SETTING

This is a single centre study. The study population will include women diagnosed with breast cancer undergoing surgical treatment with breast conserving surgery (BCS) in the Breast Unit of University Hospitals of Derby and Burton NHS Foundation Trust.

## 6. ELIGIBILITY CRITERIA

### 6.1. Inclusion Criteria

- Women with breast cancer diagnosis (both invasive and in-situ) undergoing BCS with or without oncoplastic techniques.

### 6.2. Exclusion Criteria

- Excision of skin and/or nipple-areola complex required.

## 7. STUDY PROCEDURES

### 7.1. Recruitment

#### 7.1.1. Patient Identification

- Suitable patients will be identified during the routine multi-disciplinary meetings.
- All suitable patients identified following pre-operative MDT discussion will be automatically eligible if they opt for BCS.

#### 7.1.2. Screening

Not applicable.

### 7.2. Consent

Informed consent is not required for this study, as it does not involve any deviation from standard patient care. This study does not involve sharing any identifiable patient information outside of the treating team. Only anonymous data will be used to carry out the research. The data obtained by reviewing routinely collected patient information and tests for the purpose of this study will not have any impact in patients' diagnosis and treatment.

### 7.3. Study Assessments

Participants will undergo ALL treatments as per standard local practice.
Breast conserving surgery will be performed as per standard local practice.
In the surgical theatre, following the WLE, the surgical specimen will be processed as per standard practice. This involves:

- Specimen x-ray
- Measurement of specimen weight

The specimen is subsequently sent (either fresh or in formalin) to the Pathology department for processing. There, in addition to the routine procedures, the specimen volume will be measured by water displacement using the following procedure (same for Phase 1 and Phase 2):

- The WLE specimen is placed into a graduated jug of known capacity which is subsequently filled completely with water.
- The specimen is removed, and the water is transferred into a class A measuring cylinder to measure the volume.
- The WLE specimen volume will be obtained by subtracting the measured water volume from the maximum capacity of the graduated jug.

The histology specimens will be examined and reported according to standard local practice. Additionally, the specimen volume will also be added to the report.

Full schedule of assessments is shown in Appendix 1.

### 7.4. Withdrawal Criteria

Not applicable.

### 7.5. Storage and Analysis of Samples

Surgical specimens will be processed immediately in pathology as per standard practice and the volume will be measured and recorded at that point. There will be no storage of material for research purposes.

### 7.6. End of Study

The end of study will be defined as the last data capture for the last participant. The Clinical Trials Manager will notify the Sponsor, participating sites and REC within 90 days of the end of study. The clinical study report will be written within 12 months of the end of study.

## 8. SAFETY REPORTING

### 8.1. Definitions

| Term | Definition |
|---|---|
| **Adverse Event (AE)** | Any untoward medical occurrence in a participant, including occurrences which are not necessarily caused by or related to study procedures. |
| **Related AE** | An untoward and unintended response in a participant to a study procedure. This means that a causal relationship between the study procedure and an AE is at least a reasonable possibility, i.e. the relationship cannot be ruled out. |
| **Serious Adverse Event (SAE)** | A serious adverse event is any untoward medical occurrence that:<br>• results in death<br>• is life-threatening<br>• requires inpatient hospitalisation or prolongation of existing hospitalisation<br>• results in persistent or significant disability/incapacity<br>• consists of a congenital anomaly or birth defect<br>Other 'important medical events' may also be considered serious if they jeopardise the participant or require an intervention to prevent one of the above consequences.<br>NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe. |
| **Related SAE** | An adverse event that is both serious and, in the opinion of the reporting Investigator, believed with reasonable probability to be due to one of the study procedures. |
| **Related & Unexpected SAE** | A serious adverse event that;<br>• is believed with reasonable probability to be due to one of the study procedures.<br>• the nature and severity of which is not consistent with the |

| | |
|---|---|
| | information provided in the protocol i.e. it is not listed as an expected occurrence. |

### 8.2. Operational Definitions for (S)AEs

All participants in the study will receive standard NHS treatment. This protocol does not contain investigational agent(s). Therefore, events related to the natural course of the disease and its treatment are not required to be reported.

