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TITLE

Bragatston study protocol: a multicenter cohort study on automated quantification of cardiovascular calcifications on radiotherapy planning CT-scans for cardiovascular risk prediction in breast cancer patients

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ABSTRACT

Introduction Cardiovascular disease (CVD) is an important cause of death in breast cancer survivors. Some breast cancer treatments including anthracyclines, trastuzumab and radiotherapy can increase the risk of CVD, especially for patients with pre-existing CVD risk factors. Early identification of patients at increased CVD risk may allow switching to less cardiotoxic treatments, active surveillance or treatment of CVD risk factors. One of the strongest independent CVD risk factors is the presence and extent of coronary artery calcifications (CAC). In clinical practice, CAC are generally quantified on electrocardiogram (ECG)-triggered cardiac computed tomography (CT)-scans. Breast cancer patients treated with radiotherapy routinely undergo radiotherapy planning CT-scans of the chest, and those scans could provide the opportunity to routinely assess CAC before a potentially cardiotoxic treatment. The Bragatston study aims to investigate the association between calcifications in the coronary arteries, aorta and heart valves (hereinafter called 'cardiovascular calcifications') measured automatically on planning CT-scans of breast cancer patients and CVD risk.

Methods and analysis In a first step, we will optimize and validate a deep learning algorithm for automated quantification of cardiovascular calcifications on planning CT-scans of breast cancer patients. Then, in a multicenter cohort study (University Medical Center Utrecht, Erasmus MC Cancer Institute, Radboudumc), the association between cardiovascular calcifications measured on planning CT-scans of breast cancer patients (n≈16,000) and incident (non-)fatal CVD events will be evaluated. To assess the added predictive value of these calcifications over traditional CVD risk factors and treatment characteristics, a case-cohort analysis will be performed among all cohort members diagnosed with a CVD event during follow-up (n≈200) and a random sample of the baseline cohort (n≈600).

Ethics and dissemination The Institutional Review Boards of the participating hospitals decided that the Medical Research Involving Human Subjects Act does not apply. Findings will be published in peer-reviewed journals and presented at conferences.

Trial registration number NCT03206333

STRENGTHS AND LIMITATIONS OF THIS STUDY

- For each patient, an individual cardiovascular risk score will be automatically calculated on routine radiotherapy planning CT-scans
- Cardiovascular calcifications will be measured using an automated deep learning algorithm in an objective, reproducible and fast manner
- A case-cohort design will be used to estimate absolute risks, which will facilitate clinical (shared) decision making
- Outcome data will be obtained through linkage with high quality national registries
- Due to the relatively short follow-up, the number of long-term cardiovascular disease events will be limited which may lead to an underestimation of the prognostic value of cardiovascular calcifications

INTRODUCTION

Over the past 25 years, breast cancer mortality rates have declined substantially following improvements in therapy and early detection due to screening.[1, 2] This, in combination with high breast cancer incidence rates, has resulted in a considerable number of breast cancer survivors.[3] In 2012, there were 6,2 million women worldwide who had been diagnosed with breast cancer in the previous five years and many of them are assumed to die of causes unrelated to breast cancer.[3, 4] This implies a strong need for research on prevention of breast cancer treatment-induced complications, such as cardiovascular diseases (CVDs).

CVD is the leading cause of death in women worldwide accounting for one third of all global female deaths in 2015.[5] Also in breast cancer patients it is an important cause of mortality.[4] Colzani et al. showed that in breast cancer patients 12% of all deaths within 10 years after diagnosis were attributed to CVD, and in the subgroup of elderly patients (>65 years) 24% of deaths were CVD-related.[6] Radiotherapy and some systemic therapies such as chemotherapy (anthracyclines) and immunotherapy (trastuzumab) can increase the risk of CVD, in particular in patients with pre-existing CVD risk factors.[7-13] Early and accurate identification of patients at increased risk of CVD, i.e. before breast cancer treatment is administered, is important to reduce the burden of CVD in breast cancer survivors.

One of the strongest independent CVD risk factors is the presence and extent of coronary artery calcifications (CAC).[14] In clinical practice, CAC are quantified on electrocardiogram (ECG)-synchronized cardiac computed tomography (CT) scans without contrast. All breast cancer patients that receive radiation therapy (>60% of breast cancer patients[15]) routinely undergo a radiotherapy (RT) planning CT-scan of the chest. Although these scans do not have the same image quality for the detection of CAC as cardiac scans due to the absence of ECG triggering and lower image resolution, it has been shown that CAC can still be routinely assessed on these scans.[16, 17] CAC was measured using an automated deep learning algorithm which has the advantage of being an objective, reproducible and fast method. One in four breast cancer patients had some degree of

CAC.[16, 17] Based on information from radiotherapy medical records, 28% of patients with severe CAC did not have other traditional CVD risk factors.[16] However, evidence on whether CAC measured on RT planning CT-scans is a predictor of CVD risk is still lacking. In addition, the prognostic value of CAC has not yet been investigated in patients with breast cancer.

If breast cancer patients at increased risk of CVD can be identified, these patients may benefit from less cardiotoxic treatment strategies, for example adaptation of RT target volumes or technique, chemotherapy dose reduction or switching to less harmful regimes, an intervention on CVD risk factors including lifestyle changes or pharmacoprevention, and from close monitoring for early detection of cardiotoxicity during and after breast cancer treatment using imaging techniques or biomarkers.[18-30] In that way the burden of CVD among breast cancer survivors could be reduced and lead to a better overall survival rate and improved quality of life.

The Bragatston study aims to investigate the association between CAC measured automatically on RT planning CT-scans using a deep learning algorithm and CVD risk among breast cancer patients. Furthermore, thoracic aorta calcifications (TAC), aortic valve calcifications (AVC) and mitral valve calcifications (MVC) will also be analyzed as they are also associated with CVD risk.[31-33] In this manuscript, we report the design of the Bragatston study.

METHODS AND ANALYSIS

Study aims

The Bragatston study is divided into three work packages (WP):

WP 1: This diagnostic package aims to optimize and validate an in-house developed automated deep learning algorithm to measure the presence and extent of CAC, TAC, AVC

and MVC (hereinafter called 'cardiovascular calcifications') on RT planning CT-scans of breast cancer patients.

WP 2: This etiological package will evaluate the association between cardiovascular calcifications measured automatically on RT planning CT-scans and the risk of (non-)fatal CVD events in breast cancer patients. It will also evaluate if the association is modified by type of (neo-)adjuvant breast cancer treatment.

WP 3: This prognostic package will assess the added value of cardiovascular calcifications measured automatically on RT planning CT-scans over traditional CVD risk factors and breast cancer treatment characteristics to predict (non-)fatal CVD events in breast cancer patients.

Study design and population

For WP 1 and 2, the Bragatston study uses a cohort design (Figure 1). The cohort will include all patients with non-metastatic primary breast cancer treated with radiotherapy at the University Medical Center Utrecht in Utrecht, the Erasmus MC Cancer Institute in Rotterdam and the Radboudumc in Nijmegen (n≈16,000), the Netherlands. Patients with a prevalent cancer diagnosis will be excluded. From these institutions RT planning CT-scans and clinical data will be collected starting from the time CT radiotherapy planning was introduced, which was in 2005 (University Medical Center Utrecht) and 2006 (Erasmus MC Cancer Institute and Radboudumc) until the end of 2016.

For WP 3, a case-cohort study will be conducted.[34] The case-cohort study will include all cohort members diagnosed with a CVD event during follow-up, called hereafter cases. In addition, a random sample will be selected at baseline from the cohort to serve as control. To increase statistical power, a case-to-control ratio of 1:3 will be applied leading to a random sample of approximately 600 patients.

Data collection procedures

Automatic calcification quantification

Cardiovascular calcifications will be measured automatically using a calcium scoring algorithm previously developed in our group.[35] The algorithm uses two consecutive convolutional neural networks (CNN) to label voxels as calcifications in the coronary arteries (left main coronary artery, left anterior descending artery, left circumflex artery and right coronary artery), as well as calcifications in the thoracic aorta and the aortic and mitral valve (leaflets and annulus). The first CNN is used on a large field of view to enable learning from contextual spatial information. This CNN is able to identify calcified voxels and label them according to their anatomical location. The second CNN uses a smaller field of view and analyses the detailed local texture. This CNN can differentiate the true atherosclerotic calcifications among the candidates detected by the first CNN (Figure 2).

The algorithm was initially developed to analyse low-dose lung cancer screening CT-scans.[35] Hence the algorithm has been modified to be able to measure calcifications on RT planning CT-scans of breast cancer patients. This procedure has been described in detail elsewhere.[17] The first results show that automatic calcification quantification is possible on RT planning CT-scans of breast cancer patients. Reproducibility of automatically versus manually measured calcium scores was high with linearly weighted kappa values ≥ 0.84 and intraclass correlation coefficients ≥ 0.94.[17] For the current project, we will further develop the method to ensure its robustness with respect to image acquisition parameters and thereby enable its applicability in multicenter settings.

