PROTOCOL TITLE 'Clinical evaluation of Contrast Enhanced Breast CT to improve Staging and treatment follow up in women with breast cancer'

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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

ABR	General Assessment and Registration form (ABR form), the application form that is required for submission to the accredited Ethics Committee; in Dutch: Algemeen Beoordelings- en Registratieformulier (ABR-formulier)
AE	Adverse Event
AR	Adverse Reaction
CA	Competent Authority
CCMO	Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek
CV	Curriculum Vitae
EU	European Union
GCP	Good Clinical Practice
GDPR	General Data Protection Regulation; in Dutch: Algemene Verordening Gegevensbescherming (AVG)
IC	Informed Consent
METC	Medical research ethics committee (MREC); in Dutch: medisch-ethische toetsingscommissie (METC)
(S)AE	(Serious) Adverse Event
SPC	Summary of Product Characteristics; in Dutch: officiële productinformatie IB1-tekst
Sponsor	The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical company, academic hospital, scientific organisation or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a subsidising party.
SUSAR	Suspected Unexpected Serious Adverse Reaction
UAVG	Dutch Act on Implementation of the General Data Protection Regulation; in Dutch: Uitvoeringswet AVG
WMO	Medical Research Involving Human Subjects Act; in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen

SUMMARY

Rationale: Breast CT is a novel modality that has not been largely evaluated in a clinical setting. Only recently FDA and CE marked BCT machines have been released, and the number of installed bases internationally is below 10 (although rapidly rising).
Internationally only a few studies on contrast enhanced breast CT have been performed in small numbers of patients, albeit with excellent results. Nevertheless, substantial evidence for this novel modality is still absent. In particular, the correlation of enhancement and histological grade of DCIS, correlation of CEBCT findings with histopathology, and prediction and assessment of primary systemic treatment are open fields for which more substantial evaluation is clearly needed.

Objectives:

a. Staging of women with breast cancer, particularly those with extensive carcinoma in situ (DCIS).

b. Evaluation of women treated with primary systemic therapy.

Study design: Observational cohort study

Study population: Adult women (>18 years old) with breast cancer, scheduled for a pre-surgery staging contrast enhanced breast MRI and eligible for primary systemic therapy who are able to provide informed consent will be enrolled

Intervention: All participants will undergo a bilateral CEBCT scan using a dedicated breast CT scanner (Koning, USA). This scanner creates full 3D volumes of the breast using conebeam reconstruction. The scanner is designed as an exam table, on which the patient is in prone position during image acquisition. Centrally in the table a horizontal CT gantry is positioned. A mammographic x-ray tube and an x-ray flat panel detector are mounted on the CT gantry and circle the breast during acquisition in 10 seconds. For CEBCT two acquisitions need to be obtained (1 before and 1 after contrast), and we will perform the CEBCT of both breasts.
Prior to the examination an iv-canula will be inserted for contrast administration during the procedure. Like in (contrast enhanced) mammography the breasts are imaged one at a time. The breast to be imaged is suspended through the central table opening into the imaging space. In the clinical protocol we will first image the non-affected breast prior to contrast administration. Subsequently, the patient is repositioned and the affected breast will be imaged. Thereafter an iodinated contrast agent (Iomeron 300) is administered (1.5 ml/kg body mass) using a power injector. Considering sufficient of time after contrast administration the affected breast will be scanned again. Finally, the non-affected breast is repositioned in the gantry, and a post-contrast acquisition of this breast is obtained as well

Main study parameters/endpoints:
<u>Objective a.</u>

The primary endpoint is the non-inferiority of CEBCT error rate against the golden standard, i.e. histopathology, to DCE MRI error rate against the golden standard for tumor staging.

As secondary objectives, we will record:
- the concordance of tumor extent between CEBCT and large section histopathology
- the frequency of detection of contralateral cancers with CEBCT.

Objective b.

The primary endpoint is the non-inferiority of CEBCT to predict pCR after primary systemic therapy as compared to DCE MRI computed from the area under the receiver operating characteristic (ROC) curve (AUC) (or AUROC).

As secondary objectives, we will record:
- the concordance of morphological and enhancement characteristics of cancers on CEBCT and their corresponding existing subtyping to the expected response to primary systemic therapy
- the potential of CEBCT predicting the response to primary systemic therapy early in treatment

Nature and extent of the burden and risks associated with participation, benefit and group relatedness: CT imaging is associated with risks related to the use of radiation and contrast administration. The risks of this study are reduced as much as possible. In breast CT only the breast is exposed to radiation, sparing the rest of the chest to any significant amount of radiation. These patients already get numerous mammographic views during standard clinical care, and generally receive radiotherapy to the affected breast. There is most probably no effect due to the level of additional radiation involved in breast CT imaging, considering the course of treatment of the study population (mastectomy or post-surgery radiation therapy).

The introduction of an imaging technology that will improve staging and treatment follow up of breast cancer detection, will have impact on survival rate and quality of life of breast cancer patients. By optimizing the image quality of this modality we will ensure that this impact on women’s healthcare is maximized. The research will not be directly beneficial to the subjects since the research breast CT results will not influence the subject’s treatment.