For the purposes of this study, the following events do not constitute SAEs:

- Hospitalisation for:
  - Surgical complications related to breast conserving surgery
  - Pre-planned elective procedures unless the condition worsens
  - Treatment for progression of the patient's cancer
- Progression or death as a result of the patient's cancer

### 8.3. Recording and Reporting SAEs

Collection of AEs is not required for trial analysis.
Whilst it is not anticipated there will be any serious adverse events directly related to the study, it is important that this protocol includes a process for dealing with any unexpected serious adverse events in the unlikely event they occur.

For the purpose of this study, the following are regarded as expected SAEs and should not be reported on an SAE form:

- Haematoma, wound infection, seroma or other complication of breast surgery.

Events that meet the criteria for a SAE, per protocol, must be recorded by the investigator using the 'non-CTIMP safety report to REC form' from the HRA website. The completed form should be submitted to the Sponsor and REC within 15 days of the CI becoming aware of the event.

| **UHDB contact information**: |
|---|
| **Email**: uhdb.randdsae@nhs.net |

#### 8.3.1. Assessment of SAEs

##### 8.3.1.1 Severity

The investigator should determine the severity of the SAE;

- Mild: no interference with daily activities.
- Moderate: moderate interference with daily activities.
- Severe: considerable interference with daily activities (e.g. inability to work).

**NOTE**: to avoid confusion or misunderstanding the term "severe" is used to describe the intensity of the event, which may be of relatively minor medical significance, and is NOT the same as "serious" which is described in the safety definitions.

#### 8.3.1.2 Causality

Clinical judgement should be used to determine the relationship between the study procedures and the occurrence of each SAE;

- Not-related: There is no evidence of a causal relationship between the event and study procedures.
- Related: There is evidence of a causal relationship between the event and study procedures i.e. a relationship to the study procedures cannot be completely ruled out.

Assessment of causality must be made by a medically qualified doctor (usually the principal investigator).

#### 8.3.1.3 Expectedness

The assessment of expectedness is only required if the event is deemed to be related to study procedures.

- Expected: Event previously identified and described in the protocol.
- Unexpected: Event not previously described in the protocol.

The expectedness assessment is delegated to CI.

### 8.4. Pregnancy reporting

Not required.

### 8.5. Reporting Urgent Safety Measures

If any urgent safety measure is taken the research team should inform the Sponsor within 24 hours using the Sponsors safety incident reporting form. The Sponsor will inform the REC and participating sites of the measures taken and the circumstances giving rise to those measures within 3 days on implementation of the urgent safety measure.

## 9. DATA HANDLING

### 9.1 System and Compliance

Study data will be entered at site into paper case report forms (CRF) directly or onto worksheets by study personnel. The worksheet data must be transferred into the CRF within 5 days of data collection. Participants will be identified only by their unique study number. The NHS numbers will be linked to the study numbers and this information will be stored securely locally with restricted access only to investigators who are part of the patients' treating team. Only the study number will be entered in the study database. The database will consist of a password protected Excel spreadsheet.

Processing of study data and checking for consistency, validity and quality will be undertaken by study statisticians at Derby Clinical Trials Unit prior to analysis. Data checks will include out-of-range data, cross-checks for conflicting data, missing data and data queries.

### 9.2 Source Data

Source documents are where data are first recorded, and from which participants' CRF data are obtained. These include, but are not limited to, hospital records (from which medical history and previous and concomitant medication may be summarised into the CRF), clinical and office charts, laboratory and pharmacy records, diaries, microfiches, radiographs, and correspondence.

CRF entries will be considered source data if the CRF is the site of the original recording (e.g. there is no other written or electronic record of data). All documents will be stored safely in confidential conditions. On all trial-specific documents, the participant will be referred to by the trial participant number, not by name.

Investigators should keep records of all participating patients and copies of any worksheets. It is necessary for investigators to provide access to source document for monitoring and audit purposes to Sponsor, any monitoring or regulatory authorities as deemed necessary.

| **Study variable** | **Source data** |
|---|---|
| Measured tissue sample volumes | Histopathology report |
| Measured specimen weight | Histopathology report |
| Radiological density of healthy breast tissue on specimen x-ray | CRF |
| Tumour diameter on specimen x-ray | CRF |
| Percentage of specimen occupied by tumour | CRF |
| Tumour histology | Histopathology report |
| Tumour grade | Histopathology report |
| Patient age | Hospital records |
| Patient BMI | Hospital records |

### 9.3 Workflow

Data will be collected and retained in accordance with the Caldicott Principles, UK Data Protection Act 2018 and General Data Protection Regulation (GDPR).