CAC, TAC, AVC and MVC will be expressed in volume scores (in mm^3). For routine ECG-gated cardiac CT-scans, CAC is expressed in the Agatston score which also takes the calcification density into account.[36] The CT-scans used in this project are ungated and therefore we will report CAC as modified Agatston score.[37] These modified Agatston scores will be categorized into the Agatston classification consisting of five categories: 0, 1-10, 11-100, 101-400, >400 Agatston units.

Reference library of manual calcification quantification

Reference standard for calcium scoring will be defined by manual calcium scoring. Calcifications in the coronary arteries, the aorta and heart valves will be manually identified and labelled. As is standard procedure, 3D region growing will be used with a threshold of 130 Hounsfield Units (HU).[36] Manual annotation will be performed by observers who will be trained and supervised by a radiologist (PAJ) with more than 10 years of experience in cardiac CT. Subsequently, manually annotated calcifications will be quantified to determine calcium scores. Those reference annotations will be used to train the algorithm and to evaluate its performance.

Tumor and treatment characteristics and CVD risk factors

Tumor and breast cancer treatment data will be obtained through linkage with the Netherlands Cancer Registry (NCR) hosted by the Netherlands Comprehensive Cancer Organisation.[38]

For WP 3, detailed data on breast cancer treatment and traditional CVD risk factors present at breast cancer diagnosis will be extracted from hospital and general practice medical records. The following traditional CVD risk factor data will be collected: age, sex, hypertension, hypercholesterolaemia, diabetes, rheumatoid arthritis, smoking and body mass index. Local project members of the participating hospitals will perform linkage with the NCR and will collect medical record data.

Assessment of outcome

The primary outcome is the incidence of (non-)fatal CVD events, which is defined as hospitalization or death from CVD. Death from CVD will be recorded if it is primary cause of

death, meaning this is the disease that leads to death. CVD outcomes will be captured through linkage with Dutch Hospital Data (DHD), the Dutch Heart Registration (DHR), the Dutch Population Register (PR) and the National Cause of Death Register (CDR).

DHD collects nationwide medical and administrative data for all inpatient and day hospital-care in the Netherlands (i.e. Hospital Discharge Register). The DHD uses the International Classification of Disease 9th revision (ICD-9).[39] According to this classification, CVD will be categorized as diseases of the circulatory system (ICD-codes 390-459) and will be further subcategorized into the following subcategories: hypertensive disease (401-405), ischemic heart disease (410-414), pericarditis (420), valvular dysfunction (424), cardiomyopathy (425), arrhythmia (426-427), heart failure (428) and cerebrovascular disease (430-438) and other. Linkage with the DHD will be facilitated by Statistics Netherlands using the record identification number.[40] This number is based on a combination of date of birth, sex and postal code and is assigned to each resident in the Netherlands.

For a more complete data collection on incident CVD, additional linkage with the DHR will be performed.[41] The DHR collects data on cardiac interventions (e.g. percutaneous coronary intervention) and cardiothoracic surgery (e.g. coronary artery bypass surgery, heart valve surgery). Linkage will be performed using a combination of identifiers including date of birth, sex and maiden name.

Data on vital status will be obtained from the Dutch Population Register (PR). Causes of death will be obtained from the CDR maintained by Statistics Netherlands. The register contains information on all primary and secondary causes of death from all deceased persons registered in the Netherlands. Causes of death are classified according to ICD-10.[42] CVD mortality will be categorized as diseases of the circulatory system (ICD-codes I00-I99) and will be further subcategorized into the following subcategories: hypertensive diseases (I10-13), ischemic heart diseases (I20-I25), pericarditis (I30-32), valvular dysfunction (I34-38), cardiomyopathy (I42), arrhythmia (I44-49), heart failure (I50) and cerebrovascular diseases (I60-I69) and other. Linkage with the PR and CDR will be provided by Statistics Netherlands. Linkage will be performed by local project members of the

participating hospitals. Registries are complete until the end of 2016 (DHD and DHR) or 2017 (CDR).

Power calculation

The cohort will consist of approximately 16,000 breast cancer patients (University Medical Center Utrecht: n≈8,000; Erasmus MC Cancer Institute: n≈5,000; Radboudumc: n≈3,000). A preliminary study was conducted within the prospective breast cancer cohort Utrecht cohort for Multiple BREast cancer intervention studies and Long-term evaLuAtion (UMBRELLA).[16, 43] In total, 561 UMBRELLA patients were included and 24% of them had CAC (i.e. Agatston score>0).[16] By including at least 12,000 patients with an average follow-up of 4 years, 2,880 patients will be expected to have CAC. Assuming 4.5% risk of CVD events after 4 years of follow-up, 130 CVD events among patients with CAC are expected.[44] In the 9,120 patients without CAC, with a CVD risk of 1.5% after 4 years, 137 CVD events are expected. Based on the expected number of at least 200 cases of CVD, a maximum of 20 predictor variables can be selected for predicting CVD without risk of overfitting.[45]

Statistical analysis

For WP 1, reliability and agreement will be assessed between automatically and manually determined calcium scores. Agreement between continuous calcium scores will be assessed using Bland-Altman plots and between calcium score categories using proportional agreement. To determine reliability, intraclass correlation coefficients will be calculated for continuous calcium scores. Reliability of calcium categories will be evaluated as Cohen's linearly weighted kappa.

For WP 2, (non-)fatal CVD event rates per 1,000 person years will be calculated for each calcium score category and plotted using Kaplan-Meier survival curves. Differences

between categories will be tested with log-rank tests. Cox proportional hazard models will be used to examine the association between calcium scores and (non-)fatal CVD events. Results will be expressed as hazard ratios with their corresponding 95% confidence intervals. Follow-up time will be the underlying time variable starting from the date of RT planning CT-scan and ending at the date of diagnosis of (non-)fatal CVD event or censoring. Censored observations will include non-cardiovascular death, diagnosis of other cancers or end of follow-up, whichever came first. Models will be adjusted for age at RT planning CT-scan and calendar year of RT planning CT-scan. To assess possible effect modification of the association between cardiovascular calcifications and CVD risk by cardiotoxic chemotherapy, left-sided radiotherapy or trastuzumab, stratified analysis will be performed. If evidence for effect modification will be found, models with and without the cross-product term for calcium score and cardiotoxic treatment, will be compared using a log-likelihood ratio test. In order to assess the potential effect of competing events precluding the outcome of interest, sensitivity analyses will be conducted comprising cumulative incidence analysis and competing risk survival analysis as described by Fine and Gray.[46]

For WP 3, in univariable cox regression analysis, we will identify which patient characteristics, traditional CVD risk factors or breast cancer treatment characteristics are associated with the risk of CVD events. As proposed by Prentice, a weighted cox regression model will be applied to account for the case-cohort design.[34] Subsequently, a prediction model will be developed including patient characteristics, traditional CVD risk factors and treatment characteristics. In a second prediction model, calcium scores will be added and the incremental value of calcium scores in CVD risk prediction will be evaluated by comparing discrimination (c-statistics) and reclassification (net reclassification index). To take into account the potential effect of missing data, a sensitivity analysis will be conducted imputing missing values of traditional CVD risk factor and breast cancer treatment variables using multiple imputation.[47]

Patient and public involvement

We will conduct a survey among 100 UMBRELLA patients to explore their preferences regarding disclosure of calcium scores and corresponding CVD risk. Themes that will be included in the questionnaire are patient's knowledge about CVD risk following a breast cancer diagnosis, the patient's wish to be informed about CVD risk and preferences on way of disclosure of CVD risk. The survey will be developed in collaboration with the Dutch Patient Advocacy Group, a joint initiative from the Dutch Breast Cancer Research Group (BOOG) and the Dutch Breast Cancer Association (BVN).[48,49] We will inform breast cancer patients about the results of this project by means of newsletters and presentations at patient conferences, for example at the annual UMBRELLA patient conference.

ETHICS AND DISSEMINATION

The study protocol has been reviewed by the Institutional Review Boards (IRBs) of the University Medical Center Utrecht (reference number: 16-721/C), Erasmus MC Cancer Institute Rotterdam (MEC-2017-1125) and Radboudumc (2017-3847). The IRBs decided that the Medical Research Involving Human Subjects Act does not apply to the study. The requirement for informed consent was waived in accordance with the Code of Conduct for Medical Research developed by the Federation of Medical Scientific Societies.[50] Analyses will be performed in a secure environment of Statistics Netherlands. The dataset will be anonymized by Statistics Netherlands. The results of the Bragatston study will be published in international peer-reviewed journals and presented at scientific conferences.

DISCUSSION

The Dutch Bragatston study has been set up to optimize and validate an automated deep learning algorithm for the identification of breast cancer patients at high risk of CVD based on

the presence of cardiovascular calcifications on RT planning CT. Most breast cancer treatment guidelines and survival prediction tools mainly focus on tumor characteristics while other patient characteristics are hardly taken into account. In the era of personalized medicine, given the high burden of CVD in breast cancer patients it is critical to incorporate patient CVD risk factors in treatment decisions to find an optimal balance between cancer control and cardiotoxicity. Automated measurement of cardiovascular calcifications on RT planning CT-scans may be an elegant solution, because RT planning CT-scans are readily available imaging data and therefore there is no additional (radiation exposure) harm to patients and only a minimal financial burden to society when calcium scores are measured on these scans.

