Acta Universitatis Tamperensis 2230

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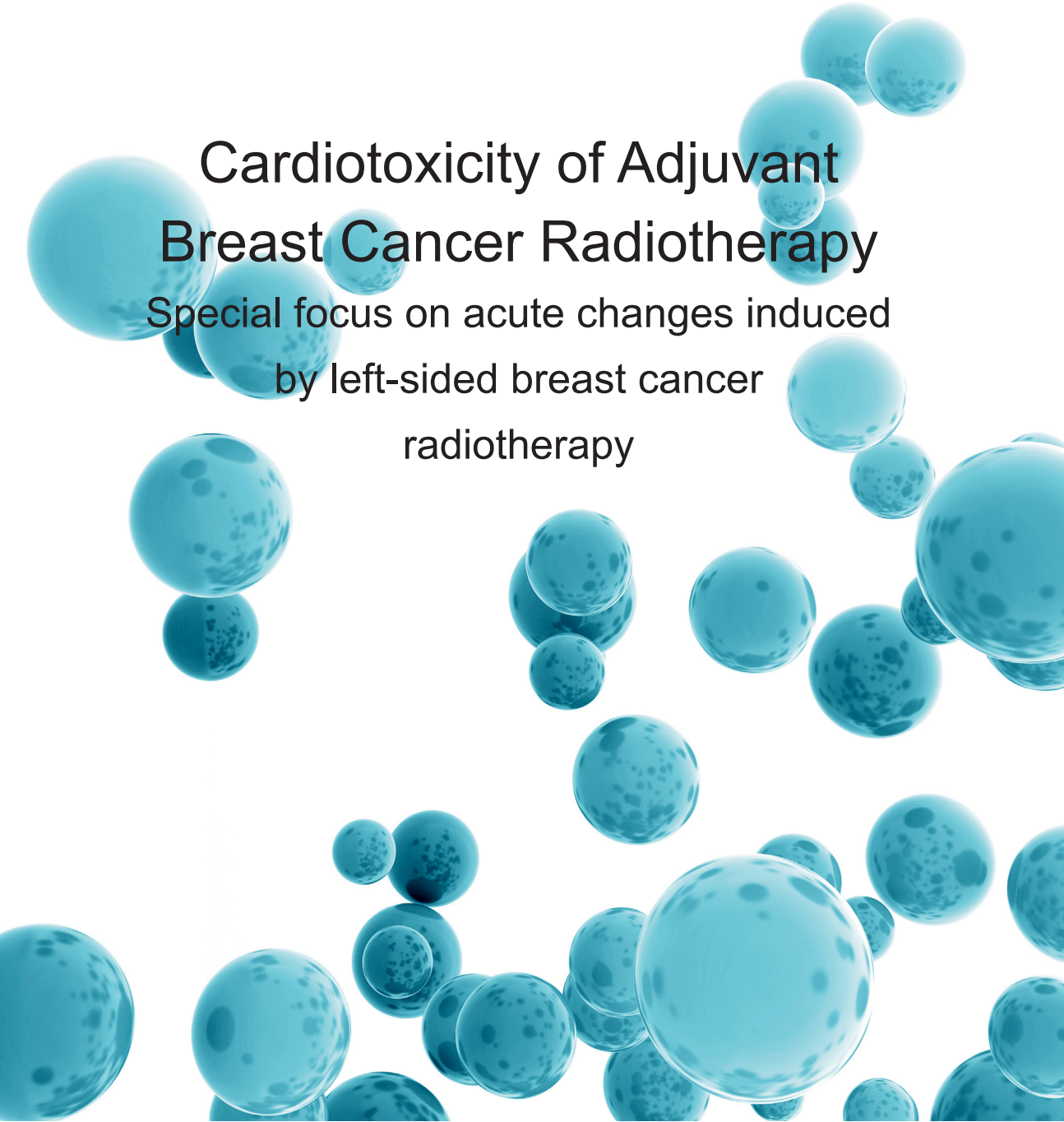
Cardiotoxicity of Adjuvant Breast Cancer Radiotherapy

Special focus on acute changes induced by left-sided breast cancer radiotherapy



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Special focus on acute changes induced by left-sided breast cancer radiotherapy

ACADEMIC DISSERTATION
To be presented, with the permission of
the Board of the School of Medicine of the University of Tampere,
for public discussion in the auditorium F115 of the Arvo building,
Lääkärinkatu 1, Tampere,
on 2 December 2016, at 12 o'clock.

UNIVERSITY OF TAMPERE

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Acta Universitatis Tamperensis 2230
Tampere University Press
Tampere 2016

UNIVERSITY OF TAMPERE

ACADEMIC DISSERTATION
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The originality of this thesis has been checked using the Turnitin OriginalityCheck service in accordance with the quality management system of the University of Tampere.

Cover design by
Mikko Reinikka

Acta Universitatis Tamperensis 2230
ISBN 978-952-03-0274-0 (print)
ISSN-L 1455-1616
ISSN 1455-1616

Acta Electronica Universitatis Tamperensis 1730
ISBN 978-952-03-0275-7 (pdf)
ISSN 1456-954X
http://tampub.uta.fi

Suomen Yliopistopaino Oy – Juvenes Print
Tampere 2016

Contents

Abstract

Breast cancer is the most common cancer in women. Due to early diagnosis, surgery and improvements in adjuvant treatment options, the prognosis of this disease today is excellent, as breast cancer has a 5-year survival rate of 90%. In Finland, more than 67 000 breast cancer survivors were alive in 2015 according to Finnish Cancer registry data.

However, adjuvant cancer treatments may cause side effects, which diminish the later quality of life. In addition, long-term treatment-related side effects cause increases in non-breast cancer-related morbidities and mortality. Adjuvant breast cancer radiotherapy, especially left-sided radiotherapy, is known to cause later cardiac morbidity and mortality. Clinically detectable radiation-induced cardiotoxicity begins emerging after 5-10 years and presents clinically as coronary artery disease, valvular dysfunction or heart failure.

Exposure of heart to adjacent cardiac radiation during left-sided adjuvant breast cancer radiotherapy is shown to dose-dependently increase the risk of later cardiovascular diseases. No safe cardiac radiation dose has been identified. In addition, other patient and treatment related factors, such as prior chemotherapy, obesity, hypertension and smoking, affect the later cardiac risk.

This study was designed to evaluate the acute effects of radiation and concomitant or prior medication therapy on cardiac function and biomarkers and to enhance cardioprotection during adjuvant breast radiotherapy. In left-sided breast cancer patients, we observed subclinical changes in echocardiographic parameters already immediately after radiotherapy. In addition, we observed that concomitant aromatase inhibitor use induced more profound changes in echocardiography parameters than radiotherapy alone. Myocardial damage, as measured via serum troponin levels, was observed in 20% of patients, and the release was correlated with higher cardiac radiation doses. Furthermore, the daily reproducibility of the heart-sparing deep inspiration breath hold radiotherapy was improved with a new marker block placement and correction of the breath hold

level by image guidance. These procedures led to a significant decrease in cardiac radiation doses.

Tiivistelmä

Rintasyöpä on länsimaiden yleisin naisten syöpäsairaus. Ennuste on kuitenkin hyvä, kiitos varhaisen diagnosoinnin, hyvän kirurgian ja tehokkaiden liitännäishoitojen. Viiden vuoden kohdalla rintasyöpädiagnoosista elossa on 90% naisista. Vuoden 2015 Syöpärekisteritietojen mukaan Suomessa olikin elossa yli 67 000 rintasyövän läpikäynyttä naista.

Rintasyövän liitännäishoidot eivät kuitenkaan ole haitattomia. Akuuttien haittavaikutusten lisäksi potilaille voi jäädä myös elämänlaatua alentavia pitkäaikaishaittoja. Haittavaikutukset voivat myös lisätä muiden sairauksien riskiä ja kuolleisuutta. Liitännäissädehoidon –erityisesti vasemman rinnan sädehoidon- on todettu lisäävän sekä sydänsairauksien esiintyvyyttä, että riskiä kuolla sydäntapahtumiin nuorempana. Sädehoidon aiheuttamat sydänhaitat tulevat oireisina esiin tyypillisesti 5-10 vuoden viiveellä itse hoidosta. Näitä sydänhaittoja ovat mm. sepelvaltimoiden ahtaumat, läppäviat ja sydämen vajaatoiminta.