### 9.4 Data Access and Security

Direct access will be granted to authorised representatives from the Sponsor, host institution and the regulatory authorities to permit study-related monitoring, audits and inspections.

### 9.5 Archiving

At the end of the study, following completion of the end of study report, UHDB will securely archive all centrally held study related documentation for a minimum of 5 years. At the end of the defined archive period arrangements for confidential destruction will be made. It is the responsibility of the PI to ensure that data and all essential documents relating to the study are retained securely for a minimum of 5 years after the end of study, and in accordance with national legislation. All archived documents must continue to be available for inspection by appropriate authorities upon request.

## 10. STATISTICS AND DATA ANALYSIS

### 10.1. Sample Size Calculation

Phase 1: Regression model.

A detailed search of the literature found no data upon which a sample size calculation could be based. However, "rules of thumb" have been established for regression models for which sample size calculations cannot be calculated. The accuracy of several of these estimations has been assessed by Green [14] and, based on his research the following estimate method was selected:

S = 104+i

Where S = Sample size; I = Number of independent variables (i.e. the number of variables we will use to predict the tissue volume). This is assuming the treatment effect would be moderate. Taking into account the number of variables this results in a sample size of 110.

Phase 2: Comparison of accuracy using limits of agreement analysis.

The sample size of 246 samples was calculated based on the precision of 95% confidence limits for 95% limits of agreement using the formulae taken from Bland and Altman [15]. The precision for the limits of agreement confidence interval was set at 5% as a difference of more than 5% could affect the accuracy of the boost radiotherapy in the CI's clinical judgement. The standard deviation of the differences using the current method was estimated at 23.26%, based on a clinical audit carried out by the CI between January and December 2022.

### 10.2. Planned Recruitment Rate

The study set-up will require 4 months. Based on our Unit's number of treated cancers (>800/year of which at least 60% are WLE), the estimated time to recruit enough patients to complete phase 1 of the study would be 4 months. the recruitment time for phase would require another 9 months. There will be no recruitment gap between phase 1 and phase 2 as the analysis of the data from phase 1 will be carried out simultaneously to phase 2 recruitment. Finally, we will require 4 months

for data analysis and study close- out. The results of this research will be presented in breast cancer conferences and published in a peer-reviewed journal.

## 10.3. Statistical Analysis

### 10.3.1. Summary of Baseline Data and Flow of Patients

The baseline variables (age and BMI) will be reported with medians & Interquartile Ranges (IQR).

A Consolidated Standards of Reporting Trials (CONSORT) flow diagram will not be required for this study.

### 10.3.2. Outcome Analysis

Study phase 1 primary outcome: A regression model to estimate the tissue volumes.

This phase will include the 6 explanatory / independent variables:

1. The diameter of the mass (mm)
2. The healthy tissue density (using a Visual Analogue Scale (VAS))
3. The area occupied by the tumour (% of tissue sample)
4. Specimen weight (g)
5. Patient age
6. Patient BMI

The outcome / dependent variable will be the tissue volume, measured using the water displacement / Archimedes principle.

The regression model will be fitted using a bi-directional stepwise regression. Variables will be included if they make an important improvement in the model's fit. They will be excluded if, after other variables have been included in the model, they no longer make an important improvement in the model's fit. The model will stop fitting variables when both of the following conditions are met:

1. None of the variables that are not already included will make an important difference to the model's fit if they are included.
2. All of the variables that are included do make an important difference, so none of them should be removed.

The definition of "important" improvements in the model's fit and how they will be assessed will be described in detail in the Statistical Analysis Plan.

Study phase 2: Assessing the accuracy of the model obtained from phase 1.

This phase of the study will use a second collection of tissue samples, that have not been used to develop the model. It is needed because the regression model has been developed to predict the tissue volumes of the first sample as accurately as possible. It may not estimate other patients' volumes as accurately as these patients and we need to demonstrate that, even if the estimates for

other patients are less accurate than for the original sample of patients, they are still more accurate than the current method.

Study phase 2 primary outcome: Comparison of the new regression-based method and the current method with the gold standard measurement (using the Archimedes/water-based method). The estimates will be compared to the measured tissue volumes using two limits of agreement analyses (Bland and Altman [15]).

