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Body composition and chemotherapy toxicity in women with early breast cancer (CANDO-3): Protocol for an observational cohort study

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TITLE PAGE

Title: **Body composition and chemotherapy toxicity in women with early breast cancer (CANDO-3): Protocol for an observational cohort study**

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ABSTRACT

INTRODUCTION: Systemic anti-cancer therapy is given to selected patients with early breast cancer (EBC) pre- or post-surgery with the aim of eradicating micrometastatic spread and reducing the risk of cancer recurrence. Chemotherapy treatment is most effective when patients receive the optimum dose, on time and without delays or reductions in their treatment doses. Most chemotherapy drugs are dosed according to body surface area calculated from a patient's height and weight. These calculations were however designed based on data from normal weight patients. This has resulted in uncertainty as to the optimal dosing for patients with different amounts of blood, muscle and fatty tissue (body composition). This study utilises segmental bioelectrical impedance analysis (sBIA; using the Seca mBCA 515) to determine whether differences in the measures of resistance and reactance, and derived estimates of body composition, are predictive of chemotherapy toxicity from in the treatment of EBC.

METHODS AND ANALYSIS: A prospective observational cohort study of women with EBC in whom adjuvant or neo-adjuvant chemotherapy is planned. A total of 300 participants will be recruited across seven UK hospital sites (~40/site). The primary outcome is to determine if higher fat mass index (FMi) is associated with increased National Cancer Institute, Common Terminology Criteria for Adverse Events (CTCAE) v5.0 grade 3 (or higher) chemotherapy toxicity.

ETHICS AND DISSEMINATION: This study has received ethical approval from the South Central Hampshire B Research Ethics Committee, England (19/SC/0596: IRAS: 263666). The Chief Investigator and Co-investigators will be responsible for publication of the study findings in a peer-reviewed journal, on behalf of all collaborators.

REGISTRATION: The study is registered with the ISRCTN (79577461).

ARTICLE SUMMARY

Strengths and limitations of this study

- Prospective study that explores the separate and combined relationships between fat mass (FM), fat free mass (FFM) and skeletal muscle mass (SMM) together with impedance measures, on chemotherapy toxicity
- Observational study designed to assess associations but not causation.
- BIA is a convenient, cost effective and quick method for looking at changes in raw values of resistance, reactance and Phase Angle, and derived estimates of FFM and FM.
- Measurements can be performed on the same days as routine clinic appointments/pre-chemotherapy blood tests meaning no extra visits for participants.
- BIA is non-invasive, does not involve radiation and can be measured on all patients with early breast cancer (EBC).

INTRODUCTION

Obesity is an established risk factor for development of post-menopausal breast cancer and a meta-analysis of over 100 case-control studies showed a 13% increased risk per 5 kg/m^2 [1]. Factors implicated include a direct effect of adipose tissue on levels of circulating hormones, such as insulin, insulin-like growth factors, and oestrogens, creating an environment that encourages carcinogenesis and discourages apoptosis, as well as stimulation of the body's inflammatory response [2]. In contrast, most studies in pre-menopausal women have concluded that obesity does not increase the risk of development of breast cancer; the mechanism for these phenomena remain unclear [3, 4].

A high body mass index (BMI) is also associated with reduced overall survival in breast cancer patients and this effect does not appear to be limited to post-menopausal women. A meta-analysis of 43 studies enrolling patients diagnosed between 1963-2005 showed obesity associated with poorer overall survival (HR 1.33) and breast cancer specific survival (HR 1.33) with a more marked effect in pre-menopausal women (HR 1.47) [5]. Ewertz *et al*. reported a significant increase in risk of developing distant metastases for patients with a BMI of 25 kg/m^2 or greater as compared with patients with a BMI less than 25 kg/m^2 (46% vs 42%) [6]. The Southampton based Prospective Study of Sporadic and Hereditary Breast Cancer in Young Women, (POSH), confirms that, in women aged 40 years and under at diagnosis, obese patients have significantly lower 8-year overall survival than healthy weight patients, (58.6% vs. 73.3%, p<0.001) [7].

The underlying mechanism is likely to be multi-factorial. Patients with a high BMI tend to present later with larger tumours due to their body habitus [6]. Some studies have also indicated an increased incidence of biologically adverse features, including oestrogen receptor (ER) negative tumours, in obese patients [8]. It is also possible that patients with a high BMI receive less effective treatment for EBC. Surgical complications occur with a higher incidence in obese patients, potentially delaying systemic therapies [9]. Ewertz *et al.* reported that both chemotherapy and endocrine therapy seemed to be less effective in patients with BMIs of 30 kg/m^2 or greater [6].

Chemotherapy dose reductions are more common in those with obesity [10], and whilst a meta-analysis showed no evidence for increased toxicity in obesity it was unclear if there was confounding by poorly specified dose capping and use of growth factors [11]. In a more recent study higher rates of toxicity were seen in obese compared to healthy weight patients when receiving dose-dense chemotherapy [12]. A review by the American Society of Clinical Oncology, (ASCO) concluded that up to 40% of obese cancer patients currently receive limited chemotherapy doses that are not based on actual weight, and hypothesised that this may explain the higher cancer mortality rates observed in overweight and obese individuals [13]. The ASCO review found no strong evidence that short or long-term toxicity is increased among obese women receiving full-weight based chemotherapy doses and has therefore recommended the use of full weight-based cytotoxic chemotherapy doses in obese patients in the curative and adjuvant setting. However, data were limited and generally limited to first cycle doses and /or haematological toxicity. Their panel therefore recommended further research to guide appropriate dosing of obese patients with cancer. Our own analysis of chemotherapy prescription records for the 77 POSH participants who were treated with adjuvant chemotherapy at the Southampton Oncology Centre indicates that obese patients did not routinely receive capped chemotherapy doses but were significantly more likely to receive one or more dose delay due to toxicity (33.3% vs. 5.9%, p=0.0068) than normal weight patients [7].

Most chemotherapy drugs are dosed according to predicted body surface area (BSA). Patients with a similar BSA or BMI may have wide variations in amount and distribution of adipose tissue and skeletal muscle (body composition). Whilst there may be an association between BMI and both fat mass (FM) and fat free mass (FFM), there is at least a two-fold variability in both FM and FFM for any given BMI or BSA over the range of values usually seen in women with breast cancer [14].