If we find that cardiovascular calcifications measured on RT planning CT-scans are predictors of CVD risk, the next step will be to investigate how to act on this information and how to disclose this information to the patient. The dilemma of disclosing calcium scores and corresponding CVD risk lies in the fact that there is no evidence yet regarding effective risk reducing interventions. Thus far, no randomized trials have been conducted on the effectiveness of calcium score-based treatment strategies with CVD morbidity and mortality reduction as outcome measure.[51] The Risk Or Benefit IN Screening for CArdiovascular diseases (ROBINSCA) trial is the first ongoing randomized controlled trial investigating the value of CAC imaging followed by preventive treatment in reducing coronary heart disease-related mortality and morbidity.[52] In the intervention arm, participants with a CAC Agatston score above 100 will be treated with statins and angiotensin converting enzyme (ACE) inhibitors, independent of their blood cholesterol level and blood pressure value. The results of the ROBINSCA trial might provide important insights potentially relevant for breast cancer patients with moderate or high CAC score.

The importance of our study lies in the possibility to introduce targeted preventive interventions to reduce treatment related CVD. Those include minimization of the mean radiotherapy heart dose, for example by application of volumetric modulated arc therapy

(VMAT) instead of the standard three dimensional conformal radiation therapy (3DCRT).[53] Furthermore, chemotherapy-induced cardiotoxicity may be reduced by switching to less harmful regimens, for example an anthracycline free regime consisting of docetaxel, carboplatin and trastuzumab has been described for human epidermal growth factor receptor 2 (HER-2) positive breast cancer as a more heart friendly alternative to the standard regimen.[19, 21, 27] Another strategy is to screen for and treat modifiable cardiovascular risk factors like high blood pressure, diabetes mellitus and high cholesterol levels.[18-20] Additionally, increased awareness needs to be generated among physicians to identify and refer breast cancer patients at high risk for CVD to a cardio-oncologist. Cardio-oncology is a new upcoming discipline focused on cardiovascular care for cancer patients which comprises CVD risk stratification, close monitoring during and after cancer treatment by means of imaging techniques or circulating biomarkers and management of a possible CVD event.[18, 20, 27]

In conclusion, over the last two decades, advances in breast cancer treatments has led to improved survival rates. However, these treatments can increase the risk of CVD. To optimize the individual benefit and risk evaluation of treatment options, we propose to evaluate the inclusion of information on patient CVD risk. Automated measurement of cardiovascular calcifications on routinely obtained RT planning CT-scans may be an inexpensive, fast and accurate solution. The Bragatston study will determine the correlation between those RT planning CT detected cardiovascular calcifications and the occurrence of CVD events.

AUTHORS' CONTRIBUTIONS

MJE, II, SGMV, HJGDB, SAMG, NL, MGAS, AJT, JP, HM, J-PP, HMV set up the study and protocols. MJE, II, SGMV, SAMG and HMV drafted the manuscript. All authors read and approved the final manuscript.

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COMPETING INTERESTS

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FIGURE TITLES AND LEGENDS

Figure 1:

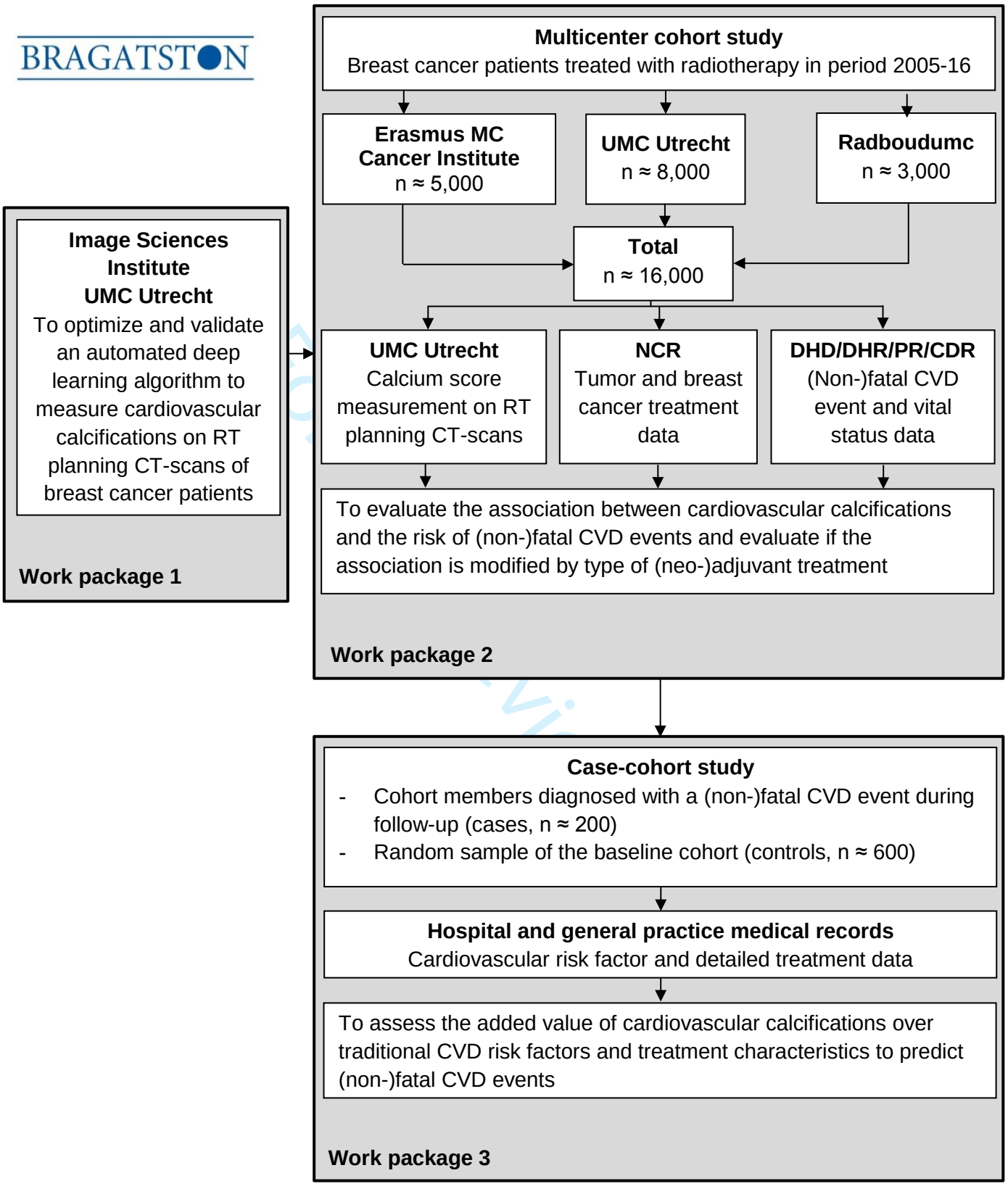
Title: Flowchart Bragatston study

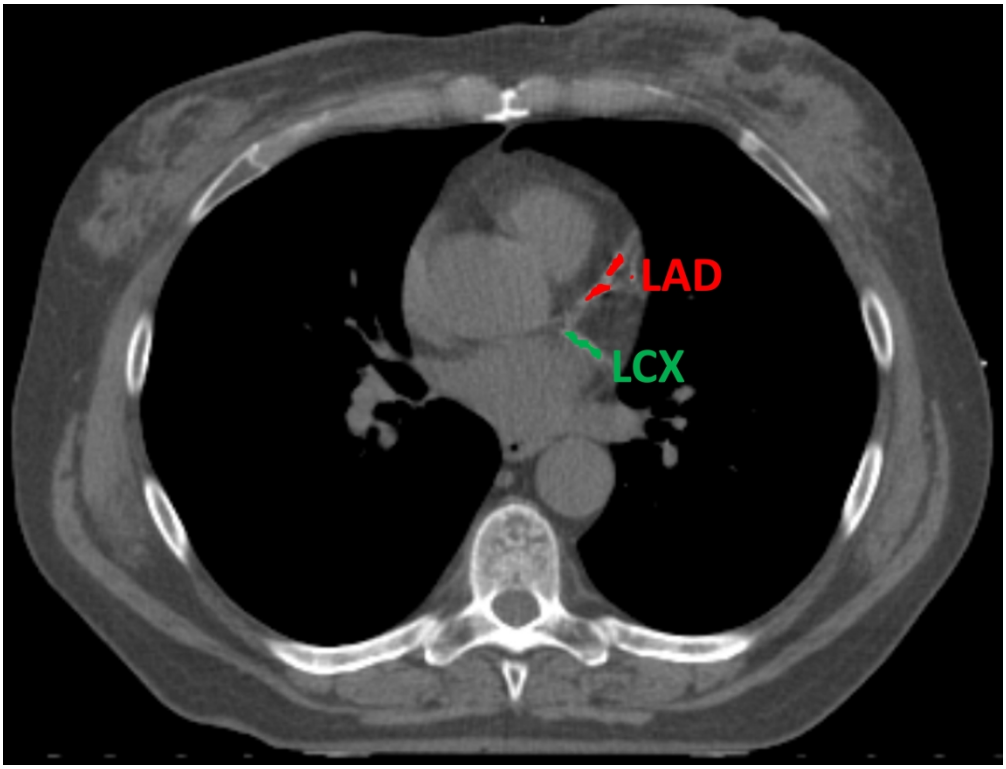
Legend: Abbreviations: CDR = National cause of death register; CT = computed tomography; CVD = cardiovascular disease; DHD = Dutch Hospital Data; DHR = Dutch Heart Registration; NCR = Netherlands Cancer Registry; PR = Dutch Population Register; RT = radiotherapy