1. INTRODUCTION AND RATIONALE

To decide on the most optimal therapy for patients with breast cancer, it is of paramount importance to understand the extent and nature of the cancer. For approximately 80% of breast cancer patients the primary therapy is surgical, followed by radiotherapy in case of breast conserving therapy, and when required systemic therapy.[1]
Knowing the extent of tumor, and how it is positioned within the breast, is extremely relevant for surgical success, which is defined as the excision of the cancer with no residual tumor/DCIS on the inked margin. This is relatively difficult to achieve, the rate of re-excisions can be, according to literature, as high as 34%.[2, 3] In the Netherlands, the presence of focal involvement (<4mm) of a margin is accepted, as it is thought that these represent the basis of spicular extensions of the tumor that are in general adequately treated by radiotherapy;[19] a theory backed by very low numbers of in-breast recurrence.[4] Still, even using this very liberal approach, the re-excision rate is about 4% in invasive cancers and as high as 19% in DCIS.[1]

Previous studies using MRI and contrast mammography have clearly shown that tumor size estimations using a contrast enhanced study correspond better to tumor size at final histopathology than clinical examination, mammography or ultrasound (fig 1).[5-11] As we previously showed, MRI still outperforms contrast mammography, likely due to its 3D nature.[12]
Despite the overwhelming evidence that staging of *all* breast cancers is improved with preoperative MRI, and that in addition approximately 4% of unexpected contra-lateral cancers are detected, the indication is worldwide supported mainly for invasive lobular breast cancers.[13-15] However, this will likely change as preliminary results of the currently running multi-institutional “Preoperative Breast MRI in Clinical Practice: Multicenter International Prospective Meta-Analysis of Individual Data (MIPA)” study unequivocally show that in centers with sufficient experience and expertise re-excisions are reduced from 13% to 8% when using pre-operative MRI for staging, whereas mastectomies do not increase.[16] This implies that surgeons can use the information obtained with 3D contrast enhanced imaging studies to improve the quality of surgical procedures, and it also shows that the correlation of tumor size on radiology and pathology is an important measure for the quality of preoperative staging (despite the fact that the vastly different shape of the breast in vivo and at pathology makes perfect correlation impossible). Still, not-withstanding the clear value of breast MRI, over- and underestimation of tumor size does occur in up to 15% of cases.[17, 18] Particularly DCIS components surrounding the primary tumor are hard to visualize.[19] Consequently, the higher spatial resolution, as well as the concurrent depiction of calcifications, may further improve the quality of pre-operative imaging when shifting from MRI to CEBCT, while associated costs and the burden to the patient (due to the open scanner design and the shorter examination times) decrease.

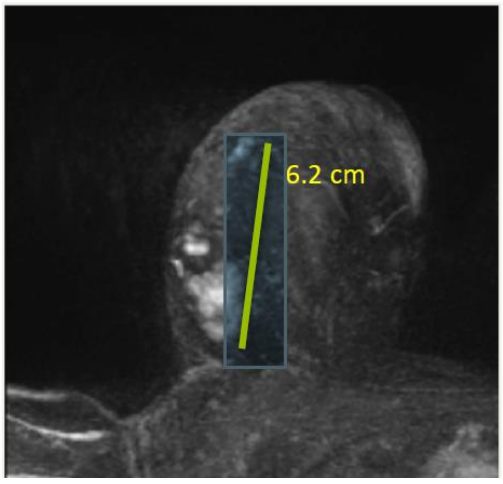

Figure 1: Preoperative evaluation of tumor extent using contrast enhanced MRI

The most optimal treatment for patients with breast cancer may not always be primary surgery. For ~20% of patients with larger tumors (≥T2) or node positive cancers primary systemic therapy (including endocrine, immuno-, and/or chemotherapy) may be used to