Rinnan liitännäissädehoidossa sydän pyritään mahdollisimman hyvin suojaamaan hoidon aikana. Se on tärkeää, sillä myöhempien sydäntapahtumien riski on annosriippuvainen: mitä suurempi on sydämen saama keskisädeannos, sitä suurempi on myöhempi riski. Täysin sydänturvallista annosrajaa ei tutkimuksissa ole voitu osoittaa. Sädeannoksen lisäksi sydänsairastuvuuteen vaikuttavat myös muut tekijät, kuten muu toteutettu syöpälääkehoito, obesiteetti, verenpainetauti tai tupakointi.

Tämän tutkimuksen tarkoituksena oli seurata rintasyövän sädehoidon aiheuttamia akuutteja muutoksia sydämen toimintaan ja kiertäviin biomarkkereihin. Lisäksi arvioimme samanaikaisten tai aiempien lääkitysten merkitystä näihin havaittuihin muutoksiin sekä tarkastelimme keinoja parantaa hoidonaikaista toistettavuutta rintasyövän hengitystahdistetussa sädehoidossa. Vasemmanpuoleisen rinnan sädehoidon saaneilla potilailla totesimme subkliinisiä muutoksia sydämen toiminnassa sydämen ultraäänitutkimuksella jo heti sädehoidon päätyttyä. Samanaikainen lääkehoito aromataasi-inhibiittorilla syvensi osaa näistä havaituista

muutoksista. Seerumin troponiini-pitoisuus nousi merkkinä sydänlihasvauriosta viidesosalla potilasta sädehoidon aikana ja näillä potilailla sydämen saama sädeannos oli merkittävästi suurempi kuin muilla potilailla. Lisäksi havaitsimme, että hengitystahdistetussa rinnan sädehoidossa päivittäinen hoidon toistettavuus parani asettamalla seurantakuutio rintalastan päälle palletason sijaan sekä korjaamalla hengityskorkeuden asetuksia hoidon aikana hoitokoneen kuvausprotokollan avulla. Näin toimien hoidon aiheuttamia sydänannoksia saatiin merkittävästi pienennettyä.

List of original communications

I

Tuohinen SS, Skyttä T, Virtanen V, Luukkaala T, Kellokumpu-Lehtinen PL, Raatikainen P. Early effects of adjuvant breast cancer radiotherapy on right ventricular systolic and diastolic function. Anticancer Res. 2015 Apr 35(4):2141-7

II

Skyttä T, Tuohinen S, Virtanen V, Raatikainen P, Kellokumpu-Lehtinen PL. The concurrent use of aromatase inhibitors and radiotherapy induces echocardiographic changes in patients with breast cancer. Anticancer Res. 2015 Mar;35(3):1559-66

III

Skyttä T, Tuohinen S, Boman E, Virtanen V, Raatikainen P, Kellokumpu-Lehtinen PL. Troponin T-release associates with cardiac radiation doses during adjuvant left-sided breast cancer radiotherapy. Radiat Oncol. 2015 Jul 10;10:141.
.

IV

Skyttä T, Kapanen M, Laaksomaa M, Peltola S, Haltamo M, Boman E, Hyödynmaa S, Kellokumpu-Lehtinen PL. Improving the reproducibility of voluntary deep inspiration breath hold technique during adjuvant left-sided breast cancer radiotherapy. Acta Oncol. 2016 Aug;55(8):970-5

Abbreviations

A	Late filling velocity in left ventricular diastole
ABC	Active Breathing Control
AC	Adriamycin and Cyclophosphamide
ACEI	Angiotensin Converting Enzyme Inhibitors
AI	Aromatase Inhibitor
AJCC	The American Joint Committee of Cancer
ALDN	Axillary Lymph Node Dissection
AMI	Acute Myocardial Infarction
ARB	Angiotensin Receptor II Blocker
ASE	American Society of Echocardiography
ASCO	American Society of Clinical Oncology
ASTRO	American Society of Radiation Oncology
AUC	Area Under Curve
BC	Breast Cancer
BHL	Breath Hold Level
BMJ	British Medical Journal
BNP	B-type Natriuretic peptide
BRCA	Breast Cancer genes
CAD	Coronary Artery Disease
CAF	Cyclophosphamide, Adriamycin, 5-Fluorouracil
CEF	Cyclophosphamide, Epirubicin, 5-Fluorouracil
CHD	Coronary Heart Disease
CHF	Congestive Heart Failure
CI	Confidence Interval (95% if not stated otherwise)
CMF	Cyclophosphamide, Methotrexan, 5-Fluorouracil
COPD	Chronic obstructive pulmonary disease
CR	Complete Response
CT	Computer Tomography
CTV	Clinical Target Volume

CV	Cardiovascular
CVD	Cardiovascular Disease
DCIS	Ductal Carcinoma In Situ
DFS	Disease Free Survival
DIBH	Deep Inspiration Breath Hold
DNA	Deoxyribo Nuclear Acid
dt	Deceleration time
E	Early filling velocity in left ventricular diastole
e´	Mitral annular velocity
EACVI	The European Association of Cardiovascular Imaging
EBCTCG	Early Breast Cancer Trialist´ Collaborative Group
ECG	Echocardiogram
EF	Ejection Fraction
EORTC	European Organization for Research and Treatment of Cancer
ER	Estrogen Receptor
ERβ	Estrogen Receptor Beeta
ESMO	European Society of Medical Oncology
ESTRO	European Society of Radiation Oncology
FDG	Fluorodeoxyglucose
GLS	Global Longitudinal Strain
Gy	Gray
HbA1C	Glycohemoglobin
HDL	High Density Lipoprotein
HER2	Human Epidermal Growth Factor 2
HF	Heart Failure
HFpEF	Heart Failure with preserved Ejection Fraction
HIF	Hypoxia Inducible Factor
HL	Hodgkin Lymphoma
HR	Hazard Ratio
HRT	Hormonal Replacement Therapy
hscTnT	High sensitivity cardiac Troponin T
ICRU	International Committee of Radiation Units and Measurements
IHC	Immunohistochemistry
IMRT	Intensity Modulated Radiotherapy
IQR	Inter Quartile Range

IR	Incidence Ratio
ISH	*In Situ* Hybridization
ITC	Isolated Tumor Cells
IVRT	Isovolumetric Relaxation Time
k	Kilo
kV	Kilovoltage
LA	Left Atrium
LAD	Left Anterior Descending artery
LDL	Low Density Lipoprotein
LKB	Lyman-Kutcher-Burman
LOD	Lowest Detection Limit
LV	Left Ventricle
LVDD	Left Ventricle`s Diastolic Dysfunction
LVEF	Left Ventricle`s Ejection Fraction
MAPSE	Mitral Annular Plane Systolic Excursion
MI	Myocardial Infarct
MRI	Magnetic Resonance Imaging
MV	Megavoltage
NOS	Nitric Oxide Species
NSABP	National Surgical Adjuvant Breast Project
NTCP	Normal Tissue Complication Probability
NT-proBNP	N-terminal proBrain Natriuretic Peptide
OAR	Organ(s) At Risk
OR	Overall Risk Ratio
OS	Overall Survival
pCR	Pathologic Complete Response
PET	Positron Emission Tomography
PR	Progesterone Receptor
PTV	Planning Target Volume
QUANTEC	Quantitative Analysis of Normal Tissue Effects in the Clinic
RAS	Renin-angiotensin System
RIHD	Radiation Induced Heart Disease
RNA	Ribonucleic Acid
RPM	Real-time Position Management
ROS	Reactive Oxygen Species
RR	Risk Ratio

RT	Radiotherapy
RV	Right Ventricle
S´	Peak systolic annular velocity
SEER	United States Surveillance Epidemiology and End Results
SERM	Selective Estrogen Receptor Modulator
SIR	Standardized Incidence Ratio
SNB	Sentinel Node Biopsy
SPECT	Single Photon Emission Computed Tomography
SRI	Strain Rate Imaging
SSO	Society of Surgical Oncology
TAPSE	Tricuspid Annular Plane Excursion
TGFβ	Transforming Growth Factor Beeta
TnI	Troponin I
TnT	Troponin T
T3	Triiodothyroid hormone
T4	Thyroxin
VD	Valvular Disease
vDIBH	Voluntary Deep Inspiration Breath Hold
VMAT	Volumetric Modulated Arc Therapy
VTI	Velocity Time Interval
WHO	World Health Organization
2D	Two Dimensional
3D	Three Dimensional
Σ	Systematic error
σ	Random error

1 Introduction

Breast cancer is the most common cancer in women, as 1,67 million new cases were diagnosed globally in 2012[1]. In Finland, over 5000 new breast cancers and additionally almost 400 new ductal carcinoma in situ (DCIS)-lesions were found in 2014, corresponding to a breast cancer incidence of 95.7/100 000 person years[2]. Although breast cancer is the most frequent cause of cancer death in Finnish women, the mortality of breast cancer is rather low and the disease has an excellent 5-years survival rate of 90%[3]. As a consequence, more than 64 000 breast cancer survivors were alive in Finland as of 2013 - constituting 1.2% of the country´s entire population[4].