Figure 2:

Title: Example of automatic calcification quantification on radiotherapy planning CT-scan using our deep learning algorithm

Legend: Radiotherapy planning CT-scan image showing calcifications in the left anterior descending artery (in red) and left circumflex artery (in green). Abbreviations: LAD = left anterior descending artery; LCX = left circumflex artery


BRAGATSTON
Image Sciences Institute
UMC Utrecht
To optimize and validate an automated deep learning algorithm to measure cardiovascular calcifications on RT planning CT-scans of breast cancer patients
Work package 1
Multicenter cohort study
Breast cancer patients treated with radiotherapy in period 2005-16
Erasmus MC Cancer Institute
n ≈ 5,000
UMC Utrecht
n ≈ 8,000
Radboudumc
n ≈ 3,000
Total
n ≈ 16,000
UMC Utrecht
Calcium score measurement on RT planning CT-scans
NCR
Tumor and breast cancer treatment data
DHD/DHR/PR/CDR
(Non-)fatal CVD event and vital status data
To evaluate the association between cardiovascular calcifications and the risk of (non-)fatal CVD events and evaluate if the association is modified by type of (neo-)adjuvant treatment
Work package 2
Case-cohort study
- Cohort members diagnosed with a (non-)fatal CVD event during follow-up (cases, n ≈ 200)
- Random sample of the baseline cohort (controls, n ≈ 600)
Hospital and general practice medical records
Cardiovascular risk factor and detailed treatment data
To assess the added value of cardiovascular calcifications over traditional CVD risk factors and treatment characteristics to predict (non-)fatal CVD events
Work package 3



Title: Example of automatic calcification quantification on radiotherapy planning CT-scan using our deep learning algorithm

Legend: Radiotherapy planning CT-scan image showing calcifications in the left anterior descending artery (in red) and left circumflex artery (in green). Abbreviations: LAD = left anterior descending artery; LCX = left circumflex artery

197x149mm (600 x 600 DPI)

STROBE Statement

Checklist of items that should be included in reports of observational studies

Section/Topic	Item No	Recommendation	Reported on Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1, 3
		(*b*) Provide in the abstract an informative and balanced summary of what ~~was~~ will be done ~~and what was found~~	3, 4
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	5,6
Objectives	3	State specific objectives, including any prespecified hypotheses	6
Methods			
Study design	4	Present key elements of study design early in the paper	7
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	7
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	7
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	8-11
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	8-11
Bias	9	Describe any efforts to address potential sources of bias	7, 8, 12
Study size	10	Explain how the study size was arrived at	11
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	8, 10
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	11,12
		(*b*) Describe any methods used to examine subgroups and interactions	12
		(*c*) Explain how missing data were addressed	12
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	12
		(*e*) Describe any sensitivity analyses	12

Section/Topic	Item No	Recommendation	Reported on Page No
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	NA**
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	NA**
		(b) Indicate number of participants with missing data for each variable of interest	
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	NA**
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	
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	NA**
		(*b*) Report category boundaries when continuous variables were categorized	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	NA**
Discussion			
Key results	18	Summarise key results with reference to study objectives	NA**
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	NA**
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	NA**
Generalisability	21	Discuss the generalisability (external validity) of the study results	NA**
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	16

**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.*

****Not applicable, because this paper is just about the study protocol**

BMJ Open

Bragatston study protocol: a multicenter cohort study on automated quantification of cardiovascular calcifications on radiotherapy planning CT-scans for cardiovascular risk prediction in breast cancer patients

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Secondary Subject Heading:	Cardiovascular medicine, Epidemiology, Oncology, Radiology and imaging
Keywords:	Breast cancer, Cardiovascular disease, Coronary artery calcifications, Prediction, Deep learning algorithm, Radiotherapy planning CT-scan

TITLE

Bragatston study protocol: a multicenter cohort study on automated quantification of cardiovascular calcifications on radiotherapy planning CT-scans for cardiovascular risk prediction in breast cancer patients

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KEYWORDS

Breast cancer; Cardiovascular disease; Coronary artery calcifications; Prediction; Deep learning algorithm; Radiotherapy planning CT-scan

ABSTRACT

Introduction Cardiovascular disease (CVD) is an important cause of death in breast cancer survivors. Some breast cancer treatments including anthracyclines, trastuzumab and radiotherapy can increase the risk of CVD, especially for patients with pre-existing CVD risk factors. Early identification of patients at increased CVD risk may allow switching to less cardiotoxic treatments, active surveillance or treatment of CVD risk factors. One of the strongest independent CVD risk factors is the presence and extent of coronary artery calcifications (CAC). In clinical practice, CAC are generally quantified on electrocardiogram (ECG)-triggered cardiac computed tomography (CT)-scans. Breast cancer patients treated with radiotherapy routinely undergo radiotherapy planning CT-scans of the chest, and those scans could provide the opportunity to routinely assess CAC before a potentially cardiotoxic treatment. The Bragatston study aims to investigate the association between calcifications in the coronary arteries, aorta and heart valves (hereinafter called 'cardiovascular calcifications') measured automatically on planning CT-scans of breast cancer patients and CVD risk.

Methods and analysis In a first step, we will optimize and validate a deep learning algorithm for automated quantification of cardiovascular calcifications on planning CT-scans of breast cancer patients. Then, in a multicenter cohort study (University Medical Center Utrecht, Erasmus MC Cancer Institute, Radboudumc), the association between cardiovascular calcifications measured on planning CT-scans of breast cancer patients (n≈16,000) and incident (non-)fatal CVD events will be evaluated. To assess the added predictive value of these calcifications over traditional CVD risk factors and treatment characteristics, a case-cohort analysis will be performed among all cohort members diagnosed with a CVD event during follow-up (n≈200) and a random sample of the baseline cohort (n≈600).

Ethics and dissemination The Institutional Review Boards of the participating hospitals decided that the Medical Research Involving Human Subjects Act does not apply. Findings will be published in peer-reviewed journals and presented at conferences.

Trial registration number NCT03206333

STRENGTHS AND LIMITATIONS OF THIS STUDY

- For each patient, an individual cardiovascular risk score will be automatically calculated on routine radiotherapy planning CT-scans
- Cardiovascular calcifications will be measured using an automated deep learning algorithm in an objective, reproducible and fast manner
- A case-cohort design will be used to estimate absolute risks, which will facilitate clinical (shared) decision making
- Outcome data will be obtained through linkage with high quality national registries
- Due to the relatively short follow-up, the number of long-term cardiovascular disease events will be limited which may lead to an underestimation of the prognostic value of cardiovascular calcifications

INTRODUCTION

Over the past 25 years, breast cancer mortality rates have declined substantially following improvements in therapy and early detection due to screening.[1, 2] This, in combination with high breast cancer incidence rates, has resulted in a considerable number of breast cancer survivors.[3] In 2012, there were 6,2 million women worldwide who had been diagnosed with breast cancer in the previous five years and many of them are assumed to die of causes unrelated to breast cancer.[3, 4] This implies a strong need for research on prevention of breast cancer treatment-induced complications, such as cardiovascular diseases (CVDs).

CVD is the leading cause of death in women worldwide accounting for one third of all global female deaths in 2015.[5] Also in breast cancer patients it is an important cause of mortality.[4] Colzani et al. showed that in breast cancer patients 12% of all deaths within 10 years after diagnosis were attributed to CVD, and in the subgroup of elderly patients (>65 years) 24% of deaths were CVD-related.[6] Radiotherapy and some systemic therapies such as chemotherapy (anthracyclines) and immunotherapy (trastuzumab) can increase the risk of CVD, in particular in patients with pre-existing CVD risk factors.[7-13] Early and accurate identification of patients at increased risk of CVD, i.e. before breast cancer treatment is administered, is important to reduce the burden of CVD in breast cancer survivors.

One of the strongest independent CVD risk factors is the presence and extent of coronary artery calcifications (CAC).[14] In clinical practice, CAC are quantified on electrocardiogram (ECG)-synchronized cardiac computed tomography (CT) scans without contrast. All breast cancer patients that receive radiation therapy (>60% of breast cancer patients[15]) routinely undergo a radiotherapy (RT) planning CT-scan of the chest. Although these scans do not have the same image quality for the detection of CAC as cardiac scans due to the absence of ECG triggering and lower image resolution, it has been shown that CAC can still be routinely assessed on these scans.[16, 17] CAC was measured using an automated deep learning algorithm which has the advantage of being an objective, reproducible and fast method. One in four breast cancer patients had some degree of CAC.[16, 17] Based on information from

radiotherapy medical records, 28% of patients with severe CAC did not have other traditional CVD risk factors.[16] However, evidence on whether CAC measured on RT planning CT-scans is a predictor of CVD risk is still lacking. In addition, the prognostic value of CAC has not yet been investigated in patients with breast cancer.