downstage the tumor.[1] The logic behind this approach is primarily that these women will have an indication for the systemic therapy in any case, and if not given before surgical treatment, it will be given after surgery.
The advantages of administering systemic therapy before surgery are that it is possible to 1) shrink the tumor, thus allowing less aggressive surgery; 2) downstage the axilla, thus allowing to prevent axillary clearance in women with initially N+ disease; 3) achieve a pathological complete response (pCR) after primary systemic therapy, which is for most tumors a good prognostic indicator for disease free survival; 4) monitor response to therapy of the primary tumor, allowing for early identification of non-response and therefore allowing for a possible switch to more effective therapies.[20, 21] These advantages obviously come at a risk, as the tumor may progress during the systemic treatment. Fortunately, this is rare (<5%), and so far no negative effects of primary systemic therapy on survival have been documented.[22] In the last decades it has become clear that different molecular subtypes of breast cancer respond differently to systemic therapy, with triple negative and HER2+ subtypes showing the best results.[23] Imaging based studies have shown that morphological characteristics of tumors, as well as enhancement characteristics correlate well with these subtypes and have a predictive value with regard to treatment response and eventual outcome.[24-32] The obvious advantage of imaging based response prediction over genomic assays is that the whole tumor is evaluated rather than a small sample. When used in conjunction with the classical subtyping, it appears that intratumoral heterogeneity is in itself an important predictor of response.[33-37] Characteristics of the peri-tumoral region also seem to be predictive of response.[38-40] Unfortunately MRI based predictors are hard to translate to clinical practice as many parameters are subjective in nature, and the non-linear relation between contrast concentration and enhancement makes quantification difficult.[41, 42] Other quantitative imaging parameters such as ‘apparent diffusion coefficient’ obtained from diffusion weighted MRI and ‘standardized uptake values’ from PET/CT are limited by the inherent low signal to noise ratio of these techniques and therefore limited spatial resolution.[26,27,30] CEBCT will allow showing morphological detail and spatial variation in enhancement within breast tumors to an unprecedented level, which may subsequently be used to select women with breast cancers for specific forms of primary systemic therapy based upon the whole tumor profile.
In practice, none of the systemic therapies are purely selective for cancer, which implies that most have significant toxic side-effects.[43-45] Early detection of non-response (which basically means that we selected the wrong treatment at the start) is therefore of paramount importance. CEBCT will allow for detailed assessment of the response of specific cancers to specific systemic therapies, which we will document. In order to use CEBCT as a therapy modulating technique a substantial knowledge base needs to be built.
A directly useful clinical application in this setting is the potentially important role for CEBCT in preoperative evaluation of patients treated with some form of primary systemic therapy. Breast MRI is not very accurate in the detection of residual disease with a sensitivity of 83% and equal specificity.[46] Unfortunately, after neoadjuvant chemotherapy, due to the softer and often fragmented tumor, surgery is much more challenging, leading to higher rates of re-excision, or unnecessary aggressive surgical procedures.[47,48] Therefore, the very high spatial resolution of CEBCT may be exploited to better document the area of residual disease; and when specific enough may potentially prevent surgery completely in women who have achieved a pCR.

Breast CT is a novel modality that has not been largely evaluated in a clinical setting. Only recently FDA and CE marked BCT machines have been released, and the number of installed bases internationally is below 10 (although rapidly rising).
Internationally only a few studies on contrast enhanced breast CT have been performed in small numbers of patients, albeit with excellent results. The first study using CEBCT included 46 lesions of which 29 were benign, showing that all malignancies were more conspicuous on CEBCT than on mammography and on BCT without contrast administration.[49] In a small study in patients presenting with calcifications Aminololama-Shakeri et al. showed that all 17 DCIS lesions enhanced and were highly suspicious, whereas benign lesions associated with calcifications were far less conspicuous.[50] Seifert et al reported on 23 CEBCT examinations of patients with known cancers, showing the detection of 3 additional lesions due to the performance of CEBCT.[51] The largest series published thus far originates from Guangzhou, He et al. reported a sensitivity of 98.7% for CEBCT in a series of 270 lesions containing 110 cancers, which compared favourably with mammography (78.4%), ultrasound (81.1%) and non-contrast breast CT (89.2%).[52]
Only Wienbeck compared CEBCT directly to breast MRI, showing in a series of 100 lesions that CEBCT in experienced hands yields equal accuracy as breast MRI, although according to their results, the sensitivity is somewhat lower, whereas the specificity is higher.[53] Finally, Uhlig et al showed in 23 patients that the mean enhancement in CEBCT is different for cancers of different molecular subtypes, thus underscoring the possibility to infer treatment selection from CEBCT images.[54,55] Consequently, there is a solid basis for the assumptions underlying this study, which is further backed by the extensive literature that is available on MRI and contrast enhanced mammography. Still substantial evidence for this novel modality is absent. In particular, the correlation of enhancement and histological grade of DCIS, correlation of CEBCT findings with histopathology, and prediction and assessment of primary systemic treatment are open fields for which more substantial evaluation is clearly needed.

2. OBJECTIVES

a. Staging of women with breast cancer, particularly those with extensive carcinoma in situ (DCIS):

Primary objective a:

1) The non-inferiority of CEBCT error rate against the golden standard, i.e. histopathology, to dynamic contrast enhanced (DCE) MRI error rate against the golden standard for tumor staging.

Secondary objective(s) a:

2) The concordance of tumor extent between CEBCT and large section histopathology
3) The frequency of detection of contralateral cancers with CEBCT in women with breast cancer

b. Evaluation of women treated with primary systemic therapy.

Primary objective b:

1) The non-inferiority of CEBCT to predict pCR after primary systemic therapy as compared to DCE MRI computed from the AUROC.

Secondary objective(s) b:

2) The concordance of morphological and enhancement characteristics of cancers on CEBCT and their corresponding existing subtyping to the expected response to primary systemic therapy?
3) The potential of CEBCT predicting the response to primary systemic therapy early in treatment?

3. STUDY DESIGN

All participants will undergo a bilateral CEBCT scan using a dedicated breast CT scanner (Koning, USA). This scanner creates full 3D volumes of the breast using conebeam reconstruction (fig 2). The scanner is designed as an exam table, on which the patient is in prone position during image acquisition. Centrally in the table a horizontal CT gantry is positioned. A mammographic x-ray tube with a 0.3 mm focal spot size, and an x-ray flat panel detector are mounted on the CT gantry and circle the breast during acquisition in 10 seconds. For CEBCT two acquisitions need to be obtained (1 before and 1 after contrast), and we will perform the CEBCT of both breasts.