The global breast cancer incidence has been steadily rising during recent decades[1]. The reasons for and epidemiological risk factors associated with this increase have been widely sought to find means of prevention. Thus far, obesity[5,6], metabolic syndrome[7], smoking[8], alcohol consumption[9], nulliparity[10], lack of breastfeeding[11] and prolonged hormonal replacement therapy with both oestrogen and progesterone[12] are recognized as factors that increase the risk of breast cancer.

Improvements in breast cancer treatment results and decreases in breast cancer-related mortality[1] despite increases in its incidence have been attributed to a combination of awareness, education and screening[13] as well as better adjuvant treatment options[14-18]. Surgery is the cornerstone of early stage breast cancer treatment. Patients treated with breast conserving surgery and adjuvant radiotherapy (RT) exhibit 10-year overall survival (OS) rates similar to those treated with total mastectomy only[19]. The excision margins, irrespective of the surgery type, must be free of tumor cells[20]. Adjuvant treatment decisions after surgery are based on the size of the primary tumor, tumor biology, local invasion, nodal status and patient´s age and comorbidities. If the risk of local or systemic cancer recurrence justifies chemotherapy, targeted therapy or endocrine therapy use in the adjuvant setting, then these treatments are offered to patients[21].

The general idea of adjuvant breast cancer RT was presented in the early 20th century[22]. At that time, RT was administered after mastectomy and axillary dissection, although the first randomized trials raised questions regarding the actual benefit[23]. As breast-conserving surgery was introduced in the 1980´s, trials were carried out whether RT would be beneficial in the treatment of the remaining breast tissue. Significant benefits were observed with adjuvant RT even in the first trials[24]. Today, adjuvant RT is routinely used after breast conserving surgery[25] and in node-positive patients after mastectomy[26].

Adjuvant breast cancer RT, especially left-sided, increases the risk of cardiac morbidity and mortality later in life[27-31]. Clinically detectable radiation-induced cardiac side effects begin emerging after 5-10 years. These side effects include coronary artery disease (CAD), acute myocardial infarction (AMI), systolic or diastolic chronic heart failure (CHF), valvular insufficiency and conduction abnormalities[32,33]. Higher cardiac radiation doses are shown to correlate with an increased risk of later cardiovascular disease (CVD) development [29]. Furthermore, this later CVD risk increases in patients with pre-existing cardiac risk factors such as obesity, smoking, diabetes, hypercholesterolemia and hypertension as well as in patients who have received previous cardiotoxic chemotherapy and adjuvant endocrine treatment[29,32].

Three-dimensional (3D) planning in RT and the implementation of new techniques such as deep inspiration breath hold (DIBH) help to decrease the cardiac doses during the adjuvant breast RT[34]. However, no safe cardiac radiation threshold has been established and complete cardiac avoidance is not possible in all patients.

In this non-randomized observational study, we aimed to characterize the acute, subclinical cardiac changes occurring immediately after RT in breast cancer patients. Cardiac serum marker measurements, electrocardiography (ECG), echocardiography and 24-hour rhythm monitoring (Holter) were performed at baseline and after RT. In addition, in patients receiving adjuvant chemotherapy, these evaluations were repeated between chemotherapy and RT. Baseline factors and concurrent medications were documented to evaluate the possible additive or, potentially, protective effects.

In addition, the implementation and the reproducibility of a new cardiac-sparing RT technique were evaluated.

2 Review of the literature

2.1 Breast cancer treatment

2.1.1 Detection

Early breast cancer can be identified either via palpation (palpable lump or other changes in breast or skin appearance) on self- or physician-administered breast examinations or via mammography screening. The benefits and harms of breast cancer screening with mammography every two years have been widely discussed during the last few years. There is, however, indisputable evidence that mammography screening reduces breast cancer mortality by 16-20%[35-38]. A new lesion detected in mammography is usually verified with an ultrasound and biopsied for histopathological analysis. Breast magnetic resonance imaging (MRI) can be used in selected patients in screening, such as childhood cancer survivors with a history of prior thoracic radiation exposure[39] or BRCA-mutation carriers[40], but a wider use of MRI in the general population is not cost-effective[41]. Furthermore, radiology is essential for verifying and marking the tumor location prior to surgery in patients with non-palpable breast lesions [42].

2.1.2 Surgery

Surgery was suggested as a treatment for breast cancer as early as in the first century[43,44]. However, before the discovery of general anesthesia in 1846[45], most patients rather chose to decline this brutal operation. Lymphatic spread of breast cancer cells to adjacent lymph nodes was recognized already in the 16th century[43]. The first systematic mastectomies were performed during the 1850´s, and surgeons soon discovered the need for lymph node dissection to improve the treatment results[44]. Halsted described a series of operated breast cancer patients in 1890[46] and the presented radical mastectomy-technique[47] was considered as a standard procedure for many decades thereafter[43].

Breast-conserving surgery combined with adjuvant RT has been shown to be as effective as total mastectomy in patients with an early stage breast cancer[19,48,49]. Breast-conservation is also shown to be safe among younger patients[50], in primary tumors exceeding 5 cm (T3)[51], in triple-negative subtype[52] and even in multifocal tumors confined in the same segment of the breast[53]. In patients eligible for breast-conserving surgery, the final surgical decision should be based on patient´s preferences[54]. However, it is shown that surgical procedures vary among institutions based on the subspecialties of surgeons[55]. The width of necessary surgical margins around the tumor has been recently evaluated by ASCO/SSO/ASTRO with a consensus that "no ink" on the margin is sufficient, e.g. surgical margins are negative of tumor cells[20]. New oncoplastic techniques may help to achieve breast conserving surgery in patients with large or multifocal tumors and to maintain breast aesthetics[56]. The St Gallen consensus panel in 2013 identified the breast cancer patient group, with whom caution should be exercised when these patients opt for breast-conserving surgery. The identified risk factors were a very young age (<35 years), an extensive DCIS component or a severe contraindication of RT[57].

2.1.3 Sentinel node and axillary lymph node dissection

Increased knowledge of the breast lymphatic drainage and the first observations of the later-named sentinel node were made as early as 1970[58]. However, it took almost 30 years until the idea of staging of breast cancer with a sentinel node biopsy became reality[59,60]. Malignant cells in the sentinel node can be either the only site of breast cancer dissemination, in which case locoregional surgery or RT can cure affected patients, or it can represent the first signs of systemic disease dissemination[61]. Omitting axillary lymph node dissection (ALND) from sentinel node negative patients saves the breast cancer patients from major arm morbidities (oedema, restricted function) without increasing the risk of cancer recurrence[62-64]. During the last two decades, sentinel node biopsy (SNB) has become the standard of care in clinically node-negative patients.

During recent years, there has been a shift in the surgical community towards omitting ALND also in SNB-positive patients when only isolated tumor cells or micrometastases are detected[65,66]. However, the presence of an occult

micrometastasis (<2 mm) is an independent risk factor for OS and disease free survival (DFS)[67] and must be taken into account during the adjuvant treatment planning. As the majority of patients in the SNB-trials[65,66] also received adjuvant breast RT, a trial was designed to compare ALND to adjuvant RT covering also the lymph node regions in the axilla and supraclavicular area in clinically node-negative, but SNB-positive patients. This AMAROS trial demonstrated excellent local control in both groups as well as significantly less arm morbidity in RT-only patients than in ALND-patients[68].

ALND is routinely performed in patients with preoperatively detected positive axillary lymph nodes (with or without neoadjuvant chemotherapy[69]) and in patients with inflammatory breast cancer[70].

2.1.4 Histo- and molecular pathology

Pathology reports aim to characterize breast tumors first by morphological features: invasive or non-invasive (in situ); ductal, lobular, papillar, medullar or other subtype carcinomas and by the tumor grade according to WHO classifications[71,72]. The tumor size is measured and the presence of lymphovascular or neuronal invasion is reported, as well as the adjacent margins in all directions. Furthermore, immunohistochemistry (IHC) is used to evaluate and quantify the estrogen receptor (ER) and progesterone receptor (PR) expression in breast cancer cells[73]. Human epidermal growth factor 2 (Her2) expression in identified either using IHC or, preferably, using *in situ* hybridization (ISH) technology[74]. The tumor mitotic activity is often characterized with Ki67-monoclonal antibodies. The Ki67-protein is associated with cell proliferation and is present in all cell cycle phases except resting phase[75]. The percentage of Ki67-stained cells therefore represents breast cancer mitotic activity, which varies from <10% in slow growing tumors to 99% in highly aggressive tumors. In addition, Ki67 expression has prognostic significance[76].