Study phase 2 secondary outcome 1: The differences between the two estimation methods and the gold standard tissue volumes will be compared using intraclass correlation. This will demonstrate how strongly related the two estimation methods are to each other. This is not a within-patient comparison but a comparison closely the results from the new estimation meths resemble the results from the current method.

Study phase 2 secondary outcome 2: The differences between the two estimation methods and the gold standard will be compared within-patient using a paired t-test. The mean difference in accuracy and its standard deviation will be reported.

### 10.4. Subgroup Analyses

As this is a proof-of-concept study, subgroup analyses will not be carried out.

### 10.5. Adjusted analyses

Not applicable

### 10.6. Interim Analysis and Criteria for the Premature Termination of the Study

The Sponsor may suspend or prematurely terminate either the entire study, or the study at an individual site, for significant reasons that must be documented (e.g. an unacceptable risk to participants or serious repeated deviations from the protocol/ regulations). If this occurs the Sponsor shall justify its decision in writing and will promptly inform any relevant parties (i.e. participants, investigators, participating sites, REC, regulatory bodies). Interim analyses are not planned for this study.

### 10.7. Analysis Groups

Patients with complete datasets will be included in the analyses for phase 1 and 2.

### 10.8. Procedure(s) to Account for Missing or Spurious Data

The tissue samples from patients with missing data will not be included in the regression model. Missing data will not be imputed. 110 patients with complete data will be required for phase 1 and 246 for phase 2.

## 11. MONITORING, AUDIT & INSPECTION

The Investigator(s) must ensure that source documents and other documentation for this study are made available to study monitors, the REC or regulatory authority inspectors. Authorised representatives of the Derby CTSU/ Sponsor may visit the participating sites to conduct audits/ inspections. Monitoring and source data verification will be conducted by the Sponsor according to their risk assessment. The extent and nature of monitoring will be determined by the study objectives, purpose, design, complexity, number of patients and sites, and endpoints. Source data and study documentation will be made available to any representatives of the Sponsor who wish to conduct audits or monitoring visits.

## 12. ETHICAL AND REGULATORY CONSIDERATIONS

### 12.1. Assessment and Management of Risk

Not applicable

### 12.2. Peer review

This study has been peer reviewed as part of the Derby and Burton Hospital Charity Small Research Grants Scheme application process.

### 12.3. Public and Patient Involvement

A Microsoft Teams meeting with three breast cancer patients of different background and age group was held to discuss the background/rationale of this research and its future objectives, as well as the proposed research method and estimated completion timescale. No changes to the application/lay abstract were deemed necessary. It was felt that, from a patient's perspective, discussing this research at the time of diagnosis would be irrelevant, as it does not alter the standard care and patients' focus would be on cancer treatment.

During this meeting, the importance of accurate radiotherapy planning has been unanimously acknowledged by all participants. In fact, both under- and overestimation of the boost target volume (and subsequent increased risk of local recurrence or impaired cosmetic outcome respectively) could have negative impact on patients' quality of life and require further treatment including surgery.

Further meetings with PPI members will be held following phase 1 and 2 to discuss the research findings and define the following steps of the research project.

### 12.4. Research Ethics Committee (REC) & Regulatory Considerations

The study will be conducted in compliance with the approved protocol and the Declaration of Helsinki. The protocol and all related documentation (e.g. informed consent form, participant information sheet, questionnaires) have been reviewed and received approval by a Research Ethics

Committee (REC). The investigator will not begin any participant activities until approval from the HRA and REC has been obtained and documented. All documentation and correspondence must be retained in the trial master file/investigator site file. Substantial amendments that require HRA and REC (where applicable) review will not be implemented until the HRA and REC grants a favourable opinion (with the exception of those necessary to reduce immediate risk to participants).

It is the responsibility of the CI to ensure that an annual progress report (APR) is submitted to the REC within 30 days of the anniversary date on which the favourable opinion was given, annually until the study is declared ended. The CI is also responsible for notifying the REC of the end of study (see Section 6.9) within 90 days. Within one year of the end of study, the Sponsor will submit a final report with the results, including any publications/abstracts to the REC.

Before any site can enroll a patient into the study confirmation of capacity must be sought from the site's research and development (R&D) department. In addition for any amendment that will potentially affect the site's permission, the research team must confirm with the site's R&D department that permission is ongoing (Section 11.10).