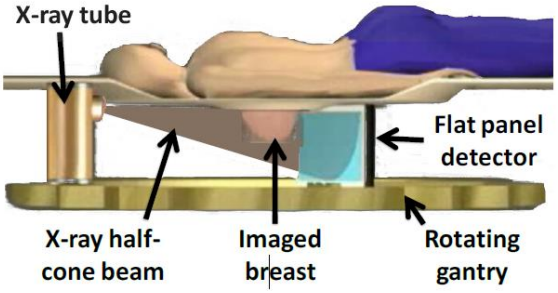

Figure 2: Schematic drawing of the dedicated breast CT scanner

Prior to the examination an iv-canula will be inserted for contrast administration during the procedure. Like in (contrast enhanced) mammography the breasts are imaged one at a time. The breast to be imaged is suspended through the central table opening into the imaging space. The maximum imaging volume is 28 × 28 × 16 cm, which allows the complete examination of most breast sizes. In the clinical protocol we will first image the non-affected breast prior to contrast administration. Subsequently, the patient is repositioned and the affected breast will be imaged. Thereafter an iodinated contrast agent (Iomeron 300) is administered (1.5 ml/kg body mass) using a power injector (flow-rate 2.5 ml/s, flush of 30 ml saline). To prevent contrast nephropathy the applicable protocols of the relevant hospital must be followed, see appendix A (Radboudumc specific) and appendix B (NKI specific). Considering sufficient of time after contrast administration the affected breast will be scanned again. Finally, the non-affected breast is repositioned in the gantry, and a post-contrast acquisition of this breast is obtained as well.

3.1 Timing of post-contrast scan

Based upon timing studies performed for contrast enhanced mammography (that uses the same type and dose of contrast agent) 2 minutes after contrast administration the affected breast will be scanned again. Since this timing is not specific for CEBCT we will optimize the timing using prior developed phantoms (fig 3) and in addition with

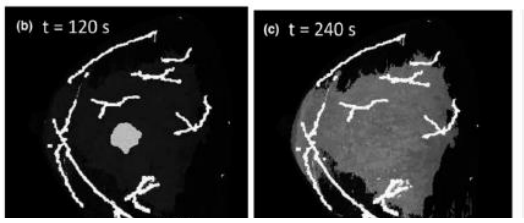

Figure 3: 2 frames form a patient based phantom for CE BCT showing the effect of timing on lesion conspicuity

the initial 30 patients participating in this study (method A).

Method A (n=30):
After the phantom based contrast timing we will verify the results in our first 30 patients. By the following scan protocol:

- Pre-contrast scan non-affected breast
- Pre-contrast scan affected breast
- Contrast injections
- Post-contrast scan affected breast after t=70sec
- Post-contrast scan affected breast after t=90sec
- Post-contrast scan affected breast after t=110sec
- Post-contrast scan affected breast after t=130sec
- Post-contrast scan affected breast after t=150sec
- Post-contrast scan non-affected breast

When 30 patients are imaged according Method A, we will perform an interim analysis (described in chapter 10.1). This interim analysis is meant to appoint the optimal post-contrast scan timing. This optimal timing will be used for the remaining participants in Method B.

3.2 CE BCT for staging of women with breast cancer (objective a)

Method B (n=383):
After the interim analysis we will continue with the study, according to the following scan protocol:

- Pre-contrast scan non-affected breast
- Pre-contrast scan affected breast
- Contrast injections
- Post-contrast scan affected breast after t= <*based on interim analysis Method A*>
- Post-contrast scan non-affected breast

Consequently, the acquisition time is <10 minutes in all patients (and <5 minutes in most).

3.3 CE BCT for evaluation of primary systemic therapy (objective b)

The contrast enhanced imaging is necessary for the cancer staging and will be used to come to an appropriate treatment plan for the patient. In general, there are two options for the patients:

1. (unilateral) mastectomy and post surgery adjuvant therapy
2. Primary systemic therapy

Patients in group 1 will reach the end point of the study after the staging imaging, since they will undergo mastectomy. Patients in group 2 will be asked to undergo a second CE BCT post systemic therapy. This will involve the following scan protocol:

- Pre-contrast scan non-affected breast
- Pre-contrast scan affected breast
- Contrast injections
- Post-contrast scan affected breast after t= <*based on interim analysis Method A*>
- Post-contrast scan non-affected breast

- ** Post-contrast scan affected breast (after ±5 min)

*** This allows for evaluation of changes in enhancement characteristics of the tumor and the fibroglandular tissue surrounding the tumor.*

Post processing, volumetric measures and reader study

From the obtained pre- and post contrast scans we will generate subtraction images for all CEBCT scans to enable detection of even very tiny areas of enhancement, as well as maximum intensity projections to provide a global overview of each breast. All CEBCT scans will subsequently be independently evaluated by a dedicated breast radiologist on a clinical reporting station using software for 3D analysis of the data, allowing MPR in all directions, as well as volume rendering and the assessment of slabs in various thicknesses.
For the studies comparing CEBCT to breast MRI, we will ensure independent blinded reading of both modalities by two different radiologists.