With the help of gene expression profiling, breast cancers can be divided into four main intrinsic molecular subtypes: Her2-enriched, luminal A, luminal B and basal-like[77] (Table 1). Combining the evidence of these subtypes[78] with clinical characteristics[79] (tumor size, grade, invasion, nodal status, age), perhaps with the use on nomograms[80] or web-based databases[81], may enable clinicians to estimate

the risk of relapse on an individual level and discuss the adjuvant treatment options with affected patients.

Subgroup	Receptor status			
	ER	PR	Her2	KI67
Luminal A	+	+		<20%
Luminal B	+	+/-	+/-	>20%
Her2-enriched	-	-	+	High
Triple negative/Basal-like	-	-	-	High

Table 1. The molecular subgroups of breast cancer based on the 2013 St. Gallen consensus[82].

2.1.5 TNM-staging

The American Joint Committee of Cancer (AJCC) updates the tumor, node and metastasis (TNM) staging system for different cancers. It is internationally accepted system used to determine prognosis and guide oncological management in different cancers. The current TNM staging for breast cancer, which is from the 7th AJCC edition[83], is presented in Supplementary table 1 (page 132).

2.1.6 Adjuvant endocrine therapy

Oophorectomy was found to have impact on inoperable advanced breast cancer in the late 19th century[84]. In 1895 doctor George Thomas Beatson performed and later reported the first oophorectomy intended to treat breast cancer[85]. Stanley Boyd followed Beatson´s lead and adopted this new therapy as well as speculated *"that internal secretion of the ovaries in some cases favors the growth of the cancer"* [86]. Boyd later reported, that one third of the oophorectorized patients had clinical benefit[87]. However, oophorectomy was associated with major surgery-related risks in the early 20th century and was somewhat discouraged for several decades with the introduction on radiation induced ovarian ablation. Finally, the idea was translated also to the adjuvant setting with positive reports[88]. An EBCTCG meta-analysis in 1992 demonstrated a clear benefit of ovarian ablation in premenopausal patients as an adjuvant treatment modality (26% reduction in recurrence and 25% reduction in mortality) [89].

As the ER and its inducing function in breast cancer cells was discovered in 1970´s [90-92], the scientific community and pharmaceutical industry quickly began to develop ER antagonizing drugs (antioestrogens) to treat advanced breast cancer[93,94]. In addition, adjuvant trials were started early on and the rationale was summarized in 1976 British Medical Journal by Basil A. Stoll, who made the five following points: 1. Clinical benefit of chemotherapy was observed in adjuvant setting 2. New antioestrogens were beneficial in advanced breast cancer 3. Hormonal treatment was more effective when carried out early in the setting of minimal tumor burden 4. Endocrine treatment has a selective mechanism of action and 5. ER-positivity (ER+) could be recognized in breast cancer cells with staining. [95] The first clinical adjuvant trials made with tamoxifen were reported a few years later[96,97], demonstrating already the benefits of adjuvant antiestrogen treatment. The 1998 EBCTCG meta-analysis regarding adjuvant tamoxifen further established the significance of the endocrine therapy: adjuvant tamoxifen trials of 1 year, 2 years and 5 years of use demonstrated proportional reductions in the 10-year recurrence rates of 21%, 29% and 47%, respectively. The proportional reductions in 10-year OS were 12%, 17% and 26%, respectively, showing a significant benefit with increasing treatment times ($p<0.01$)[98]. Similar efficacy in the adjuvant treatment setting was observed with another selective ER modulator (SERM), toremifene[99,100]. Tamoxifen was considered the standard of care in both pre- and postmenopausal patients until the era of aromatase inhibitors.

Blocking of estrogen synthesis in the adrenal cortex or extraglandular tissue was first attempted with aminoglutethimide, a novel aromatase inhibitor (AI). In trials, it was as effective as tamoxifen but induced more side-effects[101,102]. Furthermore, it was only as effective as a previously used hormonal therapy, high dose medroxyprogesterone acetate (MPA), when admistered after tamoxifen[103]. Most of the trials with aminoglutethimide were conducted in the 1980´s and more selective aromatase inhibition was sought to improve the results with less toxicity. Exemestan, an irreversible AI was reported to decrease the estrogen levels without decreasing cortisol or aldosterone levels in 1992[104]. Soon, the trials involving letrozole[105] and anastrozole[106] reported near complete aromatase enzyme inhibition in postmenopausal women. Large randomized adjuvant trials were launched to test these drugs: the ATAC trial[107], which compared anastrozole with tamoxifen, BIG1-98[108], which compared letrozole with tamoxifen and TEAM[109], which compared exemestane with sequential use of tamoxifen and exemestane. The selected setting to test exemestane did not turn out to be financially beneficial, as the two other drugs were approved earlier as the preferred adjuvant endocrine therapy in postmenopausal patients. The updated 100-month results of the ATAC trial showed an HR of 0.75 ($p<0.01$) for DFS favoring anastrozole[107] ; the early 2005 results of the BIG 1-98 trial demonstrated an HR of 0.81 ($p<0.01$) favoring letrozole after only 25.8 months of follow-up. Thus, the patients in the tamoxifen arm were offered a switch to letrozole, which complicated the subsequent analyses[110]. The TEAM study showed, that exemestane alone and a sequential use of tamoxifen 2-3 years followed by exemestane were equally effective at 5 years[109]. Later, exemestane was compared against anastrozole and neither was found to be superior in terms of breast cancer control after 4 years of follow-up[111]. The results of these studies were recently combined in the EBCTCG meta-analysis comparing AIs alone, AI+tamoxifen and tamoxifen alone, in favor of AIs in all end-points[112].

As AIs alone were insufficient to decrease the estrogen secretion from the ovaries, tamoxifen remained the standard of care in premenopausal patients. In order to potentiate the treatment, one came back with the idea of the ovarian function suppression, this time in combination with either tamoxifen or AI[113]. The first results of these SOFT and TEXT trials were reported in 2014 after a median follow-up period of 68 months[114]. DFS at 5 years was 91,1% in the exemestan+ovarian suppression group and 87.3% in the tamoxifen+ovarian suppression group with a HR of 0.72 ($p<0.01$) [114]. The benefits observed in these

trials were similar in magnitude to those observed in previous trials comparing AIs to tamoxifen in postmenopausal patients. The effect of the combined treatment vs. tamoxifen alone on OS has not been reported yet.

In conclusion: adjuvant endocrine treatment is offered to patients, that have ER positive tumors, stage II or higher or stage I patients with some adverse features. In postmenopausal patients, AI´s are the first choice treatment, if not contraindicated. Premenopausal women can be treated with tamoxifen, ovarian suppression or the combination of AI+ovarian suppression.[17,115-117]

2.1.7 Adjuvant chemotherapy

The first trials using adjuvant chemotherapy in node-positive or otherwise high-risk breast cancer patients were performed in the 1980´s. The most frequently used combination in these initial trials was six infusions of CMF (cyclophosphamide, methotrexate and 5-fluorouracil) due to the previously observed efficacy of this regimen in the treatment of metastatic breast cancer[118]. CMF was shown to be efficient with respect to improving both DFS and OS (84% vs. 69% at three years) [119,120] and was soon adopted as the standard of care.

Ten years later, the addition of anthracyclines to adjuvant therapy was shown to elicit additional increases in OS and DFS compared with the previous CMF-combination[121,122]. The overall benefits of these regimens were later compared in the Early Breast Cancer Trialist´ Collaborative Group (EBCTCG) meta-analysis: the risk ratio (RR) with anthracycline-based treatment was 0.88 for distant recurrence, 0.93 for any recurrence and 0.89 for breast cancer mortality compared with CMF[123]. CEF (cyclophosphamide, epirubicin, 5-fluorouracil), CAF (cyclophosphamide, adriamycin=doxorubicin, 5-fluorouracil) and AC (adriamycin, cyclophosphamide) were the most frequently used combinations in these analyzed trials. Furthermore, higher cumulative anthracycline dosages were observed to exert greater beneficial effects[123].

Additional improvements in the adjuvant chemotherapy regimens were made after the millennium using taxanes. Taxanes were administered either sequentially[124] or concurrently[125] with anthracyclines and demonstrated an RR of 0.87 for distant recurrence, 0.86 for any recurrence, 0.87 for breast cancer mortality

and 0.89 for overall mortality, all statistically significant ($p<0.01$) in a meta-analysis [123]. Various taxane combinations were used in the randomized trials included in this EBCTCG meta-analysis. The current adjuvant treatment choice in 2015 is to add taxanes sequentially to anthracycline-based combinations [115-117,126].