### 12.5. Protocol Compliance/ Non Compliance

The investigator is responsible for ensuring that the study is conducted in accordance with the procedures described in this protocol. Prospective, planned deviations and/or waivers to the protocol are not acceptable, however accidental protocol deviations (non-compliances) may happen and as such these must be recorded. Non-compliances should be recorded in the CRF and/or a non-compliance log kept in the ISF. All non-compliances should be reviewed and assessed by the PI (or appropriately delegated individual) to determine if they meet the criteria of a "serious breach" (Section 12.6). Non-compliances which are found to frequently recur are not acceptable, will require immediate action, and could potentially be classified as a serious breach.

### 12.6. Notification of Serious Breaches to GCP and/or the Protocol

A "serious breach" is a departure from the protocol, Sponsor procedures (i.e. SOPs), or regulatory requirements which is likely to effect to a significant degree –

(a) The safety or physical or mental integrity of the subjects of the study; or
(b) The scientific value of the study.

If the PI (or delegate) is unsure if a non-compliance meets these criteria, they should consult the Sponsor for further guidance.

If a serious breach is identified the investigator should notify the Sponsor immediately (i.e. within 1 working day) using the 'Non-CTIMP Notification of a Serious Breach' form. The report will be reviewed by the Sponsor and CI, and where appropriate, the Sponsor will notify the REC within 7 calendar days of being made aware of the breach

### 12.7. Data Protection and Patient Confidentiality

The study will be conducted in accordance with the Data Protection Act 2018. The investigator must ensure that participant's anonymity is maintained throughout the study and following completion of the study. Participants will be identified on all study specific documents only by the participants

study specific identifier (and initials if deemed necessary). This identifier will be recorded on documents, biological samples and the database. The investigator site file will hold an enrolment log detailing the study specific identifier alongside the names of all participants enrolled in the study. All documents will be stored securely with access restricted to study staff and authorised personnel.

The CI will act as the custodian of the data generated in the study.

### 12.8. Financial and Other Competing Interests for the Chief Investigator, Principal Investigators at Each Site and Committee Members for the Overall Study Management

No competing interests

### 12.9. Indemnity

As UHDB is acting as the research Sponsor for this study, NHS indemnity applies. NHS indemnity provides cover for legal liabilities where the NHS has a duty of care. Non-negligent harm is not covered by the NHS indemnity scheme. UHDB, therefore, cannot agree in advance to pay compensation in these circumstances. In exceptional circumstances an ex-gratia payment can be offered.

### 12.10. Amendments

If changes to the study are required these must be discussed with the Sponsor, who is responsible for deciding if an amendment is required and if it should be deemed substantial or non-substantial. Substantial amendments will be submitted to the relevant regulatory bodies (REC, HRA) for review and approval. The amendments will only be implemented after approval and a favourable opinion has been obtained. Non-substantial amendments will be submitted to the HRA for their approval/ acknowledgment. Amendments will not be implemented until all relevant approvals are in place.

### 12.11. Access to Final Study Dataset

Access to the trial datasets will be limited to the CI and to the trial statisticians. The datasets will be provided to the sponsor at the end of the trial for archiving purposes.

Access to all data at site will be restricted to personnel approved by the PI and recorded on a delegation log. Access will also be given to the sponsor and regulatory authorities.

## 13. DISSEMINATION POLICY

### 13.1. Dissemination Policy

The dissemination of the study results will be via a study report and research papers for publication in peer-reviewed journals, and presentation at relevant conferences.

### 13.2. Authorship Eligibility Guidelines and any Intended Use of Professional Writers

Authors and Contributors will be defined as per the International Committee of Medical Journal Editors (ICMJE) recommendations. The publication and authorship policy shall be agreed with the collaborators.

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# 15. APPENDICES

## 15.1. Appendix 1 – Schedule of Assessments

| Procedures | Baseline | Surgery admission | Month 1 post surgery |
|---|---|---|---|
| Demographics | X | | |
| Body Mass Index | X | | |
| Eligibility assessment | X | | |
| Specimen x-ray | | X | |
| Specimen weight | | X | |
| Specimen Volume (captured from histopathology report) | | | X |

## 15.2. Appendix 2 – Amendment History

| Amendment No. | Protocol version no. | Date issued | Author(s) of changes | Details of changes made |
|---|---|---|---|---|
| | | | | |