4. STUDY POPULATION

4.1 Population (base)

Women with breast cancer.

4.2 Inclusion criteria

In order to be eligible to participate in this study, a subject must meet all of the following criteria:

- Women >18 years old
- Diagnoses of breast cancer
- Scheduled for a pre-surgery staging contrast enhanced breast MRI
- Eligible for primary systemic therapy

4.3 Exclusion criteria

A potential subject who meets any of the following criteria will be excluded from participation in this study:

- Women with suspected or confirmed pregnancy
- Women with prior breast cancer
- Women who are breastfeeding
- Women who are very frail and unable to cooperate
- Women who cannot give informed consent
- Contra indication of iodine contrast (i.e. contrast allergy, renal function impairment (GFR <60 ml/min/1.73m^2))
- Contra indication for irradiation (i.e. genetic mutation that predispose to breast cancer)
- Male subjects

4.4 Sample size calculation

Objective a:

For staging of women with breast cancer, we will conduct a pilot study comparing CT findings to large section histopathology in 30 patients. This number is based upon experience obtained in previous studies and it represents a hypothesis generating question. In order to compare the accuracy of tumor size estimation of CEBCT and DCE MRI with the golden

standard we assume that CEBCT is at least capable of tumor size estimations within 1 cm of pathologic size in 74% of patients as we previously documented for breast MRI.[35] To calculate the sample size we used a one-sided non-inferiority test (McNemar's test) for the difference between two correlated proportions. For a one-sided non-inferiority test of the difference between two correlated proportions, a sample size of 344 subjects achieves 80% power at a significance level of 0,05 when the non-inferiority difference is -0,04, the treatment proportion is 0,74, the standard proportion is 0,74, and the actual difference between the proportions is 0 [1] Applying a 10% lost-to-follow up margin implies that we will include 383 patients undergoing both pre-operative MRI and CEBCT for this comparison *(objective a)*.

Objective b:
For the evaluation of women treated with primary systemic therapy, we assume that CEBCT may directly improve the quality of staging residual cancer after therapy and the selection of patients with pCR. Patients will receive both a MRI and CEBCT which means we are dealing with paired data. Using radiomics, the data become continuous responses providing a likelihood of pCR between 0 and 1. From this the AUC is computed. For MRI an AUC of 0.83 was reported.[63] In order to evaluate non-inferiority of CEBCT, we expect for CEBCT too an AUC of 0.83. According to literature, pCR after primary systemic therapy ranges from 15 to 45% based on the molecular subtypes of breast cancer and the type of systemic treatments that the patients undergo [2]. Based on these assumptions a sample of 27 pCR cases and 63 non-pCR cases results in 80% power to detect a difference of 0,15 between the AUROC of MRI and CEBCT measurements using a one-sided z-test at a significance level of 0,05. This wide confidence interval is deemed acceptable due to the explorative nature of this part of the study. Considering the longitudinal aspect of this study and the sometimes severe side-effects of chemotherapy which may lead to drop-out of patients, we will apply a margin of 20% here; thus including 113 patients.

> NOTE: these 113 cases from objective b are also included in the 383 patients described for *objective a*. 30 additional patients will be included for the interim analysis. So we aim to include a total of 413 cases in this study

5. TREATMENT OF SUBJECTS
N/A, observational study

6. INVESTIGATIONAL PRODUCT
N/A, observational study

7. NON-INVESTIGATIONAL PRODUCT
Standard clinical used iodine contrast (see D2 for SMP text).

8. METHODS

8.1 Study parameters/endpoints

8.1.1 Main study parameter/endpoint

Objective a.

The primary endpoint is the MRI and CEBCT discordance with the golden standard (i.e size estimation within 1 cm from histopathological size). The results will be summarized in the following 2x2 table format over all cases:

		CEBCT		
		No discordance with Golden standard	Discordance with Golden standard	Total
MRI	No discordance with Golden standard	A (%)	B (%)	A+B (%)
	Discordance with Golden standard	C (%)	D (%)	C+D (%)
	Total	A+C (%)	B+D (%)	A+B+C+D (100%)

Table 1 Summary table for discordance (error) data where A, B, C and D denote the number of cases in each corresponding cell. All percentages are calculated with the grand total, i.e. A+B+C+D, as the denominator. The CEBCT error rate is the percentage associated with B+D, the MRI error rate is the percentage associated with C+D, and 100%(B-C)/(A+B+C+D) is the CEBCT-MRI error rate difference.*

The following hypotheses will be evaluated for the primary analysis:

Null Hypothesis (H0): pCEBCT – pMRI ≥ Δ

Alternative Hypothesis (Ha): pCEBCT – pMRI < Δ,

pCEBCT is the error rate for the CEBCT diagnosis compared to the golden standard; pMRI is the error rate for the MRI diagnosis compared to golden standard; and Δ is the non-inferiority margin. The non-inferiority Δ will be set at an absolute 4% for this study.