As chemotherapy is known to cause a large scale of side effects (such as nausea, alopecia, fatigue, anemia, neutropenia, risk of infections, neuropathy, gastro-intestinal toxicity and long term cardiotoxicity), the risk-benefit ratio must be weighted for each patient. Increasing primary tumor size, grade 3, Her2-positivity, young age, luminal B or basal-like molecular subtypes and metastatic axillary nodes increase the risk of breast cancer relapse and thus favor the use of more aggressive adjuvant therapy[116,117,127]. On the other hand, in low risk patients with ER-positive tumors -even with a modest nodal involvement-, the chemotherapy provides only minor benefits compared with hormonal therapy[128].

2.1.8 Adjuvant therapy for Her2-positive tumors

Her2-overexpression on tumor cells was found to be a negative prognostic factor[129,130] and to induce tamoxifen resistance[131] as early as the 1990´s. The first phase I-II trials using monoclonal antibodies against Her2-receptors alone or in combination with chemotherapy were conducted in metastatic breast cancer and were reported at the end of the 20th century[132,133]. The findings of these trials quickly encouraged the use of the Her2-spesific monoclonal antibody trastuzumab in the adjuvant setting. The first successful trials were reported in 2005 (HERA trial) [134] and 2006 (FinHer trial) [135]. Both showed marked improvement in DFS compared with trastuzumab-naive patients: an absolute decrease of 8.7% at two years in the Hera study and 11% at three years in the FinHer study. The RRs for any event were 0.54 and 0.42, respectively.

The long-term follow-up results of both trials were recently updated. In the HERA trial, the original study design compared also the length of the antibody-treatment: after a follow-up period of 8 years, two years of trastuzumab-treatment were not superior to one year of treatment, but one year of treatment was significantly better than observation only: an HR of 0.76 for DFS and 0.76 for OS[136]. The 5-year results of the FinHer-study showed an HR of 0.65 for distant DFS. The best results were obtained using a combination of 9 weekly trastuzumab

infusions concurrently with 3 docetaxel infusions followed by 3 CEF infusions[137]. The 9-week FinHer-regimen is currently evaluated with and without the addition of one year of trastuzumab in the on-going SOLD-trial (NCT00593697).

Given that some Her2-positive tumors are resistant to trastuzumab, dual blockage of Her2-receptor using either antibodies or per oral tyrosine kinase inhibitors has been tested. The addition of another Her2-antibody (pertuzumab) with complimentary binding mechanisms with trastuzumab has been proven to be beneficial in treating metastatic breast cancer and has also been approved in the neoadjuvant setting in Her2-positive breast cancers. In the adjuvant setting, trastuzumab combined with chemotherapy is the current standard of care for Her2-positive breast cancer.

2.1.9 Neoadjuvant treatment

Locally advanced, inoperable or inflammatory breast cancer is first treated with neoadjuvant therapy in order to achieve curative surgery later. A chemotherapeutic approach is the preferred choice of care in most cases and optimal treatment combinations are based on tumor biology[116,117]. In hormone-positive slow growing breast tumors, neoadjuvant endocrine therapy is a valid option[117]. In Her2-positive tumors, the preferred neoadjuvant treatment consists of a combination of trastuzumab, pertuzumab and taxane as this regimen exhibits an improved pathological complete response (pCR) rate of 45.8% at the time of surgery compared with 29% in trastuzumab+taxane-combination[138]. The use of the first regimen translates into an improved 3-year DFS of 92% vs. 86%[139]. The rate of pCR at the time of surgery is shown to be a predictive factor for later clinical outcomes[140].

2.1.10 Adjuvant bone-targeted therapies

Circulating tumor cells can be detected in approximately 20% of patients with early stage breast cancer[141]. The presence of circulating tumor cells before or after adjuvant chemotherapy is associated with a worse disease free survival ($p<0.001$) as well as a worse OS ($p=0.002$) [141]. These cells are often attracted to surfaces within the bone, where they can remain dormant for years[142,143]. Activated and proliferating tumor cells may later evolve into bone macrometastases, which, in

breast cancer, are mainly osteolytic. Osteoclasts are hyperactivated during this process[142,143].

Bisphosphonates are drugs that modify the function of osteoclasts as well as affect the T-cell function. For years, bisphosphonates have been used to treat bone metastasis in breast cancer[144]. Incorporating these drugs into adjuvant treatment was expected to improve metastasis-free survival, particularly by reducing bone relapses. Adjuvant trials were conducted with mixed results. Finally, the EBCTCG-meta-analysis in 2015 shed some light on this matter as the data of 18 766 women enrolled in adjuvant bisphosphonate trials was analyzed. Among premenopausal women, bisphosphonate use did not demonstrate any apparent effect; however, among postmenopausal patients, 2-5 years use of bisphosphonates produced significant reductions in breast cancer recurrence (RR 0.86, p=0.002) and breast cancer mortality (RR 0.82, p=0.002)[145].

In addition, adjuvant trials involving other drugs, such as the anti-RANK ligand antibody denosumab, are on-going. Preliminary reports regarding the ability of denosumab to reduce fractures and prevent osteoporosis are published[146], but data regarding the breast cancer recurrence rate or survival is not mature yet.

2.1.11 Adjuvant radiotherapy

At the beginning of the 20th century, irradiation of the lymph node regions after mastectomy using high voltage X rays or Radium-packs was initiated. In 1933, Pfahler[22] reported the results for 263 patients treated with postmastectomy radiation between 1902-1928. Among patients with axillary involvement, 58% were disease-free at 5 years - which at that time was 2.5 times more than among patients without radiation. Pfahler also proposed, that radiation should be initiated as soon as possible after surgery. Preoperative irradiation was also investigated, with rather good results but with high toxicity[147]. The idea of selecting patients for irradiation based on their mastectomy results was proposed and the first medical tumor boards were created in the 1930´s[148]. The general consensus already at that time was that irradiation should be used as an adjunct to mastectomy in breast cancer patients rather than as an alternative to surgery.

Increases in the availability of RT, as well as the advent of new technologies, such as the introduction of betatron in the 1940 ´s[149,150] and the linear accelerator in the 1950´s[151], enabled the launch of multi-institutional randomized prospective clinical trials. The National Surgical Adjuvant Breast Project (NSABP) was one of the largest trials of its time to evaluate the benefits of postoperative RT after mastectomy. It reported the results of 1103 patients in 1970: the RT arm had fewer local recurrences than the surgery-only arm, but no difference in OS was observed[23]. In addition, other trials reported that RT after mastectomy exerted even negative effects on OS[152]. On the contrary, RT induced beneficial effects on DFS and OS in a Norwegian study involving over 1000 patients[153]. The benefit was more pronounced in patients with a more advanced stage after mastectomy. Finally, a definitive answer regarding the role of RT after mastectomy and axillary surgery was addressed by the 2014 EBCTCG meta-analysis, which demonstrated that RT clearly benefitted all node-positive patients: HRs of 0.80 (1-3 nodes, $p=0.01$) and 0.87 (4 or more nodes, $p=0.04$) for breast cancer mortality and 0.68 ($p<0.01$) and 0.67 ($p<0.01$) for overall breast cancer recurrence, respectively[26]. However, the addition of RT did not exert any positive effect in node-negative breast cancer patients after mastectomy. Discussions are still ongoing, whether there is a subgroup of node-negative patients, who would benefit from the adjuvant RT[154].

In late 1970´s and early 1980´s, surgeons gradually began performing more partial or segmental mastectomies for smaller tumors[155]. It soon became obvious, that breast conserving surgery should always be followed by breast RT[24]. In Finland, the RT pioneer Sakari Mustakallio favored the breast conserving surgery approach very early on combined with adjuvant RT[156]. DFS and OS were shown to be at least equal to, if not even better, with breast conserving surgery+RT vs. mastectomy alone[157]. During the 1990´s, breast conserving surgery became widely adopted, mainly because it was associated with better quality of life among patients[158,159]. The EBCTCG meta-analysis in 2011 summarized all available trials regarding breast conserving surgery and RT[25]. In this meta-analysis, the use of adjuvant RT halved the risk of breast cancer recurrence in every group, whether stratified by age, tumor size, tumor grade, nodal status or the additional use of chemotherapy or endocrine treatment with an overall reduction of breast cancer death rate by approximately one-sixth.