There is accumulating evidence that differences in body composition are important determinants of chemotherapy outcomes. Using estimates of body composition derived from single-slice CT (Computerised Tomography) scans obtained in routine clinical care, Baracos and colleagues have repeatedly demonstrated that a lack of lean tissue, and skeletal muscle mass is associated with chemotherapy toxicity across a number of differing types of cancers most typically associated with a low BMI, wasting or cachexia [15], and a meta-analysis of two studies suggested that sarcopenia predicts chemotoxicity in patients with metastatic breast cancer [16]. It has been proposed that lean body mass may in some instances be a better predictor of drug dosage possibly since it is a predictor of volume of distribution for some drugs and correlates with liver volume and blood flow [17]. In contrast to the relatively large number of studies in wasting conditions, few studies have looked at the effect of body composition on chemotherapy tolerance in women with EBC. Two small retrospective series using body composition data derived from computerised tomography (CT) images indicate that sarcopenia (low muscle mass) is associated with increased chemotherapy toxicity, regardless of overall BMI [18, 19]. Del Fabro *et al*. recently reported that overweight patients had a lower pCR rate to neo-adjuvant chemotherapy for EBC, and found that in normal BMI patients with sarcopenia the pCR rate was higher [20]. Whilst the lack of lean tissue appears to be an important determinant of toxicity, this study also demonstrated that sarcopenic patients who were overweight or obese had an inferior outcome than sarcopenic normal weight patients.

Whilst much work has focussed on lack of lean mass being associated with treatment-related toxicities, relatively little attention has been directed towards determining the association with increased fat mass. Van den Berg and colleagues assessed body composition by Dual-energy X-ray Absorptiometry (DXA) scan in women with EBC receiving chemotherapy and observed a higher absolute and higher relative fat mass was associated with an increased risk of toxicity-induced modifications of treatment, but not absolute lean mass, with the greatest risk observed in those with high fat and low lean mass [21]. Prado and colleagues investigated the associations between body composition derived from single slice CT scans and chemotherapy toxicity in advanced ovarian cancer. They reported that a clear association between both FM and lean body mass (primarily driven by FM) emerged, in explaining toxicity. This association was only noted in individuals with excess body weight, with a lower ratio predicting higher exposure and risk for toxicity [22]. Shachar and colleagues using single slice CT image analysis found that low skeletal muscle area and muscle attenuation was associated with increased chemotherapy treatment-related toxicities but found no association with subcutaneous or visceral adipose tissue area [23].

These observations highlight the need to better understand the impact of differences in body composition on chemotherapy prescribing and toxicity in women with EBC. What remains unclear is whether the increased chemotoxicity associated with higher BMI in women with breast cancer is associated with a lack of FFM (sarcopenia), greater FM (excess adiposity), or some interaction between the two. In contrast to the work of Baracos' group, it is not possible to assess body composition from single-slice CT images in most women with EBC as the majority of patients would not undergo staging

investigations for distant metastatic disease in the absence of symptoms [24] and so these images would not be routinely available. Moreover, the clinical utility of determining body composition from single slice CT images has been challenged after poor accuracy and precision was demonstrated in comparison to that found with DXA [25]. Whilst it is possible to use DXA to derive estimates of body composition such measures are not readily utilised in oncology patients in routine clinical practice and it cannot be used repeatedly over time to follow the changes in body composition due to constraints of radiation exposure. This is possible with segmental bioelectrical impedance analysis (sBIA), which will be used in this study to determine whether differences in the measures of resistance and reactance, and derived estimates of body composition, are predictive of chemotherapy toxicity in the treatment of breast cancer.

RATIONALE

All of the methods used to measure body composition in patients with and receiving treatment for breast cancer have technical and practical limitations, most notably associated with the assumptions that underlie the prediction of body composition, or their accessibility for application in routine clinical care [6]. BIA is readily accessible and measures the biophysical properties of the body on terms of resistance and reactance from which estimates of total body water, either at the whole body level or with sBIA at the level of individual limbs [7, 8]. Resistance is largely determined by the distance the current has to travel (e.g. hence the practice of expressing values adjusted for height) and the ability of the current to pass through tissue which is largely dependent of water content. Reactance is directly measured and principally reflects the capacitance of tissue and the integrity of the cells through which the current flows. Phase Angle is a summative statement that reflects the relationship between resistance and reactance at different frequencies. BIA is not a direct assessment of body composition and its utility as an indicator of body composition relies on the relationship between measures of resistance and reactance and total body water and the generation of regression models against other methods of body composition, preferably multicomponent modelling and/or whole body MRI. These regression equations are both device and population specific and dependent on several key assumptions including homogenous composition, fixed cross-sectional area, consistent distribution of current density and constant tissue hydration. The raw measured values of resistance and reactance and the derived parameter of Phase Angle are not affected by the factors that affect or assumptions used in the estimation of body composition and have both excellent accuracy and precision. Where device-specific reference ranges of healthy individuals are available, both the measured impedance values, Phase Angle and derived estimates of body composition can be standardised and expressed as Standard Deviation Scores (or Z scores) adjusted for age, gender and BMI.

The 2021 ASCO guideline update on appropriate systemic therapy dosing for obese adult patients with cancer [26] recommends prospective studies to “explore the role of body composition in predicting dose limiting toxicities”. This study design directly addresses that recommendation and we have previously demonstrated the feasibility and acceptability of incorporation of sBIA measurements into routine clinical care using a phase-sensitive sBIA instrument (Seca mBCA 515) in a pilot study (CANDO-2) which provided preliminary data on the correlation between CT and BIA, and on changes in impedance measures during systemic chemotherapy [27]. This particular device has been validated extensively and shown to yield directly comparable measures of body composition obtained by both four compartment models and whole body MRI in healthy cohorts [28, 29]. The central proposition

that underlies this program of work is that it is possible to use sBIA to mark differences between women with EBC in their resilience to chemotherapy. This may be related either to differences in relative chemotherapy dosing arising from differing amounts and proportions of lean and fat tissue and/or differences in physiological and metabolic state relating to both structure (amounts and proportions of lean and fat) and the quality of tissue (reflected in the bioelectrical properties of the body). The study is designed with broad inclusion criteria, minimal exclusions and is multicentre in nature with a view to minimising bias by recruitment of a representative population of EBC patients receiving chemotherapy.

HYPOTHESIS:

Differences in bioelectrical impedance measures of resistance, reactance and phase angle and/or derived estimates of elevated FMi and low Fat FFMi (Fat Free Mass index) are individually and jointly predictive of chemotherapy toxicity.

OBJECTIVES:

Primary objective:

- To determine if higher FMi is associated with increased Common Terminology Criteria for Adverse Events (CTCAE, National Cancer Institute; v5.0) grade 3 or higher chemotherapy toxicity [30] in women receiving neo/adjuvant chemotherapy for EBC.

Secondary objectives:

- To determine the prognostic potential of sBIA derived body composition measures including FFMi in predicting grade 3 or higher chemotherapy toxicity.
- To determine whether EBC patients with different body composition patterns:
  i) Are planned to receive the same neo/adjuvant chemotherapy dose intensity
  ii) Receive the planned neo/adjuvant chemotherapy dose intensity.
- To determine the relationship between sBIA-measured physical properties e.g. phase angle and reactance and grade 3 or higher chemotherapy toxicity.
- To determine in exploratory analyses the association of body composition measures across all grades of chemotherapy toxicities.
- In an exploratory optional sub-study (Southampton only) determine if functional markers of physical fitness (e.g. Cardiopulmonary Exercise Testing; CPET) provide additional information over and above structural measures of body composition including DXA.
- To determine the concurrent validity of sBIA, with (where available) DXA and where performed as part of routine clinical care, CT approaches to determining body composition at baseline in these women.