A 2-sided 90% confidence for the CEBCT-MRI error rate difference will be estimated using Wald Z statistic. If the upper bound of the 90% CI is less than the non-inferiority margin, CEBCT will be considered non-inferior to MRI.

Objective b.

The primary endpoint of objective B is the non-inferiority of CEBCT to predict pCR after neo-adjuvant chemotherapy as compared to DCE MRI computed from the AUROC. We will estimate AUCs from the radiomics features of both CEBCT and MRI as compared to the results of pathologic resection of the tumor (gold standard) in predicting pCR (Yes/No). All analysis will be performed for all cases.

We will perform a non-inferiority test for the difference between the paired AUCs of CEBCT and MRI across all readers and all cases.

Null Hypothesis (H0): $AUC_{CEBCT} - AUC_{MRI} \leq -\Delta$

Alternative Hypothesis (Ha): $AUC_{CEBCT} - AUC_{MRI} > -\Delta$

AUC is the area under the curve, Δ is the non-inferiority margin. The non-inferiority Δ will be set at an absolute 0.15 for this study.

The objective will be evaluated at statistical significance level $\alpha = 0.05$. A two-sided 90% confidence interval for the difference between paired AUCs will be computed. If the lower bound of the 90% confidence interval is higher than -0.15 we will conclude non-inferiority for CEBCT images versus breast MRI images in this pilot study.

8.1.2 Secondary study parameters/endpoints (if applicable)

As secondary objectives, we will record the frequency of contralateral lesions and their final pathological outcome, morphological and enhancement characteristics of cancers on CEBCT complementary to existing subtyping.

The concordance rates according morphological and enhancement characteristics of cancers will be compared using the Fisher exact test for unpaired data and McNemar's test for paired data.

Participant characteristics such as age, ethnicity, race, cup size, family history for breast cancer, level of comfort during BCT examination and level of comfort of BCT compared to other image modality.

8.2 Randomisation, blinding and treatment allocation

NA

8.3 Study procedures

The diagnostic work-up will be performed as per standard clinical practice and therefore, in addition to the CEBCT examination, will include:
- *objective a:* pre-operative breast MRI for breast cancer staging
- *objective b:* MRI for evaluation of residual tumor after systemic therapy

CEBCT imaging:
The CEBCT acquisition will be performed using the Koning dedicated breast computed tomography clinical prototype system that is installed at the Radboudumc/NKI. All CEBCT acquisitions will be obtained using the standard imaging technique as recommended by the manufacturer (49 kVp, 300 projections in 10 seconds covering 360°, 8 ms per projection), with the tube current set to a maximum of 100 mA, depending on breast size and density, set by using the system's automatic exposure control (AEC). The tube current used for each acquisition will be recorded. For CEBCT two acquisitions need to be obtained (1 before and 1 after contrast), and CEBCT examination of both breasts will be performed, one at a time. Prior to the examination an iv-canula will be inserted for contrast administration during the procedure. The breast to be imaged is suspended through the central table opening into the imaging space. In the clinical protocol we will first image the non-affected breast prior to contrast administration. Subsequently, the patient is repositioned and the affected breast will

be imaged. Thereafter an iodinated contrast agent (Iomeron 300) is administered (1.5 ml/kg body mass) using a power injector (flow-rate 2.5 ml/s, flush of 30 ml saline). Considering sufficient time after contrast administration the affected breast will be scanned again. Finally, the non-affected breast is repositioned in the gantry, and a post-contrast acquisition of this breast is obtained as well.

Assessment for incidental findings:
Within 5 days after CEBCT acquisition, the BCT image will be reviewed by one of the breast radiologists at Radboudumc/NKI to ensure that no additional lesions previously undetected are present. Even though BCT imaging is presently in the research stage, we must ensure that any potential additional lesions that might have not been visible in all previous clinical images but are visible in the CEBCT image are detected in an appropriate timeframe, and not at the delayed stage of the study. If any potential additional lesions are seen, the patient will be informed and the appropriate clinical procedures will be followed to validate this finding with standard diagnostic techniques (e.g. additional workup, US, biopsy, etc.), as appropriate.
The incidence of these cases and their final outcome will be recorded. Aside from this possibility of incidental findings, the results of this research, specifically, the CEBCT imaging, will not affect in any way the care provided to the subject.

8.4 Withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they wish to do so without any consequences. The investigator can decide to withdraw a subject from the study for urgent medical reasons.

8.4.1 Specific criteria for withdrawal

The patient will be removed from the study if the BCT images are deemed technically unsuitable to use in the study and the scan can be not redone (for the same timepoint).

8.5 Replacement of individual subjects after withdrawal

The sample size calculation already accounts for possible drop out. Therefor subjects will not be replaced in case of withdrawal.

8.6 Follow-up of subjects withdrawn from treatment

Standard clinical care, not a specific follow-up for this study.