The current guidelines recommend adjuvant RT after breast conserving surgery and after mastectomy in node-positive patients. In addition, in some patients with T3-T4 N0 tumors with adverse features (young age, an extensive DCIS component, a triple-negative subtype or a medial location) RT can be offered after mastectomy. [115-117]

In many countries adjuvant external breast RT has been partly replaced by the application of local brachytherapy to the resection cavity. This method is suitable for early stage, low risk patients with tumors <3 cm, negative margins and no other adverse features, such as hormone receptor negativity, lymphovascular invasion or extensive intraductal component[116,160,161]. Brachytherapy can be performed either with specific balloon shaped catheters or via multiple catheters implanted in the lumpectomy cavity either during or after surgery[162]. Furthermore, brachytherapy can be administered either intraoperatively with one fraction or later with one to a few fractions using either low dose rate- or high dose rate-energy sources[162]. Definitive long-term data regarding the effectiveness of local brachytherapy compared with that of external whole breast RT is still awaited[163].

2.1.12 Follow-up

The European Society of Medical Oncology (ESMO) consensus guideline[115] states that the aims of the breast cancer follow-up are:

- To detect local recurrences or contralateral new breast cancers at early stage
- To evaluate and treat therapy-related complications (such as menopausal symptoms, osteoporosis and secondary cancers).
- To motivate patients to continue endocrine therapy.
- To provide psychological support and information in order to enable a return to normal life after breast cancer.

Only small randomized trials have compared the benefits and cost-effectiveness of one follow-up protocol to those of another[164-166]. Different countries, as well as different cancer centers within a country, tend to have their own follow-up protocols based on the available resources. There is, however, a general consensus that approximately a yearly mammography is cost-effective in the follow-up in detecting local recurrences[167,168]. In general, the majority of breast cancer patients

are followed for 5 years after the operation. The ESMO guideline proposes having visits every 3-4 months during the first two years and twice a year thereafter until 5 years[115]. In Finland, the current national guideline suggests having visits at 3 months and 1, 3 and 5 years and mammography scans at 1-2 year intervals[169]. A Korean study[170] characterized the time and the location of recurrence according to molecular subtypes. In this study, the highest risk of recurrence was within the first two years in triple-negative, Her2-enriched (before the era of monoclonal Her2-antibodies) and in luminal B subtypes. For luminal A, the risk of recurrence was highest at three years post-surgery. Such findings could be exploited into clinical practice to design individualized follow-up schemes.

Regular exercise is shown to be beneficial in preventing breast cancer recurrence[171,172]. On the other hand, obesity increases the risk of recurrence[173]. Thus, life-style guidance is essential during the later follow-up. Suggestions regarding cardiac follow-up after breast cancer treatments are discussed later in this thesis.

2.2 Radiotherapy

2.2.1 Mechanism of action

The radiation used in therapeutic RT is called ionizing. This type of electromagnetic, high energy, short wavelength radiation in clinical use is usually produced with linear accelerators (linacs). Therapeutic radiation is either electron beams or, more commonly, photon beams. Ionizing radiation has enough energy, when in contact with an atom, to remove tightly bound electrons from the orbit of an atom, causing these atoms to become charged or ionized. Therapeutic RT utilizes the cellular effects of radiation; the straight action of bouncing electrons to DNA-strands and other cell structures, the radiolysis of water molecules to DNA-damaging highly toxic free radicals and the generation of reactive chemical species- to treat cancer[174]. The formed DNA-strand damages can manifest as single-strand breaks, which usually are reversible due to the damage-activated repair-enzymes in the cell, or double-strand breaks, which can lead to cell death if 1) the break cannot be corrected or 2) the broken DNA site is essential for cell survival and mitosis.

Radiation-induced processes in cells require oxygen. It is shown, that hypoxia in tumors decreases the effects of RT[175,176]. Oxygen is needed for water radiolysis of water, as the formed free oxygen radicals attach to the DNA damage site and prevent the attempted repair-processes. In addition, the amount of reactive oxygen species (ROS) may continue to rise days after the initial radiation exposure affecting other cellular and physiological properties[174,177]. This explains the by-stander effect: not only the cells that have had the radiation "hit", but often also the adjacent cells will be damaged later in the radiation process[178].

Radiotherapy is a complex process, which comprises of physical, chemical and biological interactions within a time-scale ranging from nano-seconds to years (Figure 1). Changes in the microenvironment of the irradiated area and activated immunological processes play an essential role in the final efficacy of RT[179]. The biological processes are not all known in detail and a lot of research is ongoing, both to enhance the radiation effectiveness and to protect the normal cells from damage.

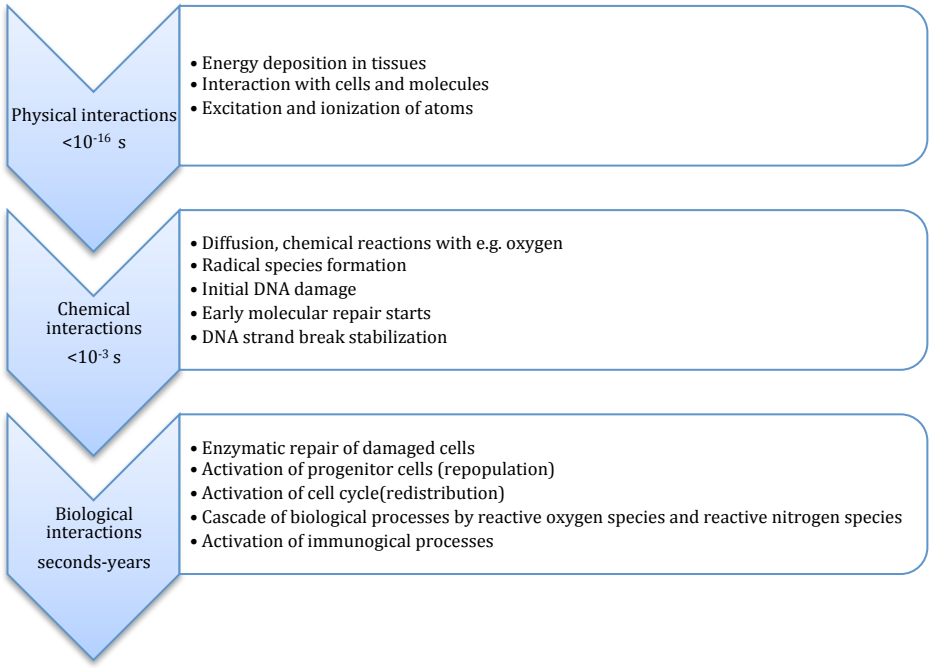

Figure 1. The time-line of different cellular and molecular interactions triggered by ionizing radiation.

2.2.2 The process of adjuvant breast cancer radiotherapy

The process of adjuvant breast cancer RT should start immediately after the surgery by referring the patients to oncological consultation. A multidisciplinary tumor board is preferred in the process. Among patients not receiving adjuvant chemotherapy, the time from the surgery to the start of RT should not exceed 12 weeks, as longer delays increase the risk of breast cancer specific mortality (HR 3.84) [180]. The currently preferred window to start RT is 4-8 weeks[181].

Adjuvant RT is indicated after the breast conserving surgery regardless of tumor biology, tumor size or patients´ age, as presented in the EBCTCG meta-analysis[25]. In elderly patients with T1N0 ER-positive tumors, RT can be omitted with the use of endocrine therapy[182]. After mastectomy, RT is indicated if the sentinel nodes were positive, irrespective of axillary dissection, and in T3-T4N0 patients with high-risk features[26,154].

RT should not be given to patients with scleroderma[183,184]. Furthermore, RT should not be given trough a pacemaker or an implantable cardioverter defibrillator because radiation may cause severe failures of these devices[185].

2.2.2.1 Planning CT

Current breast RT planning is based on a computer tomography (CT) scan of the patient[186,187]. This planning CT (or reference image) is acquired in pre-specified position. The patient is usually in supine position with both hands above the head. Special fixation devices, such as breast boards and head cuffs, are used (Figure 2.) to stabilize the position. Special attention should be made to ensure the vertical straightness of the spine. Once a comfortable and stable posture is reached, only then can the actual scanning begin. [188] The CT scan should be performed using the minimum dose required to provide accurate organ contours with a slice thickness of $\leq$3 mm[189].

After the scan, in the exact position, the reference marks are tattooed to patient´s skin guided by the in-room laser beam positioning system[187]. These marks will guide the patient set-up later in the treatment phase. The acquired CT images are sent to a RT treatment planning system for the next steps.