SUMMARY OF METHODS AND ANALYSIS

Study Design

A multicentre observational (non-interventional) investigator-led academic prospective cohort study. University Hospital Southampton NHS Foundation Trust is study sponsor and a recruiting site. The cohort will consist of a minimum of 300 women referred to breast oncology clinics with a diagnosis of early invasive breast cancer and recommended to receive neo/adjuvant chemotherapy. The study is currently planned to run for 18 months (23 July 2020 until 30 November 2022). This will likely be

extended, as permitted by available funding, to compensate for interruptions caused by the COVID-19 pandemic.

The study's inclusion and exclusion criteria are listed in table 1.

Table 1. Inclusion and exclusion criteria

Inclusion Criteria	Exclusion Criteria
• Early invasive breast carcinoma • Stage I-III disease • Tumour grade, ER and HER 2 status available • Clinical or pathological tumour size and lymph node status available • Neo-adjuvant or adjuvant systemic chemotherapy recommended by local breast multi-disciplinary meeting • No prior systemic anti-cancer treatment • No evidence of distant metastatic disease • Patient agrees to receive neo/adjuvant chemotherapy • Planned to receive 4-8, 21 day cycles of anthracycline or taxane based combination chemotherapy (*patients receiving anti-HER 2 agents in combination with 21 day taxanes are eligible*) • Aged≥ 18 years and< 80 years • Female • Able to complete written records in English	• Previous invasive malignancy (with the exception of non-melanomatous skin cancer) • Any other medical conditions preventing physical participation in the study procedures • Patients receiving single agent or weekly neo/adjuvant chemotherapy regimens e.g. weekly paclitaxel with trastuzumab • Patients with existing conditions known to affect body water or cause oedema or muscle conditions that may affect muscle mass such as muscular dystrophies. • Pregnancy

Table 1*. Inclusion and exclusion criteria for the CANDO-3 study*

Approximately 40 patients will be recruited over an 18 month period from each of seven UK hospital sites (The Christie Hospital, Manchester; Southampton General Hospital, Southampton; Churchill Hospital, Oxford; Royal Hampshire County Hospital, Winchester; Royal Devon and Exeter Hospital, Exeter; Salisbury District Hospital, Salisbury; and Queen Alexandra Hospital, Portsmouth). The patients will be recruited to the study from Breast Cancer Clinics and Breast Oncology Clinics, screened for eligibility through multidisciplinary meetings and introduced to the study when recommended to receive chemotherapy.

Weight, resistance, reactance and Phase Angle together with derived estimates of FM, FFM and SMM will be obtained using the Seca mBCA 515 and its associated Seca 115 software, at all study centres at each study visit, together with measures of maximal voluntary contraction using a hydraulic hand dynamometer. Impedance and body composition variables will be expressed as absolute values, adjusted for height, and standardised as Z scores against an age, gender and BMI device-specific reference population [31]. Participants will also be asked to complete quality of life and lifestyle (EORTC QLQ-C30 and EORTC QLQ-BR23 [32], physical activity (IPAQ-SF) [33], appetite and eating habits (CNAQ) [34] and alcohol use (AUDIT-C) [35] questionnaires at baseline and three other study visits. Routine clinical data will be obtained from electronic patient records and include a height measurement as recorded at the first clinic visit, blood test results, patient and tumour characteristics,

co-morbidities, cancer treatments and progress through treatment pathway. Chemotherapy toxicity will be recorded at each clinic visit according to the CTCAE v5.0, (National Cancer Institute), together with full details of chemotherapy, including any dose adjustments, delays or changes to the treatment regimen, to determine chemotherapy delivery (of planned dose density).

Staging and radiology planning CT scan images obtained in routine clinical care, where available prior to visit 1, will be accessed directly from the hospital picture archiving and communication system (PACS) department and link-anonymised using a unique patient study identification number. Images from external centres will be sent using standard NHS image transfer processes between hospitals to the Southampton PACS department. SliceOmatic (v5.0 Rev-7, TomoVision, Magog, Canada) software will be used to determine tissue cross-sectional areas and attenuation according to the modified Alberta protocol (Alberta protocol, TomoVision, Magog, Canada).

In an optional sub-study, patients in Southampton will be invited to undertake further body composition analysis by DXA to determine FM, FFM and appendicular FFM, and to donate one extra 10ml blood sample which will be biobanked for future analysis and research.

The study schedule is shown in table 2.

Table 2. Study schedule

Visit	V0	V1	V2	V3	V4	V5	V6	V7*	V8**	End of chemo	End of study
Description of visit	Breast clinic	Chemo clinic 1	Chemo clinic 2	Chemo clinic 3	Chemo clinic 4	Chemo clinic 5	Chemo clinic 6	Chemo clinic 7	Chemo clinic 8	Final chemo clinic	Follow up visit
Relationship to chemo cycles	~ 2 weeks prior	Up to 7 days prior cycle 1	Within the 3 days prior cycle 2	Within the 3 days prior cycle 3	Within the 3 days prior cycle 4	Within the 3 days prior cycle 5	Within the 3 days prior cycle 6	Within the 3 days prior cycle 7	Within the 3 days prior cycle 8	3 weeks post final cycle +/- 7 days	3 months post final cycle +/- 14 days
Patient information sheet	X										
Recruitment		X									
Consent		X									
Grip strength		X	X	X	X	X	X	X	X	X	X
sBIA***		X	X	X	X	X	X	X	X	X	X
CT scan†		X									
Chemotoxicity assessments			X	X	X	X	X	X	X	X	X
Questionnaires: • EORTC QLQ-C30		X			X					X	X
• EORTC QLQ-BR23		X			X					X	X
• IPAQ-SF		X			X					X	X
• CNAQ		X			X					X	X
• AUDIT-C		X									

Table 2. Study schedule

†Baseline staging CT scan would usually be at some point prior to chemotherapy, although timing can be variable but on entry to the study the patient will consent to use of the data for body composition analysis.

*only required if >6 cycles of chemotherapy planned; **only required if > 7 cycles of chemotherapy planned
Please name visits to correspond to the chemotherapy cycle that follows that visit: i.e. visit 1 = pre-chemotherapy cycle 1; visit 2 = pre-chemotherapy cycle 2, etc.