If breast cancer patients at increased risk of CVD can be identified, these patients may benefit from less cardiotoxic treatment strategies, for example adaptation of RT target volumes or technique, chemotherapy dose reduction or switching to less harmful regimes, an intervention on CVD risk factors including lifestyle changes or pharmacoprevention, and from close monitoring for early detection of cardiotoxicity during and after breast cancer treatment using imaging techniques or biomarkers.[18-30] In that way the burden of CVD among breast cancer survivors could be reduced and lead to a better overall survival rate and improved quality of life.

The Bragatston study aims to investigate the association between CAC measured automatically on RT planning CT-scans using a deep learning algorithm and CVD risk among breast cancer patients. Furthermore, thoracic aorta calcifications (TAC), aortic valve calcifications (AVC) and mitral valve calcifications (MVC) will also be analyzed as they are also associated with CVD risk.[31-33] In this manuscript, we report the design of the Bragatston study.

METHODS AND ANALYSIS

Study aims

The Bragatston study is divided into three work packages (WP):

WP 1: This diagnostic package aims to optimize and validate an in-house developed automated deep learning algorithm to measure the presence and extent of CAC, TAC, AVC and MVC (hereinafter called 'cardiovascular calcifications') on RT planning CT-scans of breast cancer patients.

WP 2: This etiological package will evaluate the association between cardiovascular calcifications measured automatically on RT planning CT-scans and the risk of (non-)fatal CVD events in breast cancer patients. It will also evaluate if the association is modified by type of (neo-)adjuvant breast cancer treatment.

WP 3: This prognostic package will assess the added value of cardiovascular calcifications measured automatically on RT planning CT-scans over traditional CVD risk factors and breast cancer treatment characteristics to predict (non-)fatal CVD events in breast cancer patients.

Study design and population

For WP 1 and 2, the Bragatston study uses a cohort design (Figure 1). The cohort will include all patients with non-metastatic primary breast cancer treated with radiotherapy at the University Medical Center Utrecht in Utrecht, the Erasmus MC Cancer Institute in Rotterdam and the Radboudumc in Nijmegen (n≈16,000), the Netherlands. Patients with a prevalent cancer diagnosis will be excluded. From these institutions RT planning CT-scans and clinical data will be collected starting from the time CT radiotherapy planning was introduced, which was in 2005 (University Medical Center Utrecht) and 2006 (Erasmus MC Cancer Institute and Radboudumc) until the end of 2016. The RT planning CT-scans that will be collected are acquired as part of clinical routine (no contrast enhancement, no ECG-triggering, 120 kVp, in-plane resolution 0.78-1.37 mm, 3.0 mm slice thickness, 3.0 mm increment).

For WP 3, a case-cohort study will be conducted.[34] The case-cohort study will include all cohort members diagnosed with a CVD event during follow-up, called hereafter cases. In addition, a random sample will be selected at baseline from the cohort to serve as control. To increase statistical power, a case-to-control ratio of 1:3 will be applied leading to a random sample of approximately 600 patients. The power gained by including more than three controls to one case is little.

Data collection procedures

Automatic calcification quantification

Cardiovascular calcifications will be measured automatically using a calcium scoring algorithm previously developed in our group.[35] The algorithm uses two consecutive convolutional neural networks (CNN) to label voxels as calcifications in the coronary arteries (left main coronary artery, left anterior descending artery, left circumflex artery and right coronary artery), as well as calcifications in the thoracic aorta and the aortic and mitral valve (leaflets and annulus). The first CNN is used on a large field of view to enable learning from contextual spatial information. This CNN is able to identify calcified voxels and label them according to their anatomical location. The second CNN uses a smaller field of view and analyses the detailed local texture. This CNN can differentiate the true atherosclerotic calcifications among the candidates detected by the first CNN (Figures 2-5).

The algorithm was initially developed to analyse low-dose lung cancer screening CT-scans.[35] Hence the algorithm has been modified to be able to measure calcifications on RT planning CT-scans of breast cancer patients. This procedure has been described in detail elsewhere.[17] The first results show that automatic calcification quantification is possible on RT planning CT-scans of breast cancer patients. Reproducibility of automatically versus manually measured calcium scores was high with linearly weighted kappa values ≥ 0.84 and intraclass correlation coefficients ≥ 0.94.[17] For the current project, we will further develop the method to ensure its robustness with respect to image acquisition parameters and thereby enable its applicability in multicenter settings.

CAC, TAC, AVC and MVC will be expressed in volume scores (in mm^3). For routine ECG-gated cardiac CT-scans, CAC is expressed in the Agatston score which also takes the calcification density into account.[36] The CT-scans used in this project are ungated and therefore we will report CAC as modified Agatston score.[37] These modified Agatston scores will be calculated by multiplying the calcification area (in mm^2) by the density score (1, 130–199 Hounsfield Units (HU); 2, 200–299 HU; 3, 300–399 HU; 4, > 399 HU) of the area

(calcification density) and summing the lesion scores, in which a minimal lesion definition of 1.5 mm^3 will be maintained to eliminate noise. Based on these scores, patients will be categorized into the Agatston classification consisting of five categories: 0, 1-10, 11-100, 101-400, >400 Agatston units.

Reference library of manual calcification quantification

Reference standard for calcium scoring will be defined by manual calcium scoring. Manual calcium scoring will be done in a subset of planning CT-scans randomly selected per hospital (UMC Utrecht: n=500; the Erasmus MC Cancer Institute: n=300; Radboudumc: n=200). Calcifications in the coronary arteries, the aorta and heart valves will be manually identified and labelled. As is standard procedure, 3D region growing will be used with a threshold of 130 HU.[36] Manual annotation will be performed by observers who will be trained and supervised by a radiologist (PAJ) with more than 10 years of experience in cardiac CT. Subsequently, manually annotated calcifications will be quantified to determine calcium scores. In line with the automatic calcification quantification, CAC, TAC, AVC and MVC will be expressed in volume scores (in mm^3). The modified Agatston scores will be calculated as described in the previous section. Those reference annotations will be used to train the algorithm and to evaluate its performance.

Tumor and treatment characteristics and CVD risk factors

Tumor and breast cancer treatment data will be obtained through linkage with the Netherlands Cancer Registry (NCR) hosted by the Netherlands Comprehensive Cancer Organisation.[38] Tumor data variables include tumor stage, grade and receptor status and treatment data variables include type of surgery (breast conserving therapy, mastectomy), radiotherapy (laterality and radiation fields (if available)), chemotherapy (yes, no), hormonal therapy (yes, no) and immunotherapy (yes, no).

For WP 3, detailed data on breast cancer treatment and traditional CVD risk factors present at breast cancer diagnosis will be extracted from hospital and general practice medical records. The following traditional CVD risk factor data will be collected: age, sex, hypertension, hypercholesterolaemia, diabetes, smoking and body mass index. Regarding hypertension, hypercholesterolaemia, diabetes and smoking, a patient will be scored positive when the risk factor is documented in the hospital medical record or reported by the GP by means of a questionnaire. Local project members of the participating hospitals will perform linkage with the NCR and will collect medical record data.

Assessment of outcome

The primary outcome is the incidence of (non-)fatal CVD events, which is defined as hospitalization or death from CVD. Death from CVD will be recorded if it is primary cause of death, meaning this is the disease that leads to death. CVD outcomes will be captured through linkage with Dutch Hospital Data (DHD), the Dutch Heart Registration (DHR), the Dutch Population Register (PR) and the National Cause of Death Register (CDR).

DHD collects nationwide medical and administrative data for all inpatient and day hospital-care in the Netherlands (i.e. Hospital Discharge Register). The DHD uses the International Classification of Disease 9th revision (ICD-9).[39] According to this classification, CVD will be categorized as diseases of the circulatory system (ICD-codes 390-459) and will be further subcategorized into the following subcategories: hypertensive disease (401-405), ischemic heart disease (410-414), pericarditis (420), valvular dysfunction (424), cardiomyopathy (425), arrhythmia (426-427), heart failure (428) and cerebrovascular disease (430-438) and other. Linkage with the DHD will be facilitated by Statistics Netherlands using the record identification number.[40] This number is based on a combination of date of birth, sex and postal code and is assigned to each resident in the Netherlands.

For a more complete data collection on incident CVD, additional linkage with the DHR will be performed.[41] The DHR collects data on cardiac interventions (e.g. percutaneous coronary

intervention) and cardiothoracic surgery (e.g. coronary artery bypass surgery, heart valve surgery). Linkage will be performed using a combination of identifiers including date of birth, sex and maiden name.

Data on vital status will be obtained from the Dutch Population Register (PR). Causes of death will be obtained from the CDR maintained by Statistics Netherlands. The register contains information on all primary and secondary causes of death from all deceased persons registered in the Netherlands. Causes of death are classified according to ICD-10.[42] CVD mortality will be categorized as diseases of the circulatory system (ICD-codes I00-I99) and will be further subcategorized into the following subcategories: hypertensive diseases (I10-13), ischemic heart diseases (I20-I25), pericarditis (I30-32), valvular dysfunction (I34-38), cardiomyopathy (I42), arrhythmia (I44-49), heart failure (I50) and cerebrovascular diseases (I60-I69) and other. Linkage with the PR and CDR will be provided by Statistics Netherlands. Linkage will be performed by local project members of the participating hospitals. Registries are complete until the end of 2016 (DHD and DHR) or 2017 (CDR).