8.7 Premature termination of the study

NA

9. SAFETY REPORTING

9.1 Temporary halt for reasons of subject safety

In accordance to section 10, subsection 4, of the WMO, the sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardise subject health or safety. The sponsor will notify the accredited METC without undue delay of a temporary halt including the reason for such an action. The study will be suspended pending a further

positive decision by the accredited METC. The investigator will take care that all subjects are kept informed.

9.2 AEs, SAEs and SUSARs

9.2.1 Adverse events (AEs)

Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to trial procedure. All adverse events reported spontaneously by the subject or observed by the investigator or his staff will be recorded.

9.2.2 Serious adverse events (SAEs)

A serious adverse event is any untoward medical occurrence or effect that

- results in death;
- is life threatening (at the time of the event);
- requires hospitalization or prolongation of existing inpatients' hospitalization;
- results in persistent or significant disability or incapacity;
- is a congenital anomaly or birth defect; or
- any other important medical event that did not result in any of the outcomes listed above due to medical or surgical intervention but could have been based upon appropriate judgement by the investigator.

An elective hospital admission will not be considered as a serious adverse event.

The investigator will report all SAEs to the sponsor without undue delay after obtaining knowledge of the events.

The sponsor will report the SAEs through the web portal *ToetsingOnline* to the accredited METC that approved the protocol, within 7 days of first knowledge for SAEs that result in death or are life threatening followed by a period of maximum of 8 days to complete the initial preliminary report. All other SAEs will be reported within a period of maximum 15 days after the sponsor has first knowledge of the serious adverse events.

9.2.3 Suspected unexpected serious adverse reactions (SUSARs)

NA

9.3 Annual safety report

NA

9.4 Follow-up of adverse events

All AEs will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist.
SAEs need to be reported till end of study within the Netherlands, as defined in the protocol

10. STATISTICAL ANALYSIS

10.1 Statistics regarding objective a:

We intend to perform a prospective study to compare the accuracy of contrast enhanced dedicated breast CT for staging of women with breast cancer. First, we will conduct a pilot study comparing CT findings to large section histopathology in 30 patients. This number is based upon experience obtained in previous studies and it represents a hypothesis generating question. Second, based on the result from the pilot study, we will decide the optimal timing for the main prospective study. In order to compare the accuracy of tumor size estimation of CEBCT and MRI with the golden standard, we assume that CEBCT is at least capable of tumor size estimations within 1 cm of pathologic size in 74% of patients, as we previously documented for breast MRI.[52] To calculate the sample size we used a one-sided non-inferiority test (McNemar's test) for the difference between two correlated proportions. For a one-sided non-inferiority test of the difference between two correlated proportions, a sample size of 344 subjects achieves 80% power at a significance level of 0,05 when the non-inferiority difference is 4%, the treatment proportion is 0,74, the standard proportion is 0,74, and the actual difference between the proportions is 0 [1]. Applying a 10% lost-to-follow up margin implies that we will include 383 patients undergoing both pre-operative MRI and CEBCT for this comparison.
Based on the distribution of the data, we will apply parametric (paired t-test) or non-parametric (wilcoxon signed rank test) statistical tests. To compare tumor size measurements of CEBCT and large section histopathology pearson's correlation coefficients and a Bland-Altman method will be used. The frequency of detection of contralateral cancers with CEBCT is observational and will be of descriptive nature (Contralateral cancer detection rate will be expressed as the cancer detection rate per 1000 investigations). All statistical analysis will be performed using SPSS statistical software (version 25) or R studio.

10.2 Statistics regarding objective b:

For the evaluation of women treated with primary systemic therapy, we assume that radiomics obtained from CEBCT may directly improve the quality of staging residual cancer after therapy and the selection of patients with pCR. Patients will receive both a MRI and CEBCT which means we are dealing with paired data. For MRI radiomics an area under the curve of 0.83 was reported.[46] In order to evaluate non-inferiority of radiomics obtained from CEBCT, we expect for CEBCT an area under the curve of 0.83. The non-inferiority Δ will be set at an absolute 0.15 for this pilot study.
The objective will be evaluated at statistical significance level $\alpha = 0.05$. A two-sided 90% confidence interval for the difference between paired AUCs will be computed. If the lower bound of the 90% confidence interval is higher than -0.15 we will conclude non-inferiority for CEBCT images versus breast MRI images.
According to literature, pCR after primary systemic therapy ranges from 15 to 45% based on the molecular subtypes of breast cancer and the consequent systemic treatments that the patients undergo [2]. Based on these assumptions, a sample of 27 from the positive group and 63 from the negative group achieves 80% power to detect a difference of 0,15 between the AUC under the null hypothesis of 0,68 and an AUC under the alternative hypothesis of 0,83 using a one-sided z-test at a significance level of 0,05. Considering the longitudinal aspect of this study and the sometimes severe side-effects of chemotherapy which may lead to drop-out of patients, we will apply a margin of 20% here; thus including 113 patients.
All statistical analysis will be performed using SPSS statistical software or R studio.

10.3 Interim analysis

Temporary hold of the study will be done after inclusion of 30 patients. Interim analysis will be performed to select the best contrast timing. Based on the outcome of the analysis the optimal timing will be applied during the remainder of the study.