*** weight, impedance and derived estimates of body composition by sBIA

Sample size

A University of Southampton, University Hospital Southampton NHS Foundation Trust-sponsored pilot study (CANDO-2) found that study mean FM corrected for differences in height (fat mass index; FMi) was 11.30 (SD 4.93) kg/m^2 in patients without toxicity (grade 3 or above) and 13.31 (difference of 2.01) in patients with a grade 3 or above toxicity [22]. Based on simulations, a difference of this magnitude would lead to an area under the curve (AUC; as a measure of discrimination) of over 0.6 based on FMi alone, suggesting at least modest discriminative ability of FMi for predicting toxicity. A sample of size 256 would ensure 90% power for a two-group comparison on FMi (with alpha of 5%), and inflating this to 300 maintains this level of power when the groups are unbalanced in size, up to 30-70 in favour of non-toxicity (where such a split has been in related literature) [23]. As such, a statistically significant result would be an indication that FMi shows predictive ability that would be worth exploring in further research since this magnitude of FMi difference is likely to be clinically significant if proven.

Statistical analysis plan

For the primary objective analyses, descriptive statistics will be used to describe the FMi in patients with versus without a grade 3 or above chemotherapy toxicity, and a formal comparison carried out using a t-Test in order determine if the mean FMi is different between groups. Additional pre-specified primary objective analyses will include fitting a logistic regression model to analyse the FMi data according to grade 3 or above toxicity outcome, adjusting for potential confounding variables e.g. BMI. AUCs will also be produced to determine the predictive ability of FMi. Unadjusted and adjusted relative risks (RR) and corresponding 95% confidence intervals (CIs), derived using Poisson regression models, will also be reported for associations between FMi and grade 3 or above toxicity outcome. Trial measurements will be made at routine clinic visits and there is no ongoing follow-up so it is anticipated that there will be minimal missing data.

Data management plan, data collection and ALEA eCRF®

A data management plan for the CANDO-3 study has been written and approved. Linked anonymised data will be kept on a secure and trusted validated ALEA eCRF® (FormsVision BV, Netherlands), certified by registered auditors to be in compliance with regulations such as the FDA's CFR 21 Part 11 and ICH GCP. FormsVision BV support University Hospital Southampton NHS Foundation Trust and many other organisations, including the University of Cambridge, Leicester and Bristol, and the World Health Organisation, with clinical trials data management.

All anonymized clinical, prescribing and toxicity data, and results of the DXA and CT image analysis will be recorded on paper CRFs and centrally entered into a common dataset in accordance with local GCP and Data Protection governance requirements. The BIA data will be recorded electronically at source and uploaded electronically into the common dataset.

Data will be cleaned and dataset secured under guidance and management by the University of Southampton Clinical Informatics Research Unit Data Management team prior to analyses. The dataset will then be analysed in accordance with the Statistical Analysis Plan under the direction of statistical analysis team, PI and co-applicants.

PATIENT AND PUBLIC INVOLVEMENT

A patient advocate (LT) is involved in the design, management and undertaking of this study as a member of the study steering group.

This protocol has been developed with advice from the patient advocate who has extensive experience both with Independent Cancer Patients' Voice (ICPV) and on a number of breast cancer clinical trials. The patient advocate is a member of the Breast Cancer Now tissue bank management board, the NCRI Breast Clinical Studies Group and is the lead patient representative for the NIHR Cancer and Nutrition Collaboration. Additional areas of involvement include: review of study documentation; attendance at Trial Steering Group meetings and ongoing advice, guidance and patient perspective both at these meetings and as required on an ongoing basis; providing guidance on the use and content of study newsletters; and advise on materials and methods to disseminate the final study outcomes.

ETHICS AND DISSEMINATION

This is an observational cohort study and patient clinical management will not change as a result of the study. The study was approved on 30/01/2020 by the Hampshire B Research Ethics Committee (Level 3 Block B, Whitefriars, Lewins Mead, Bristol, BS1 2NT; Ref: 19/SC/0596. IRAS: 263666)

The study is listed on the ISRCTN registry (ISRCTN: 79577461).

The Chief Investigator and Co-investigators will be responsible for publication of the study findings in a peer-reviewed scientific journal, on behalf of all collaborators. The manuscript will be prepared by a writing group, and participating investigators will be selected to join the writing group on the basis of contribution and following standard protocols for authorship.

Anonymous data will be available for request from three months after publication of the primary endpoint manuscript until the end of the archive and storage period. It will be available to researchers who provide a completed Data Sharing request form that describes a methodologically sound proposal, for the purpose of the approved proposal and if appropriate, signed a Data Sharing Agreement. Proposals will be reviewed for approval by the study steering committee. Data for approved applications will be shared once all parties have signed relevant data sharing documentation, covering the study steering committee conditions for sharing and if required, an additional Data Sharing Agreement from Sponsor. Proposals should be directed to the Chief Investigator.

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AUTHOR CONTRIBUTIONS

Ramsey I Cutress: Chief Investigator for study funding application. Study conceptualisation, manuscript and protocol writing

Ellen Copson and Stephen Wootton: Co-investigators for study funding application. Study conceptualisation, manuscript and protocol writing

Kesta Durkin: Manuscript and protocol writing. Manuscript review and editing

Adam Heetun: Manuscript review

Sean Ewings: Power calculations, statistics and manuscript review

Richard Munday: Study clinical informatics and manuscript review

Lesley Turner: Patient advocate, manuscript review

CANDO-3 steering Group: Manuscript review and approval

COMPETING INTERESTS

SAW holds an investigator-led Collaborative Research Agreement between Seca Medical Measuring Systems and Scales and the University of Southampton/University Hospital Southampton NHS Foundation Trust. Seca played no part in the design, conduct or interpretation of the work reported in this publication.

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STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3-5
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6-7
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	6-7
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	N/A
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6-7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	7
Bias	9	Describe any efforts to address potential sources of bias	5
Study size	10	Explain how the study size was arrived at	8
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	N/A protocol paper
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	8
		(*b*) Describe any methods used to examine subgroups and interactions	8
		(*c*) Explain how missing data were addressed	8
		(*d*) If applicable, explain how loss to follow-up was addressed	N/A
		(*e*) Describe any sensitivity analyses	N/A
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	N/A
		(b) Give reasons for non-participation at each stage	N/A
		(c) Consider use of a flow diagram	N/A
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	N/A
		(b) Indicate number of participants with missing data for each variable of interest	N/A
		(c) Summarise follow-up time (eg, average and total amount)	N/A
Outcome data	15*	Report numbers of outcome events or summary measures over time	N/A
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	N/A
		(*b*) Report category boundaries when continuous variables were categorized	N/A
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	N/A
Discussion			N/A
Key results	18	Summarise key results with reference to study objectives	N/A
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	N/a
Generalisability	21	Discuss the generalisability (external validity) of the study results	2-5
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	9

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Body composition and chemotherapy toxicity in women with early breast cancer (CANDO-3): Protocol for an observational cohort study