Power calculation

The cohort will consist of approximately 16,000 breast cancer patients (University Medical Center Utrecht: n≈8,000; Erasmus MC Cancer Institute: n≈5,000; Radboudumc: n≈3,000). A preliminary study was conducted within the prospective breast cancer cohort Utrecht cohort for Multiple BREast cancer intervention studies and Long-term evaLuAtion (UMBRELLA).[16, 43] In total, 561 UMBRELLA patients were included and 24% of them had CAC (i.e. Agatston score>0).[16] By including at least 12,000 patients with an average follow-up of 4 years, 2,880 patients will be expected to have CAC. Assuming 4.5% risk of CVD events after 4 years of follow-up, 130 CVD events among patients with CAC are expected.[44] In the 9,120 patients without CAC, with a CVD risk of 1.5% after 4 years, 137 CVD events are expected. Based on the expected number of at least 200 cases of CVD, a maximum of 20 predictor variables can be selected for predicting CVD without risk of overfitting.[45]

Statistical analysis

For WP 1, reliability and agreement will be assessed between automatically and manually determined calcium scores. Results will be presented for the total sample of 1,000 manually and automatically assessed planning CT-scans. In addition, results will be stratified by participating hospital. Agreement between continuous calcium scores will be assessed using Bland-Altman plots and between calcium score categories using proportional agreement. To determine reliability, intraclass correlation coefficients will be calculated for continuous calcium scores. Reliability of calcium categories will be evaluated as Cohen's linearly weighted kappa.

For WP 2, (non-)fatal CVD event rates per 1,000 person years will be calculated for each calcium score category and plotted using Kaplan-Meier survival curves. Differences between categories will be tested with log-rank tests. Cox proportional hazard models will be used to examine the association between calcium scores and (non-)fatal CVD events. Results will be expressed as hazard ratios with their corresponding 95% confidence intervals. Follow-up time will be the underlying time variable starting from the date of RT planning CT-scan and ending at the date of diagnosis of (non-)fatal CVD event or censoring. Censored observations will include non-cardiovascular death, diagnosis of other cancers or end of follow-up, whichever came first. Models will be adjusted for age at RT planning CT-scan and calendar year of RT planning CT-scan. To assess possible effect modification of the association between cardiovascular calcifications and CVD risk by cardiotoxic chemotherapy, left-sided radiotherapy or trastuzumab, stratified analysis will be performed. If evidence for effect modification will be found, models with and without the cross-product term for calcium score and cardiotoxic treatment, will be compared using a log-likelihood ratio test. In order to assess the potential effect of competing events precluding the outcome of interest, sensitivity analyses will be conducted comprising cumulative incidence analysis and competing risk survival analysis as described by Fine and Gray.[46]

For WP 3, in univariable cox regression analysis, we will identify which patient characteristics, traditional CVD risk factors or breast cancer treatment characteristics are associated with the risk of CVD events. As proposed by Prentice, a weighted cox regression model will be applied to account for the case-cohort design.[34] Subsequently, a prediction model will be developed including patient characteristics, traditional CVD risk factors and treatment characteristics. In a second prediction model, calcium scores will be added and the incremental value of calcium scores in CVD risk prediction will be evaluated by comparing discrimination (c-statistics) and reclassification (net reclassification index). To take into account the potential effect of missing data, a sensitivity analysis will be conducted imputing missing values of traditional CVD risk factor and breast cancer treatment variables using multiple imputation.[47]

Patient and public involvement

We will conduct a survey among 100 UMBRELLA patients to explore their preferences regarding disclosure of calcium scores and corresponding CVD risk. Themes that will be included in the questionnaire are patient's knowledge about CVD risk following a breast cancer diagnosis, the patient's wish to be informed about CVD risk and preferences on way of disclosure of CVD risk. The survey will be developed in collaboration with the Dutch Patient Advocacy Group, a joint initiative from the Dutch Breast Cancer Research Group (BOOG) and the Dutch Breast Cancer Association (BVN).[48,49] We will inform breast cancer patients about the results of this project by means of newsletters and presentations at patient conferences, for example at the annual UMBRELLA patient conference.

Timeline

Data collection started in January 2017 and we expect to complete data collection in December 2019. The estimated end date of the study is March 2020.

ETHICS AND DISSEMINATION

The study protocol has been reviewed by the Institutional Review Boards (IRBs) of the University Medical Center Utrecht (reference number: 16-721/C), Erasmus MC Cancer Institute Rotterdam (MEC-2017-1125) and Radboudumc (2017-3847). The IRBs decided that the Medical Research Involving Human Subjects Act does not apply to the study. The requirement for informed consent was waived in accordance with the Code of Conduct for Medical Research developed by the Federation of Medical Scientific Societies.[50] All data, with the exception of data provided by Statistics Netherlands, will be stored centrally at the University Medical Center Utrecht. This dataset will be sent to Statistics Netherlands for additional linkage. Analyses will be performed in a secure environment of Statistics Netherlands. The dataset will be anonymized by Statistics Netherlands. The results of the Bragatston study will be published in international peer-reviewed journals and presented at scientific conferences.

DISCUSSION

The Dutch Bragatston study has been set up to optimize and validate an automated deep learning algorithm for the identification of breast cancer patients at high risk of CVD based on the presence of cardiovascular calcifications on RT planning CT. Most breast cancer treatment guidelines and survival prediction tools mainly focus on tumor characteristics while other patient characteristics are hardly taken into account. In the era of personalized medicine, given the high burden of CVD in breast cancer patients it is critical to incorporate patient CVD risk factors in treatment decisions to find an optimal balance between cancer control and

cardiotoxicity. Automated measurement of cardiovascular calcifications on RT planning CT-scans may be an elegant solution, because RT planning CT-scans are readily available imaging data and therefore there is no additional (radiation exposure) harm to patients and only a minimal financial burden to society when calcium scores are measured on these scans.

If we find that cardiovascular calcifications measured on RT planning CT-scans are predictors of CVD risk, the next step will be to investigate how to act on this information and how to disclose this information to the patient. The dilemma of disclosing calcium scores and corresponding CVD risk lies in the fact that there is no evidence yet regarding effective risk reducing interventions. Thus far, no randomized trials have been conducted on the effectiveness of calcium score-based treatment strategies with CVD morbidity and mortality reduction as outcome measure.[51] The Risk Or Benefit IN Screening for CArdiovascular diseases (ROBINSCA) trial is the first ongoing randomized controlled trial investigating the value of CAC imaging followed by preventive treatment in reducing coronary heart disease-related mortality and morbidity.[52] In the intervention arm, participants with a CAC Agatston score above 100 will be treated with statins and angiotensin converting enzyme (ACE) inhibitors, independent of their blood cholesterol level and blood pressure value. The results of the ROBINSCA trial might provide important insights potentially relevant for breast cancer patients with moderate or high CAC score.

The importance of our study lies in the possibility to introduce targeted preventive interventions to reduce treatment related CVD. Those include minimization of the mean radiotherapy heart dose, for example by application of volumetric modulated arc therapy (VMAT) instead of the standard three dimensional conformal radiation therapy (3DCRT).[53] Furthermore, chemotherapy-induced cardiotoxicity may be reduced by switching to less harmful regimens, for example an anthracycline free regime consisting of docetaxel, carboplatin and trastuzumab has been described for human epidermal growth factor receptor 2 (HER-2) positive breast cancer as a more heart friendly alternative to the standard regimen.[19, 21, 27] Another strategy is to screen for and treat modifiable cardiovascular risk

factors like high blood pressure, diabetes mellitus and high cholesterol levels.[18-20] Additionally, increased awareness needs to be generated among physicians to identify and refer breast cancer patients at high risk for CVD to a cardio-oncologist. Cardio-oncology is a new upcoming discipline focused on cardiovascular care for cancer patients which comprises CVD risk stratification, close monitoring during and after cancer treatment by means of imaging techniques or circulating biomarkers and management of a possible CVD event.[18, 20, 27]

In conclusion, over the last two decades, advances in breast cancer treatments has led to improved survival rates. However, these treatments can increase the risk of CVD. To optimize the individual benefit and risk evaluation of treatment options, we propose to evaluate the inclusion of information on patient CVD risk. Automated measurement of cardiovascular calcifications on routinely obtained RT planning CT-scans may be an inexpensive, fast and accurate solution. The Bragatston study will determine the correlation between those RT planning CT detected cardiovascular calcifications and the occurrence of CVD events.

AUTHORS' CONTRIBUTIONS

MJE, II, SGMV, HJGDB, SAMG, NL, MGAS, AJT, JP, HM, J-PP, HMV set up the study and protocols. MJE, II, SGMV, SAMG and HMV drafted the manuscript. All authors read and approved the final manuscript.