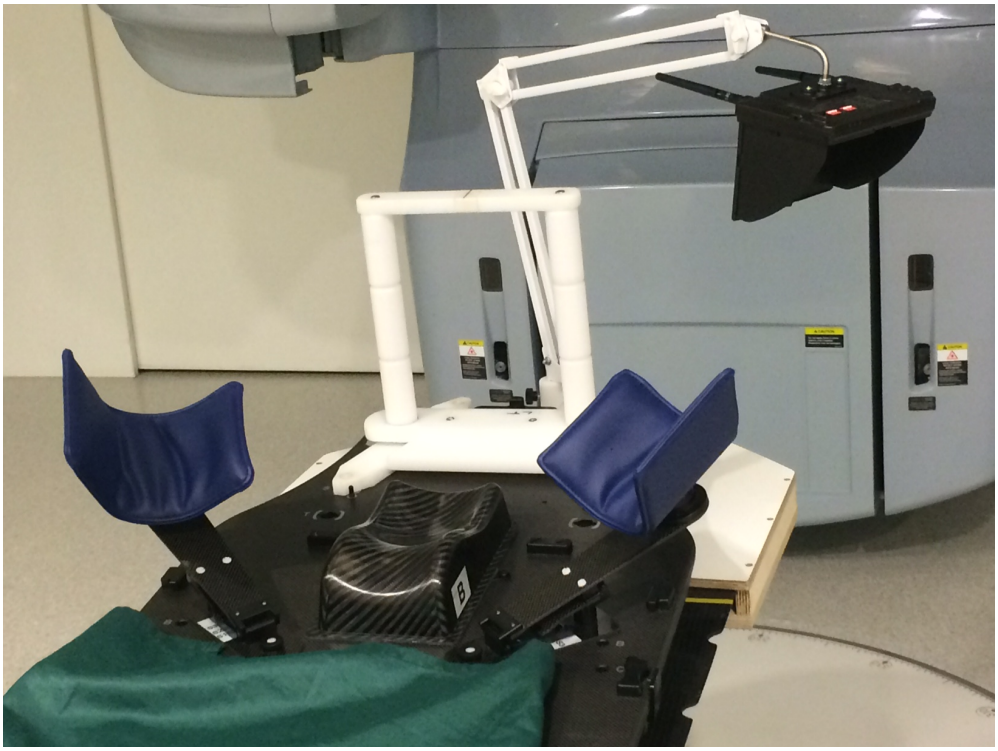

Figure 2. An example of a breast board used for the patient position stabilization. A head cuff is individually chosen to enable a firm but comfortable position. Arms rest on the blue crutches and hands grip the white rods.

2.2.2.2 Clinical target volume of the breast and lymph node regions

The clinical target volume (CTV) [190] is contoured to the CT slices by a radiation oncologist (Figure 3.). After breast conserving surgery, the CTV of the breast is the remaining breast tissue and after mastectomy the breast-CTV consists of the chest wall[191,192]. If lymph node regions are treated, the nodal-CTV is contoured based on anatomical structures[193,194]. The nodal-CTV covers typically the axillary and supraclavicular regions. The addition of parasternal region is indicated in selected cases (see section 2.2.2.6).

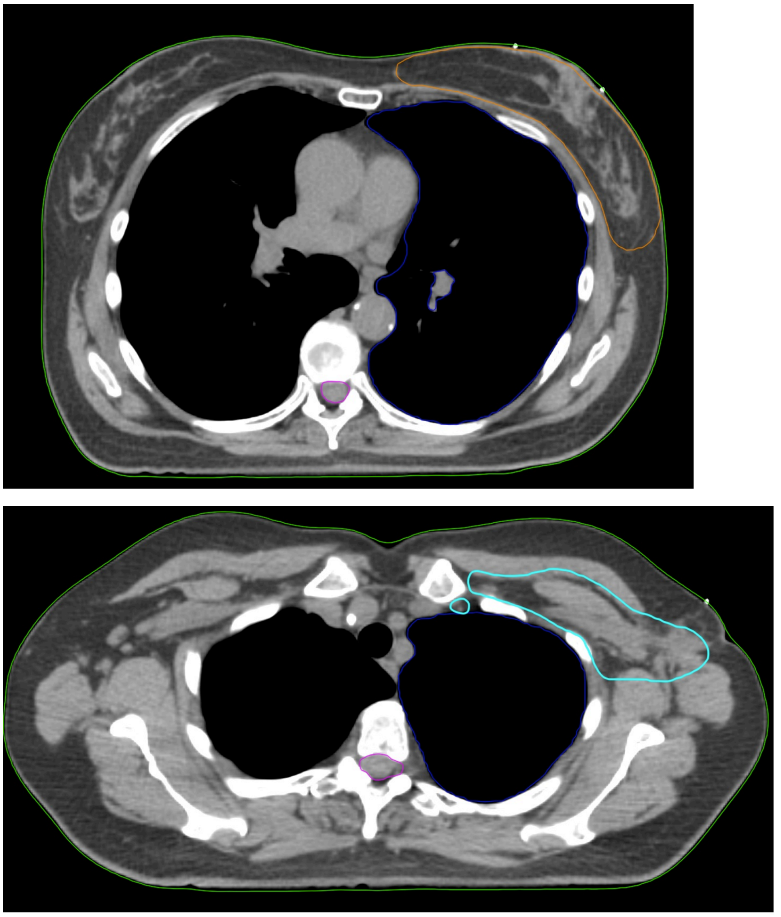

Figure 3. A) The clinical target volume (CTV) of the breast tissue contoured to planning CT slice (orange) B) CTV of the lymph node regions above the sternum level (interpectoral, axilla III, internal mammary lymph node regions; cyan).

2.2.2.3 Isolated tumor cells and micrometastasis in SN

In the case of isolated tumor cells (ITC) in the sentinel node, the use of RT is unestablished. As axillary dissection is usually omitted in these patients based on randomized trials and current guidelines[65,66,70], there are no definitive studies addressing this population. In the AMAROS trial[68], which compared axillary RT to surgery, only 10% of patients in the RT arm and 12% in the surgery arm had ITC in the sentinel node - numbers too small to perform a subgroup analysis. Other studies are contradictory: ITC is regarded to be a negative prognostic marker in some[195] and not significant in others[196-198]. Likewise, a micrometastasis (<2mm) in a sentinel node does not require further axillary surgery[64,65,70]. The risk of additional metastatic lymph nodes in the presence of micrometastatic lymph node in SNB varies from 13 to 27%[198-200]. Whether adjuvant axillary RT is indicated in these cases, is under discussion. Again, no specific subgroup analysis is done from the AMAROS trial[68] and no other randomized trials involving this subgroup have been reported to date.

Individual decision-making based on patients age and tumor characteristics are essential, when discussing the extension of RT field to cover the lymph node regions in addition to breast RT after breast conserving surgery or the use of adjuvant RT after mastectomy in patients with ITC or micrometastasis.

2.2.2.4 One to three positive nodes

The addition of RT after mastectomy is recommended in patients with 1-3 positive nodes based on the EBCTCG meta-analysis[26]. After breast conserving surgery, the addition of nodal RT in this subgroup improves the disease free survival at 10 years: 80.9% vs. 83.5 % in patients with one node and 67.6% vs. 74.8% in patients with 2-3 nodes. The use of RT was especially effective in ER-negative patients. However, at 10 years the effect on OS was not statistically significant in the whole study population.[201]

2.2.2.5 Four or more positive nodes

Adjuvant RT to lymph node regions is indicated after mastectomy in patients with 4 or more positive lymph nodes[26]. RT decreases the locoregional recurrences (13% vs. 32.1%), any recurrences (66.3% vs. 75.1%) and breast cancer mortality (70.7% vs. 80.0%) at 10 years. After breast conserving surgery, the addition of nodal RT improves the DFS at 10 years (60.3% vs. 69.8)[201].

2.2.2.6 Parasternal lymph nodes

The parasternal lymph node region, also known as the internal mammary lymph node region, is alongside the axillary region the other main lymphatic drainage checkpoint. The incidence of internal mammary lymph node metastases are shown to be more than 20% in patients with 1) 4 or more positive axillary nodes, 2) medial tumor and positive axillary lymph node, 3) young patients with T3 tumors, 4) T2 tumor and positive axillary node and 5) medial T2 tumor[202]. The need of parasternal lymph node RT has been under discussion, as it coincidentally increases the radiation dose to organs at risk (OAR)- especially heart. A recent EORTC study addressed this subject in a clinical trial, in which 4004 patients with medial or centrally located breast tumors (N0) or lateral tumors with axillary dissection (N+) were randomized to groups receiving either RT encompassing the internal mammary nodes, medial subclavian nodes as well as the upper part of axilla or to breast only RT. The results at 10 years demonstrated benefits with nodal RT, as the HR for disease progression or death was 0.89[203]. Similar results were observed in a Danish DBCG-IMN-study, in which internal mammary RT was added for right-sided breast cancer patients but not for left-sided due to the increased risk of RT induced heart disease[204]. After 7 years of follow-up, the OS in the right-sided patients was 78% vs. 75% in the left-sided ($p=0.04$). In the light of these studies, internal mammary node irradiation can be recommended for patients with high-risk features, whilst bearing in mind the increased dose to OAR[192]. In all patients, the OARs - lungs, heart, plexus brachialis, thyroid gland, and contralateral breast tissue- are contoured to the CT slices based on radiation oncologist discretion to evaluate the radiation dose to these structures.