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TITLE PAGE

Title: **Body composition and chemotherapy toxicity in women with early breast cancer (CANDO-3): Protocol for an observational cohort study**

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Keywords: Body composition, breast cancer, chemotherapy toxicity, bioelectrical impedance analysis, obesity

ABSTRACT

INTRODUCTION: Systemic anti-cancer therapy is given to selected patients with early breast cancer (EBC) pre- or post-surgery with the aim of eradicating micrometastatic spread and reducing the risk of cancer recurrence. Chemotherapy treatment is most effective when patients receive the optimum dose, on time and without delays or reductions in their treatment doses. Most chemotherapy drugs are dosed according to body surface area calculated from a patient's height and weight. These calculations were however designed based on data from normal weight patients. This has resulted in uncertainty as to the optimal dosing for patients with different amounts of blood, muscle and fatty tissue (body composition). This study utilises segmental bioelectrical impedance analysis (sBIA; using the Seca mBCA 515) to determine whether differences in the measures of resistance and reactance, and derived estimates of body composition, are predictive of chemotherapy toxicity from in the treatment of EBC.

METHODS AND ANALYSIS: A prospective observational cohort study of women with EBC in whom adjuvant or neo-adjuvant chemotherapy is planned. A total of 300 participants will be recruited across seven UK hospital sites (~40/site). The primary outcome is to determine if higher fat mass index (FMi) is associated with increased National Cancer Institute, Common Terminology Criteria for Adverse Events (CTCAE) v5.0 grade 3 (or higher) chemotherapy toxicity.

ETHICS AND DISSEMINATION: This study has received ethical approval from the South Central Hampshire B Research Ethics Committee, England (19/SC/0596: IRAS: 263666). The Chief Investigator and Co-investigators will be responsible for publication of the study findings in a peer-reviewed journal, on behalf of all collaborators.

REGISTRATION: The study is registered with the ISRCTN (79577461).

ARTICLE SUMMARY

Strengths and limitations of this study

- Prospective study that explores the separate and combined relationships between fat mass (FM), fat free mass (FFM) and skeletal muscle mass (SMM) together with impedance measures, on chemotherapy toxicity
- Observational study designed to assess associations but not causation.
- BIA is a convenient, cost effective and quick method for looking at changes in raw values of resistance, reactance and Phase Angle, and derived estimates of FFM and FM.
- Measurements can be performed on the same days as routine clinic appointments/pre-chemotherapy blood tests meaning no extra visits for participants.
- BIA is non-invasive, does not involve radiation and can be measured on all patients with early breast cancer (EBC).

INTRODUCTION

Obesity is an established risk factor for development of post-menopausal breast cancer and a meta-analysis of over 100 case-control studies showed a 13% increased risk per 5 kg/m^2 [1]. Factors implicated include a direct effect of adipose tissue on levels of circulating hormones, such as insulin, insulin-like growth factors, and oestrogens, creating an environment that encourages carcinogenesis and discourages apoptosis, as well as stimulation of the body's inflammatory response [2]. In contrast, most studies in pre-menopausal women have concluded that obesity does not increase the risk of development of breast cancer; the mechanism for these phenomena remain unclear [3, 4].

A high body mass index (BMI) is also associated with reduced overall survival in breast cancer patients and this effect does not appear to be limited to post-menopausal women. A meta-analysis of 43 studies enrolling patients diagnosed between 1963-2005 showed obesity associated with poorer overall survival (HR 1.33) and breast cancer specific survival (HR 1.33) with a more marked effect in pre-menopausal women (HR 1.47) [5]. Ewertz *et al*. reported a significant increase in risk of developing distant metastases for patients with a BMI of 25 kg/m^2 or greater as compared with patients with a BMI less than 25 kg/m^2 (46% vs 42%) [6]. The Southampton based Prospective Study of Sporadic and Hereditary Breast Cancer in Young Women, (POSH), confirms that, in women aged 40 years and under at diagnosis, obese patients have significantly lower 8-year overall survival than healthy weight patients, (58.6% vs. 73.3%, p<0.001) [7].

The underlying mechanism is likely to be multi-factorial. Patients with a high BMI tend to present later with larger tumours due to their body habitus [6]. Some studies have also indicated an increased incidence of biologically adverse features, including oestrogen receptor (ER) negative tumours, in obese patients [8]. It is also possible that patients with a high BMI receive less effective treatment for EBC. Surgical complications occur with a higher incidence in obese patients, potentially delaying systemic therapies [9]. Ewertz *et al.* reported that both chemotherapy and endocrine therapy seemed to be less effective in patients with BMIs of 30 kg/m^2 or greater [6].

Chemotherapy dose reductions are more common in those with obesity [10], and whilst a meta-analysis showed no evidence for increased toxicity in obesity it was unclear if there was confounding by poorly specified dose capping and use of growth factors [11]. In a more recent study higher rates of toxicity were seen in obese compared to healthy weight patients when receiving dose-dense chemotherapy [12]. A review by the American Society of Clinical Oncology, (ASCO) concluded that up to 40% of obese cancer patients currently receive limited chemotherapy doses that are not based on actual weight, and hypothesised that this may explain the higher cancer mortality rates observed in overweight and obese individuals [13]. The ASCO review found no strong evidence that short or long-term toxicity is increased among obese women receiving full-weight based chemotherapy doses and has therefore recommended the use of full weight-based cytotoxic chemotherapy doses in obese patients in the curative and adjuvant setting. However, data were limited and generally limited to first cycle doses and /or haematological toxicity. Their panel therefore recommended further research to guide appropriate dosing of obese patients with cancer. Our own analysis of chemotherapy prescription records for the 77 POSH participants who were treated with adjuvant chemotherapy at the Southampton Oncology Centre indicates that obese patients did not routinely receive capped chemotherapy doses but were significantly more likely to receive one or more dose delay due to toxicity (33.3% vs. 5.9%, p=0.0068) than normal weight patients [7].

Most chemotherapy drugs are dosed according to predicted body surface area (BSA). Patients with a similar BSA or BMI may have wide variations in amount and distribution of adipose tissue and skeletal muscle (body composition). Whilst there may be an association between BMI and both fat mass (FM) and fat free mass (FFM), there is at least a two-fold variability in both FM and FFM for any given BMI or BSA over the range of values usually seen in women with breast cancer [14].