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COMPETING INTERESTS

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FIGURE TITLES AND LEGENDS

Figure 1:

Title: Flowchart Bragatston study

Legend: Abbreviations: CDR = National cause of death register; CT = computed tomography; CVD = cardiovascular disease; DHD = Dutch Hospital Data; DHR = Dutch Heart Registration; NCR = Netherlands Cancer Registry; PR = Dutch Population Register; RT = radiotherapy

Figure 2:

Title: Example of automatic calcification quantification on radiotherapy planning CT-scan using our deep learning algorithm

Legend: Radiotherapy planning CT-scan image showing calcifications in the left anterior descending artery (in red) and left circumflex artery (in green). Abbreviations: LAD = left anterior descending artery; LCX = left circumflex artery

Figure 3:

Title: Example of automatic calcification quantification on radiotherapy planning CT-scan using our deep learning algorithm

Legend: Radiotherapy planning CT-scan image showing thoracic aorta calcifications (in yellow).

Figure 4:

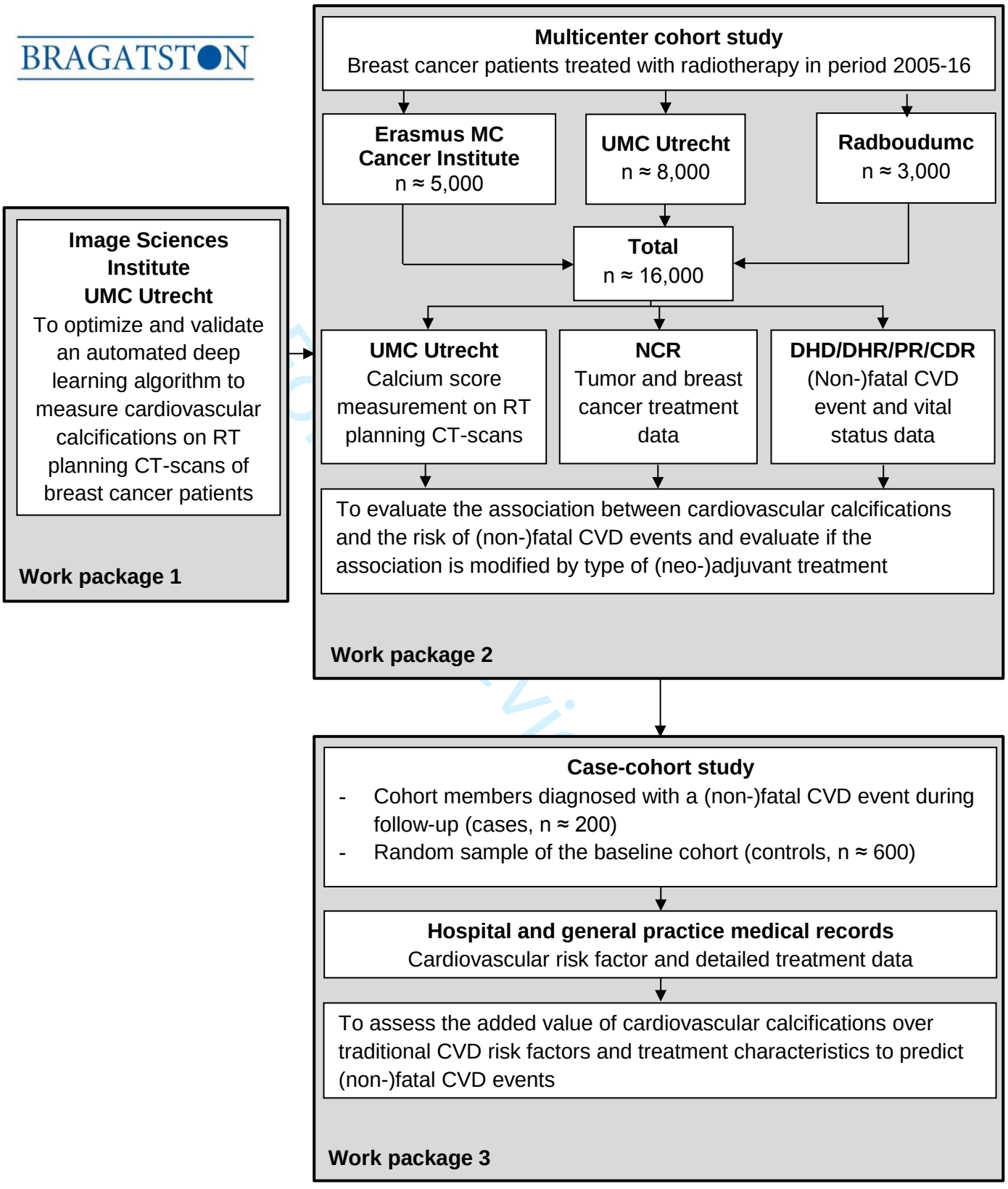
Title: Example of automatic calcification quantification on radiotherapy planning CT-scan using our deep learning algorithm

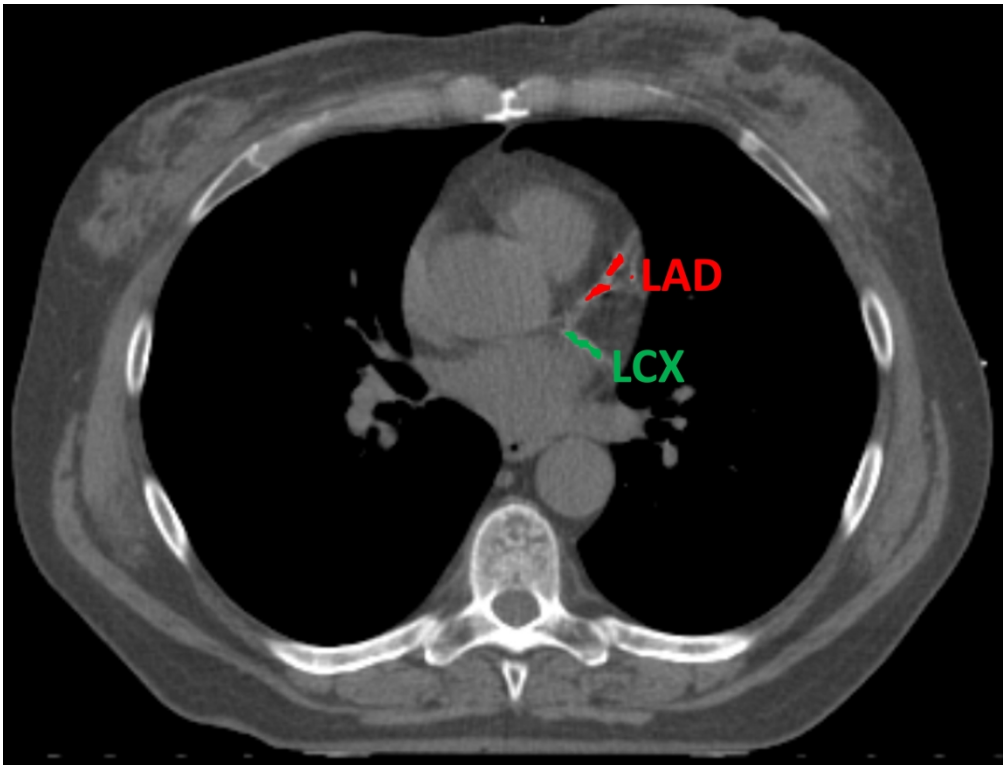
Legend: Radiotherapy planning CT-scan image showing mitral valve calcifications (in orange) and thoracic aorta calcifications (in yellow). Abbreviation: MV = mitral valve

Figure 5:

Title: Example of automatic calcification quantification on radiotherapy planning CT-scan using our deep learning algorithm

Legend: Radiotherapy planning CT-scan image showing aortic valve calcifications (in purple) and thoracic aorta calcifications (in yellow). Abbreviation: AV = aortic valve