11. ETHICAL CONSIDERATIONS

11.1 Regulation statement

This study will be conducted according to the principles of the Declaration of Helsinki (64th WMA General Assembly, Seoul, Korea, October 2013) and in accordance with the Medical Research Involving Human Subjects Act (WMO).

11.2 Recruitment and consent

Patients diagnosed with breast cancer will be informed by their care giver about this study. If interested they will receive written information and are asked permission that the research coordinator or designee will contact the patient about the willingness to participate. Questions arising will be answered and if interested the study visit will be scheduled. Patients will have time (2-5 days) to consider participation between first informing by care giver and scheduling of pre-surgery staging contrast enhanced breast MRI. When the patients visits the hospital: the written informed consent procedure will take place, eligibility evaluated and the patient will be enrolled in the study. *NOTE:* Possible pregnancy is an exclusion condition for the study. No pregnancy test will be administered; this criteria will be based on the verbal answer of the patient if there is a possibility of pregnancy.
To inform possible patients about this study, recruitment activities may involve a poster in the waiting room, and/or advertisement on web sites.

11.3 Objection by minors or incapacitated subjects

Minors and/or incapacitated adults will not be asked to participate in this study.

11.4 Benefits and risks assessment, group relatedness

The introduction of an imaging technology that will improve staging and treatment follow up of breast cancer detection, will have impact on survival rate and quality of life of breast cancer patients. By optimizing the image quality of this modality we will ensure that this impact on women’s healthcare is maximized. The research will not be directly beneficial to the subjects since the research breast CT results will not influence the subject’s treatment, if any.

11.5 Compensation for injury

The sponsor/investigator has a liability insurance which is in accordance with article 7 of the WMO.

The sponsor (also) has an insurance which is in accordance with the legal requirements in the Netherlands (Article 7 WMO). This insurance provides cover for damage to research subjects through injury or death caused by the study.

The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study.

11.6 Incentives

No incentives for participation are planned.

12. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

12.1 Handling and storage of data and documents

Please refer to the Data Management Plan.

12.2 Monitoring and Quality Assurance

In accordance with the risk-based approach of performing human research as published by the NFU, the proposed risk classification for this study is negligible or “verwaarloosbaar”. Monitoring will be performed by a certified person, with no relation to this particular study and will begin as soon as possible after start of the study. Monitoring is performed for the purpose of overseeing the progress of the study and ensuring that it is conducted, recorded and reported in accordance with the study protocol, local SOPs, GCP and other applicable regulatory requirements. For more details, please refer to the Monitoring Plan.

12.3 Amendments

Amendments are changes made to the research after a favourable opinion by the accredited METC has been given. All amendments will be notified to the METC that gave a favourable opinion.
All substantial amendments will be notified to the METC. Non-substantial amendments will not be notified to the accredited METC, but will be recorded and filed by the sponsor.

12.4 Annual progress report

The sponsor/investigator will submit a summary of the progress of the trial to the accredited METC once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

12.5 Temporary halt and (prematurely) end of study report

The investigator/sponsor will notify the accredited METC of the end of the study within a period of 8 weeks. The end of the study is defined as the last patient’s last visit.

The sponsor will notify the METC immediately of a temporary halt of the study, including the reason of such an action.

In case the study is ended prematurely, the sponsor will notify the accredited METC within 15 days, including the reasons for the premature termination.

Within one year after the end of the study, the investigator/sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC.

12.6 Public disclosure and publication policy

The results of this study can be published by the study team during scientific meetings and through publications. The anonymized breast CT and clinical diagnostic work-up images along with pertinent clinical information (e.g. biopsy outcome) may be used in future studies after anonymization with no possibility of reversing the anonymization process, both by the PI and others.

13. STRUCTURED RISK ANALYSIS

The mechanism involved in this research is the acquisition of a dedicated breast CT image. Dedicated breast CT is based on the same basic physics and technology as body CT, which is used millions of times a year world-wide. The x-ray tube voltage of the BCT is ~49 kVp to allow separation between fat and fibroglandular tissue, whereas the tube current values are chosen depending on breast size and density. The given x-ray dose for one acquisition is on average 8.2 mGy, which is about double that of 2-view mammography (~4mGy). Considering the different scan protocols, the radiation dose differs:

- Method A: 2 acquisitions of the non-affected breast, and 6 acquisitions of the affected breast)
- Method B, gr1: 2 acquisitions of each breast
- Method B, gr2: 2 acquisitions of each breast, and
  2 acquisitions of the non-affected breast, 3 of the affected breast (after systemic therapy)

Given the clinical nature of the described potential applications, this dose seems very acceptable as it may prevent biopsies in women with microcalcifications, and is negligible compared to radiotherapy in women with breast cancer.

13.1 Synthesis

The risks of this study are reduced as much as possible. In breast CT only the breast is exposed to radiation, sparing the rest of the chest to any significant amount of radiation. Although, these patients already get numerous mammographic views during standard clinical care. There is most probably no effect due to the level of additional radiation involved in breast
CT imaging, considering the course of treatment of the study population (mastectomy or post-surgery radiation therapy).

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