There is accumulating evidence that differences in body composition are important determinants of chemotherapy outcomes. Using estimates of body composition derived from single-slice CT (Computerised Tomography) scans obtained in routine clinical care, Baracos and colleagues have repeatedly demonstrated that a lack of lean tissue, and skeletal muscle mass is associated with chemotherapy toxicity across a number of differing types of cancers most typically associated with a low BMI, wasting or cachexia [15], and a meta-analysis of two studies suggested that sarcopenia predicts chemotoxicity in patients with metastatic breast cancer [16]. It has been proposed that lean body mass may in some instances be a better predictor of drug dosage possibly since it is a predictor of volume of distribution for some drugs and correlates with liver volume and blood flow [17]. In contrast to the relatively large number of studies in wasting conditions, few studies have looked at the effect of body composition on chemotherapy tolerance in women with EBC. Two small retrospective series using body composition data derived from computerised tomography (CT) images indicate that sarcopenia (low muscle mass) is associated with increased chemotherapy toxicity, regardless of overall BMI [18, 19]. Del Fabro *et al*. recently reported that overweight patients had a lower pCR rate to neo-adjuvant chemotherapy for EBC, and found that in normal BMI patients with sarcopenia the pCR rate was higher [20]. Whilst the lack of lean tissue appears to be an important determinant of toxicity, this study also demonstrated that sarcopenic patients who were overweight or obese had an inferior outcome than sarcopenic normal weight patients.

Whilst much work has focussed on lack of lean mass being associated with treatment-related toxicities, relatively little attention has been directed towards determining the association with increased fat mass. Van den Berg and colleagues assessed body composition by Dual-energy X-ray Absorptiometry (DXA) scan in women with EBC receiving chemotherapy and observed a higher absolute and higher relative fat mass was associated with an increased risk of toxicity-induced modifications of treatment, but not absolute lean mass, with the greatest risk observed in those with high fat and low lean mass [21]. Prado and colleagues investigated the associations between body composition derived from single slice CT scans and chemotherapy toxicity in advanced ovarian cancer. They reported that a clear association between both FM and lean body mass (primarily driven by FM) emerged, in explaining toxicity. This association was only noted in individuals with excess body weight, with a lower ratio predicting higher exposure and risk for toxicity [22]. Shachar and colleagues using single slice CT image analysis found that low skeletal muscle area and muscle attenuation was associated with increased chemotherapy treatment-related toxicities but found no association with subcutaneous or visceral adipose tissue area [23].

These observations highlight the need to better understand the impact of differences in body composition on chemotherapy prescribing and toxicity in women with EBC. What remains unclear is whether the increased chemotoxicity associated with higher BMI in women with breast cancer is associated with a lack of FFM (sarcopenia), greater FM (excess adiposity), or some interaction between the two. In contrast to the work of Baracos' group, it is not possible to assess body composition from single-slice CT images in most women with EBC as the majority of patients would not undergo staging

investigations for distant metastatic disease in the absence of symptoms [24] and so these images would not be routinely available. Moreover, the clinical utility of determining body composition from single slice CT images has been challenged after poor accuracy and precision was demonstrated in comparison to that found with DXA [25]. Whilst it is possible to use DXA to derive estimates of body composition such measures are not readily utilised in oncology patients in routine clinical practice and it cannot be used repeatedly over time to follow the changes in body composition due to constraints of radiation exposure. This is possible with segmental bioelectrical impedance analysis (sBIA), which will be used in this study to determine whether differences in the measures of resistance and reactance, and derived estimates of body composition, are predictive of chemotherapy toxicity in the treatment of breast cancer.

RATIONALE

All of the methods used to measure body composition in patients with and receiving treatment for breast cancer have technical and practical limitations, most notably associated with the assumptions that underlie the prediction of body composition, or their accessibility for application in routine clinical care [6]. BIA is readily accessible and measures the biophysical properties of the body on terms of resistance and reactance from which estimates of total body water, either at the whole body level or with sBIA at the level of individual limbs [7, 8]. Resistance is largely determined by the distance the current has to travel (e.g. hence the practice of expressing values adjusted for height) and the ability of the current to pass through tissue which is largely dependent of water content. Reactance is directly measured and principally reflects the capacitance of tissue and the integrity of the cells through which the current flows. Phase Angle is a summative statement that reflects the relationship between resistance and reactance at different frequencies. BIA is not a direct assessment of body composition and its utility as an indicator of body composition relies on the relationship between measures of resistance and reactance and total body water and the generation of regression models against other methods of body composition, preferably multicomponent modelling and/or whole body MRI. These regression equations are both device and population specific and dependent on several key assumptions including homogenous composition, fixed cross-sectional area, consistent distribution of current density and constant tissue hydration. The raw measured values of resistance and reactance and the derived parameter of Phase Angle are not affected by the factors that affect or assumptions used in the estimation of body composition and have both excellent accuracy and precision. Where device-specific reference ranges of healthy individuals are available, both the measured impedance values, Phase Angle and derived estimates of body composition can be standardised and expressed as Standard Deviation Scores (or Z scores) adjusted for age, gender and BMI.

The 2021 ASCO guideline update on appropriate systemic therapy dosing for obese adult patients with cancer [26] recommends prospective studies to “explore the role of body composition in predicting dose limiting toxicities”. This study design directly addresses that recommendation and we have previously demonstrated the feasibility and acceptability of incorporation of sBIA measurements into routine clinical care using a phase-sensitive sBIA instrument (Seca mBCA 515) in a pilot study (CANDO-2) which provided preliminary data on the correlation between CT and BIA, and on changes in impedance measures during systemic chemotherapy [27]. This particular device has been validated extensively and shown to yield directly comparable measures of body composition obtained by both four compartment models and whole body MRI in healthy cohorts [28, 29]. The central proposition

that underlies this program of work is that it is possible to use sBIA to mark differences between women with EBC in their resilience to chemotherapy. This may be related either to differences in relative chemotherapy dosing arising from differing amounts and proportions of lean and fat tissue and/or differences in physiological and metabolic state relating to both structure (amounts and proportions of lean and fat) and the quality of tissue (reflected in the bioelectrical properties of the body). The study is designed with broad inclusion criteria, minimal exclusions and is multicentre in nature with a view to minimising bias by recruitment of a representative population of EBC patients receiving chemotherapy.

HYPOTHESIS:

Differences in bioelectrical impedance measures of resistance, reactance and phase angle and/or derived estimates of elevated FMi and low Fat FFMi (Fat Free Mass index) are individually and jointly predictive of chemotherapy toxicity.

OBJECTIVES:

Primary objective:

- To determine if higher FMi is associated with increased Common Terminology Criteria for Adverse Events (CTCAE, National Cancer Institute; v5.0) grade 3 or higher chemotherapy toxicity [30] in women receiving neo/adjuvant chemotherapy for EBC.

Secondary objectives:

- To determine the prognostic potential of sBIA derived body composition measures including FFMi in predicting grade 3 or higher chemotherapy toxicity.
- To determine whether EBC patients with different body composition patterns:
  i) Are planned to receive the same neo/adjuvant chemotherapy dose intensity
  ii) Receive the planned neo/adjuvant chemotherapy dose intensity.
- To determine the relationship between sBIA-measured physical properties e.g. phase angle and reactance and grade 3 or higher chemotherapy toxicity.
- To determine in exploratory analyses the association of body composition measures across all grades of chemotherapy toxicities.
- To determine the concurrent validity of sBIA, with (where available) DXA and where performed as part of routine clinical care, CT approaches to determining body composition at baseline in these women.