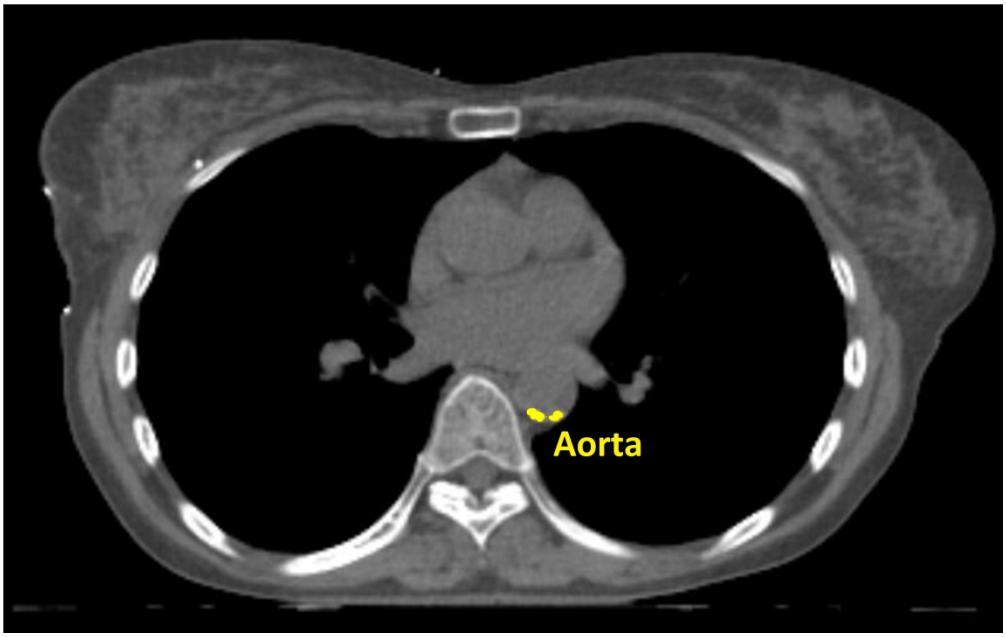

BRAGATSTON
Image Sciences Institute
UMC Utrecht
To optimize and validate an automated deep learning algorithm to measure cardiovascular calcifications on RT planning CT-scans of breast cancer patients
Work package 1
Multicenter cohort study
Breast cancer patients treated with radiotherapy in period 2005-16
Erasmus MC Cancer Institute
n ≈ 5,000
UMC Utrecht
n ≈ 8,000
Radboudumc
n ≈ 3,000
Total
n ≈ 16,000
UMC Utrecht
Calcium score measurement on RT planning CT-scans
NCR
Tumor and breast cancer treatment data
DHD/DHR/PR/CDR
(Non-)fatal CVD event and vital status data
To evaluate the association between cardiovascular calcifications and the risk of (non-)fatal CVD events and evaluate if the association is modified by type of (neo-)adjuvant treatment
Work package 2
Case-cohort study
- Cohort members diagnosed with a (non-)fatal CVD event during follow-up (cases, n ≈ 200)
- Random sample of the baseline cohort (controls, n ≈ 600)
Hospital and general practice medical records
Cardiovascular risk factor and detailed treatment data
To assess the added value of cardiovascular calcifications over traditional CVD risk factors and treatment characteristics to predict (non-)fatal CVD events
Work package 3



Title: Example of automatic calcification quantification on radiotherapy planning CT-scan using our deep learning algorithm

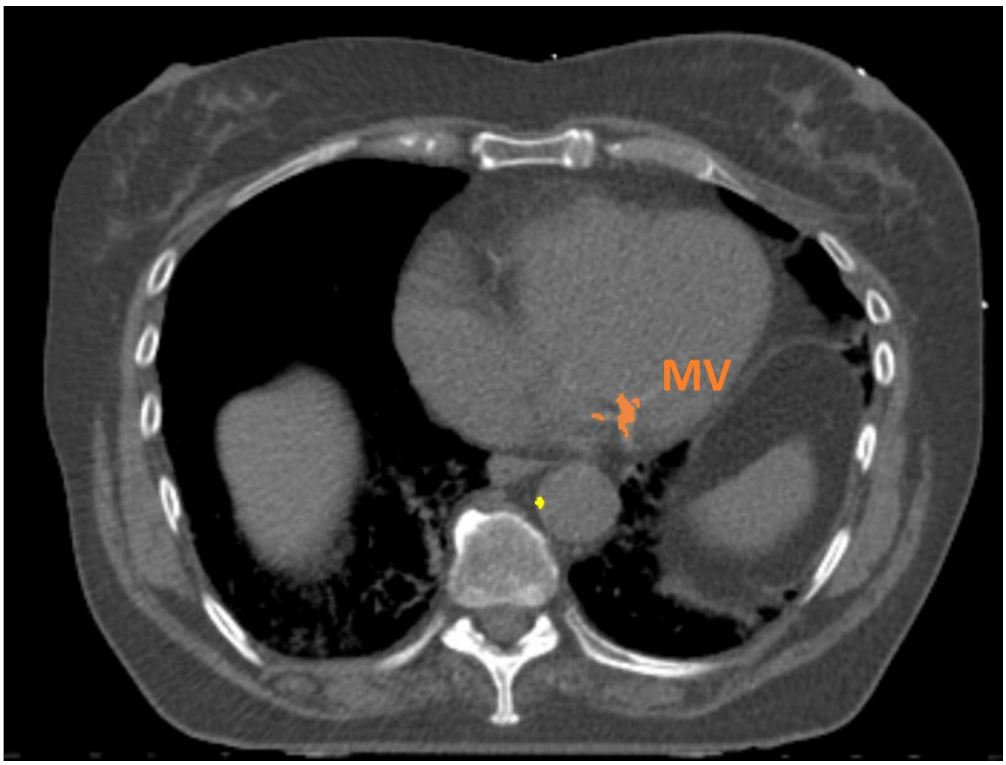
Legend: Radiotherapy planning CT-scan image showing calcifications in the left anterior descending artery (in red) and left circumflex artery (in green). Abbreviations: LAD = left anterior descending artery; LCX = left circumflex artery

197x149mm (600 x 600 DPI)



Example of automatic calcification quantification on radiotherapy planning CT-scan using our deep learning algorithm
Legend: Radiotherapy planning CT-scan image showing thoracic aorta calcifications (in yellow).

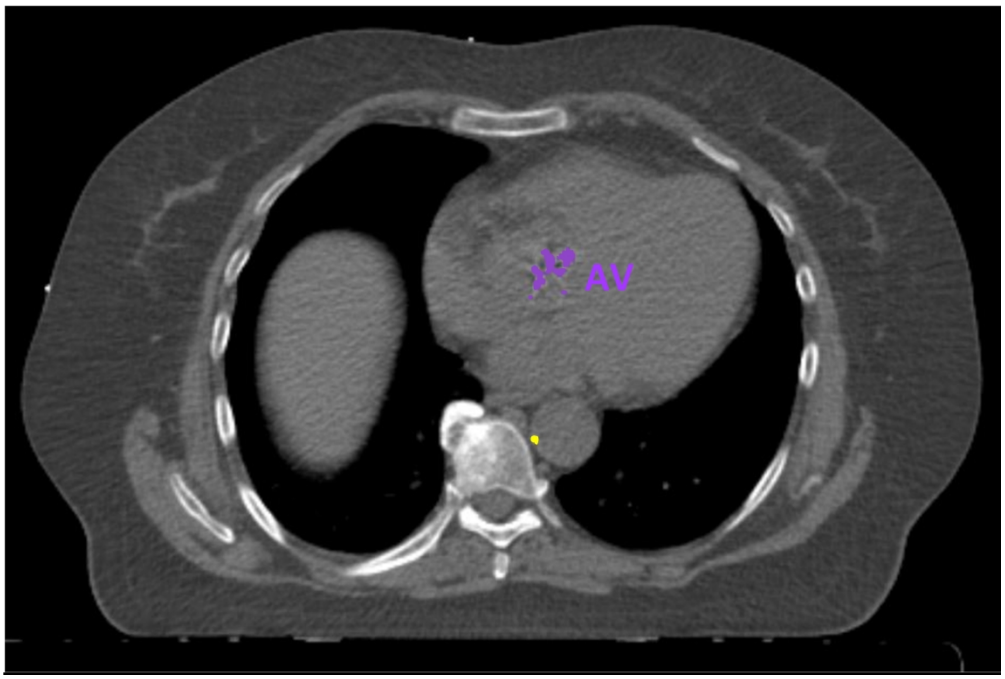
206x129mm (600 x 600 DPI)



Title: Example of automatic calcification quantification on radiotherapy planning CT-scan using our deep learning algorithm

Legend: Radiotherapy planning CT-scan image showing mitral valve calcifications (in orange) and thoracic aorta calcifications (in yellow). Abbreviation: MV = mitral valve

164x124mm (600 x 600 DPI)



Title: Example of automatic calcification quantification on radiotherapy planning CT-scan using our deep learning algorithm
Legend: Radiotherapy planning CT-scan image showing aortic valve calcifications (in purple) and thoracic aorta calcifications (in yellow). Abbreviation: AV = aortic valve

192x128mm (600 x 600 DPI)

STROBE Statement

Checklist of items that should be included in reports of observational studies

Section/Topic	Item No	Recommendation	Reported on Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1, 3
		(*b*) Provide in the abstract an informative and balanced summary of what ~~was~~ will be done ~~and what was found~~	3, 4
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	5,6
Objectives	3	State specific objectives, including any prespecified hypotheses	6
Methods			
Study design	4	Present key elements of study design early in the paper	7
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	7,13,14
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	7
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	8-11
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	8-11
Bias	9	Describe any efforts to address potential sources of bias	7, 8, 12
Study size	10	Explain how the study size was arrived at	11
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	8, 10
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	12,13
		(*b*) Describe any methods used to examine subgroups and interactions	12,13
		(*c*) Explain how missing data were addressed	13
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	12,13

		(*e*) Describe any sensitivity analyses	13

Section/Topic	Item No	Recommendation	Reported on Page No
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	NA**
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	NA**
		(b) Indicate number of participants with missing data for each variable of interest	
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	NA**
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	
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	NA**
		(*b*) Report category boundaries when continuous variables were categorized	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	NA**
Discussion			
Key results	18	Summarise key results with reference to study objectives	NA**
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	NA**
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	NA**
Generalisability	21	Discuss the generalisability (external validity) of the study results	NA**
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	16

**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.*

**Not applicable, because this paper is just about the study protocol