SUMMARY OF METHODS AND ANALYSIS

Study Design

A multicentre observational (non-interventional) investigator-led academic prospective cohort study. University Hospital Southampton NHS Foundation Trust is study sponsor and a recruiting site. The cohort will consist of a minimum of 300 women referred to breast oncology clinics with a diagnosis of early invasive breast cancer and recommended to receive neo/adjuvant chemotherapy. The study opened on 16th March 2020 and is currently planned to run until (30th June 2023)..

The study's inclusion and exclusion criteria are listed in table 1.

Table 1. Inclusion and exclusion criteria

Inclusion Criteria	Exclusion Criteria
• Early invasive breast carcinoma • Stage I-III disease • Tumour grade, ER and HER 2 status available • Clinical or pathological tumour size and lymph node status available • Neo-adjuvant or adjuvant systemic chemotherapy recommended by local breast multi-disciplinary meeting • No prior systemic anti-cancer treatment within the past 10 years • No evidence of distant metastatic disease • Patient agrees to receive neo/adjuvant chemotherapy • Planned to receive greater than 4 x 21-day cycles of anthracycline or taxane-based combination chemotherapy. 21-day combination regimens including weekly treatments are allowed e.g. 1. carboplatin D1/paclitaxel D1, D8, D15 2. EC-weekly paclitaxel • Aged≥ 18 years and< 80 years • Female • Able to complete written records in English	• Previous invasive malignancy (with the exception of non-melanomatous skin cancer) within the past 10 years • Any other medical conditions preventing physical participation in the study procedures • Patients receiving only single agent or weekly neo/adjuvant chemotherapy regimens e.g. weekly paclitaxel with trastuzumab • Patients with existing conditions known to affect body water or cause oedema or muscle conditions that may affect muscle mass such as muscular dystrophies. • Pregnancy

***Table 1**. Inclusion and exclusion criteria for the CANDO-3 study*

Approximately 40 patients will be recruited over an 18 month period from each of eight UK hospital sites (The Christie Hospital, Manchester; Southampton General Hospital, Southampton; Churchill Hospital, Oxford; Royal Hampshire County Hospital, Winchester; Royal Devon and Exeter Hospital, Exeter; Salisbury District Hospital, Salisbury; Royal Cornwall Hospitals NHS Trust, Truro; and Queen Alexandra Hospital, Portsmouth). The patients will be recruited to the study from Breast Cancer Clinics and Breast Oncology Clinics, screened for eligibility through multidisciplinary meetings and introduced to the study when recommended to receive chemotherapy. They will be introduced to the study at their clinic appointment and if interested will be handed a patient information sheet. After a minimum of 24 hours to study the patient information sheet, they will be contacted to confirm whether they want to take part. Patients will provide informed consent in the form of a paper consent form, on the day of the first study visit (V1).

Weight, resistance, reactance and Phase Angle together with derived estimates of FM, FFM and SMM will be obtained using the Seca mBCA 515 and its associated Seca 115 software, at all study centres at each study visit, together with measures of maximal voluntary contraction using a hydraulic hand dynamometer. Impedance and body composition variables will be expressed as absolute values, adjusted for height, and standardised as Z scores against an age, gender and BMI device-specific reference population [31]. Participants will also be asked to complete quality of life and lifestyle (EORTC QLQ-C30 and EORTC QLQ-BR23 [32], physical activity (IPAQ-SF) [33], appetite and eating habits (CNAQ) questionnaires [34] at baseline and at 3 other study visits, and alcohol use (AUDIT-C)

[35] questionnaires at baseline only.. Routine clinical data will be obtained from electronic patient records and include a height measurement as recorded at the first clinic visit, blood test results, patient and tumour characteristics, co-morbidities, cancer treatments and progress through treatment pathway. Chemotherapy toxicity will be recorded at each clinic visit according to the CTCAE v5.0, (National Cancer Institute), together with full details of chemotherapy, including any dose adjustments, delays or changes to the treatment regimen, to determine chemotherapy delivery (of planned dose density).

Staging and radiology planning CT scan images obtained in routine clinical care, where available prior to visit 1, will be accessed directly from the hospital picture archiving and communication system (PACS) department and link-anonymised using a unique patient study identification number. Images from external centres will be sent using standard NHS image transfer processes between hospitals to the Southampton PACS department. SliceOmatic (v5.0 Rev-7, TomoVision, Magog, Canada) software will be used to determine tissue cross-sectional areas and attenuation according to the modified Alberta protocol (Alberta protocol, TomoVision, Magog, Canada).

In an optional sub-study, patients in Southampton will be invited to undertake further body composition analysis by DXA to determine FM, FFM and appendicular FFM, and to donate one extra 10ml blood sample which will be biobanked for future analysis and research.

The study schedule is shown in table 2.

Table 2. Study schedule

Visit	V0	V1	V2	V3	V4	V5	V6	V7*	V8**	End of chemo	End of study
Description of visit	Breast clinic	Chemo clinic 1	Chemo clinic 2	Chemo clinic 3	Chemo clinic 4	Chemo clinic 5	Chemo clinic 6	Chemo clinic 7	Chemo clinic 8	Final chemo clinic	Follow up visit
Relationship to chemo cycles	~ 2 weeks prior	Up to 7 days prior cycle 1	Within the 3 days prior cycle 2	Within the 3 days prior cycle 3	Within the 3 days prior cycle 4	Within the 3 days prior cycle 5	Within the 3 days prior cycle 6	Within the 3 days prior cycle 7	Within the 3 days prior cycle 8	2-6 weeks post final cycle	3 months post final cycle +/- 31 days
Patient information sheet	X										
Recruitment		X									
Consent		X									
Grip strength		X	X	X	X	X	X	X	X	X	X
sBIA***		X	X	X	X	X	X	X	X	X	X
CT scan†		X									
Chemotoxicity assessments			X	X	X	X	X	X	X	X	X
Questionnaires: • EORTC QLQ-C30		X			X					X	X
• EORTC QLQ-BR23		X			X					X	X
• IPAQ-SF		X			X					X	X
• CNAQ		X			X					X	X
• AUDIT-C		X									

Table 2. Study schedule

†Baseline staging CT scan would usually be at some point prior to chemotherapy, although timing can be variable but on entry to the study the patient will consent to use of the data for body composition analysis.

**only required if >6 cycles of chemotherapy planned; **only required if > 7 cycles of chemotherapy planned*
Please name visits to correspond to the chemotherapy cycle that follows that visit: i.e. visit 1 = pre-chemotherapy cycle 1; visit 2 = pre-chemotherapy cycle 2, etc.

**** weight, impedance and derived estimates of body composition by sBIA*

Sample size

A University of Southampton, University Hospital Southampton NHS Foundation Trust-sponsored pilot study (CANDO-2) found that study mean FM corrected for differences in height (fat mass index; FMi) was 11.30 (SD 4.93) kg/m^2 in patients without toxicity (grade 3 or above) and 13.31 (difference of 2.01) in patients with a grade 3 or above toxicity [22]. Based on simulations, a difference of this magnitude would lead to an area under the curve (AUC; as a measure of discrimination) of over 0.6 based on FMi alone, suggesting at least modest discriminative ability of FMi for predicting toxicity. A sample of size 256 would ensure 90% power for a two-group comparison on FMi (with alpha of 5%), and inflating this to 300 maintains this level of power when the groups are unbalanced in size, up to 30-70 in favour of non-toxicity (where such a split has been in related literature) [23]. As such, a statistically significant result would be an indication that FMi shows predictive ability that would be worth exploring in further research since this magnitude of FMi difference is likely to be clinically significant if proven.

Statistical analysis plan

For the primary objective analyses, descriptive statistics will be used to describe the FMi in patients with versus without a grade 3 or above chemotherapy toxicity, and a formal comparison carried out using a t-Test in order determine if the mean FMi is different between groups. Additional pre-specified primary objective analyses will include fitting a logistic regression model to analyse the FMi data according to grade 3 or above toxicity outcome, adjusting for potential confounding variables e.g. BMI. AUCs will also be produced to determine the predictive ability of FMi. Unadjusted and adjusted relative risks (RR) and corresponding 95% confidence intervals (CIs), derived using Poisson regression models, will also be reported for associations between FMi and grade 3 or above toxicity outcome. Trial measurements will be made at routine clinic visits and there is no ongoing follow-up so it is anticipated that there will be minimal missing data.

Data management plan, data collection and ALEA eCRF®

A data management plan for the CANDO-3 study has been written and approved. Linked anonymised data will be kept on a secure and trusted validated ALEA eCRF® (FormsVision BV, Netherlands), certified by registered auditors to be in compliance with regulations such as the FDA's CFR 21 Part 11 and ICH GCP. FormsVision BV support University Hospital Southampton NHS Foundation Trust and many other organisations, including the University of Cambridge, Leicester and Bristol, and the World Health Organisation, with clinical trials data management.

All anonymized clinical, prescribing and toxicity data, and results of the DXA and CT image analysis will be recorded on paper CRFs and centrally entered into a common dataset in accordance with local GCP

and Data Protection governance requirements. The BIA data will be recorded electronically at source and uploaded electronically into the common dataset.

Data will be cleaned and dataset secured under guidance and management by the University of Southampton Clinical Informatics Research Unit Data Management team prior to analyses. The dataset will then be analysed in accordance with the Statistical Analysis Plan under the direction of statistical analysis team, PI and co-applicants.

PATIENT AND PUBLIC INVOLVEMENT

A patient advocate (LT) is involved in the design, management and undertaking of this study as a member of the study steering group.

This protocol has been developed with advice from the patient advocate who has extensive experience both with Independent Cancer Patients' Voice (ICPV) and on a number of breast cancer clinical trials. The patient advocate is a member of the Breast Cancer Now tissue bank management board, the NCRI Breast Clinical Studies Group and is the lead patient representative for the NIHR Cancer and Nutrition Collaboration. Additional areas of involvement include: review of study documentation; attendance at Trial Steering Group meetings and ongoing advice, guidance and patient perspective both at these meetings and as required on an ongoing basis; providing guidance on the use and content of study newsletters; and advise on materials and methods to disseminate the final study outcomes.

ETHICS AND DISSEMINATION

This is an observational cohort study and patient clinical management will not change as a result of the study. The study was approved on 30/01/2020 by the Hampshire B Research Ethics Committee (Level 3 Block B, Whitefriars, Lewins Mead, Bristol, BS1 2NT; Ref: 19/SC/0596. IRAS: 263666)

The study is listed on the ISRCTN registry (ISRCTN: 79577461).

The Chief Investigator and Co-investigators will be responsible for publication of the study findings in a peer-reviewed scientific journal, on behalf of all collaborators. The manuscript will be prepared by a writing group, and participating investigators will be selected to join the writing group on the basis of contribution and following standard protocols for authorship.

Anonymous data will be available for request from three months after publication of the primary endpoint manuscript until the end of the archive and storage period. It will be available to researchers who provide a completed Data Sharing request form that describes a methodologically sound proposal, for the purpose of the approved proposal and if appropriate, signed a Data Sharing Agreement. Proposals will be reviewed for approval by the study steering committee. Data for approved applications will be shared once all parties have signed relevant data sharing documentation, covering the study steering committee conditions for sharing and if required, an additional Data Sharing Agreement from Sponsor. Proposals should be directed to the Chief Investigator.

FUNDING STATEMENT

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AUTHOR CONTRIBUTIONS

Ramsey I Cutress: Chief Investigator for study funding application. Study conceptualisation, manuscript and protocol writing

Ellen R Copson and Stephen A Wootton: Co-investigators for study funding application. Study conceptualisation, manuscript and protocol writing

Kesta Durkin: Manuscript and protocol writing. Manuscript review and editing

Adam Heetun: Manuscript review

Sean Ewings: Power calculations, statistics and manuscript review

Richard Munday: Study clinical informatics and manuscript review

Lesley Turner: Patient advocate, manuscript review

CANDO-3 steering Group: Manuscript review and approval

COMPETING INTERESTS

SAW holds an investigator-led Collaborative Research Agreement between Seca Medical Measuring Systems and Scales and the University of Southampton/University Hospital Southampton NHS Foundation Trust. Seca played no part in the design, conduct or interpretation of the work reported in this publication.

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STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3-5
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6-7
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	6-7
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	N/A
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6-7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	7
Bias	9	Describe any efforts to address potential sources of bias	5
Study size	10	Explain how the study size was arrived at	8
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	N/A protocol paper
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	8
		(*b*) Describe any methods used to examine subgroups and interactions	8
		(*c*) Explain how missing data were addressed	8
		(*d*) If applicable, explain how loss to follow-up was addressed	N/A
		(*e*) Describe any sensitivity analyses	N/A
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	N/A
		(b) Give reasons for non-participation at each stage	N/A
		(c) Consider use of a flow diagram	N/A
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	N/A
		(b) Indicate number of participants with missing data for each variable of interest	N/A
		(c) Summarise follow-up time (eg, average and total amount)	N/A
Outcome data	15*	Report numbers of outcome events or summary measures over time	N/A
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	N/A
		(*b*) Report category boundaries when continuous variables were categorized	N/A
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	N/A
Discussion			N/A
Key results	18	Summarise key results with reference to study objectives	N/A
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	N/a
Generalisability	21	Discuss the generalisability (external validity) of the study results	2-5
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	9

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.