2.2.2.7 Planning target volume

The planning target volume (PTV) is the final RT planning target. The PTV takes into account the inter- and intra-fraction variability of the patient´s posture and movement as well as allows other uncertainties during planning and treatment delivery. It is a geometric area around the CTV (Figure 4), so that the planned dose is *de facto* delivered to the desired area[190]. The margin between the CTV to PTV should be evaluated in every clinic by measuring set-up accuracy. In reported trials, the required margin is shown to vary between 4-8 mm[205-207].

A) B)

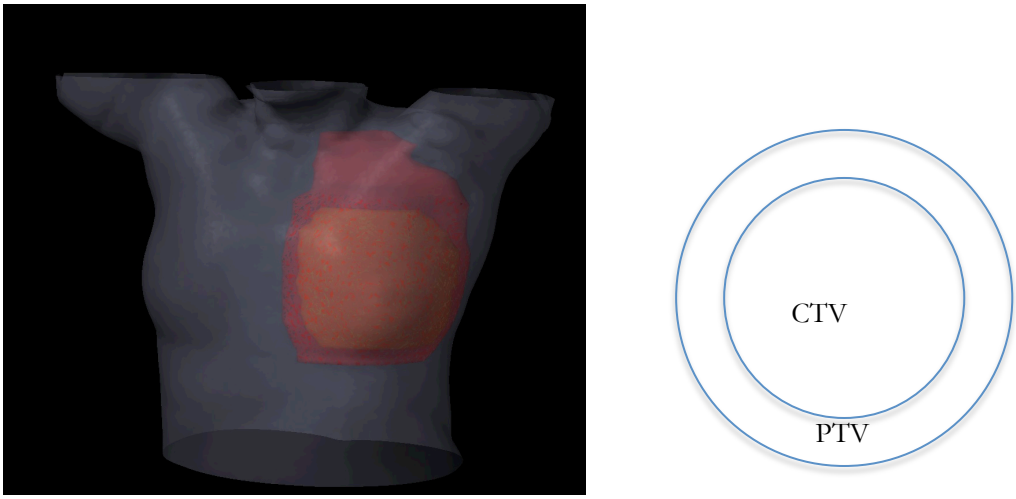

Figure 4. A) A body surface view showing the final PTV covering the breast and lymph node regions (red). CTV of the breast tissue is shown (orange). B) An illustration of clinical target volume (CTV) and planning target volume (PTV)

2.2.2.8 Boost to the tumor bed

An additional radiation dose (a boost) to the tumor bed after breast conserving surgery was thought to be beneficial with respect to reducing local relapses. The EORTC boost vs. no-boost trial tested this concept of adding 16 Gy (in 2 Gy fractions) to the resection cavity after 50 Gy whole-breast RT. The 20-year follow-up results of this trial were updated in 2015. These results indicated, that the addition of boost reduced the local recurrence rate from 13% to 9%, but had no effect on breast cancer mortality in the entire study population[208]. Subgroup analyses demonstrated that the boost was beneficial in younger patients and in patients with an extensive DCIS component or grade III tumors[208-210]. Tumor margin width had no effect on recurrence, if the operation margins were negative of tumor cells[208]. These findings as well as other trials have encouraged the recent ASCO/ASTRO guidelines: if the resection margins are free of tumor cells ("no ink on margin"), it is regarded sufficient and the margin width should not influence the decision to administer a boost[211,212]. Likewise, in the EORTC trial analysis, the guideline stresses the fact that tumor biology as well as patient´s age, is an important risk factor for local recurrence[211,212]. The boost volume should comprise the tumor bed with small margins. This outcome is most reliably achieved when the surgeon implants surgical clips in the resection cavity[213].

2.2.2.9 External beam planning

In adjuvant breast RT, as in all modern RT, the dose to the PTV should be as conformal and homogeneous as possible without compromising the OAR. The beam planning is based on the International Committee of Radiation Units and Measurements (ICRU) report 50 principles[214].

After breast conserving surgery, two opposing tangential photon fields are usually chosen to treat the breast-PTV. The homogeneity can be improved using forward planned field-in-field intensity modulated RT (IMRT) compared with the traditional wedged 3D planning[215-217]. If the lymph node regions are treated, additional 2-3 anterior-posterior opposing fields are added[218,219]. After mastectomy, beam planning can be done with a combined use of electron fields for the thoracic wall and photon beams for the node-PTV or with photon fields alone[220-223].

In select cases, the PTV dose coverage can be improved and the OAR dose can be reduced with volumetric modulated arc therapy (VMAT) or multiple field IMRT techniques. However, the doses to contralateral breast as well as to the contralateral lung can increase with these techniques and clinical judgment should be used to select an optimal plan for each patient[224,225]. The boost-PTV can be covered using either sequential use of electrons or photons[226] or with simultaneous integrated boost (SIB)-technique[227,228].

2.2.3 Fractionation

For decades, breast cancer adjuvant RT has been delivered in daily 1.8-2 Gy fractions 5 days a week to a total dose of 45-50 Gy. This, so called conventional fractionation, is based on the idea of better normal tissue tolerance and the historical experiments with various fractionation models[229-233]. The idea of hypofractionation for breast cancer RT started to gain popularity in 1980´s, first in the United Kingdom (UK) and Canada. The reasons were derived both from radiobiological models[234-236] and from practical issues, such as long distances to RT units. The first reports regarding hypofractionation were encouraging[237] and two large randomized trials (START A and B) were launched in the UK to compare hypofractionated regimens (41.6 Gy in 13 fractions, 39 Gy in 13 fractions or 40 Gy in 15 fractions) to the conventional 2 Gy fractionation. The 10 year results were presented in 2013 and showed that hypofractionation achieved an equal local control rate with less long term skin toxicity[238]. Based on the START-trials and the Canadian long-term results[239], modest hypofractionation is now a widely accepted modality in the adjuvant breast RT[115,116]. Some discussion is still on-going regarding the treatment of lymph node regions with hypofractionated RT, because the numbers of N+ patients in these trials have been limited.

2.2.4 Cardiac sparing techniques

Radiation-induced long-term cardiotoxicity has been a major concern in left-sided breast cancer patients[27,29]. A retrospective study has shown, that the risk of a major cardiac event increased linearly with each additional Gy to the heart[29]. In addition, increasing doses to left ventricle (LV) have also shown to correlate with perfusion defects[240,241]. Therefore, several methods of reducing cardiac radiation doses have been sought.

2.2.4.1 Prone vs. supine

The idea of the prone position technique is to allow the breast tissue to fall away from the thoracic wall, thereby reducing the dose to the lungs and heart. Especially patients with large and pendulous breasts were observed to experience less cardiac radiation exposure in the prone position than in a supine position[242]. On the other hand, the prone positioning simultaneously moves the heart anteriorly and is shown to increase the cardiac dose in patients with smaller breasts[243,244]. Although the prone position has gained acceptance in the United States, questions regarding the daily treatment reproducibility[245] as well the need of specific breast boards during treatment has slowed its wider use in Europe.

2.2.4.2 Free breathing vs. breath hold techniques

In 1997, Chen et al[246] published a study showing that simple inspiratory maneuvers caused marked decreases (mean - 40.2%) in the cardiac volume within the radiation portal. They suggested, that such an approach would allow a simple way to reduce cardiac involvement. This finding was rapidly accepted and the first clinical trials comparing breath hold techniques with free breathing (FB) in left sided breast cancer patients were reported a few years later[247,248]. The first studies were conducted using an active breathing control (ABC)-device, which pre-specifies the lung volume and blocks the breathing to a certain moderate deep inspiration-phase. Later, the voluntary deep inspiration breath hold (vDIBH)-method was introduced[249,250]. In vDIBH, an infrared camera tracks the movement of an external marker block during the breathing cycle (Figure 5). This marker block can be placed either on the sternum or on the abdominal wall. A real-time position management (RPM) system uses the data required from the infrared camera to demonstrate patient breathing patterns, allowing individual estimations of the perfect breath hold window. In addition, the RPM system further interacts with the actual RT by stopping the beam, if the breath hold level is not within the predefined limits.

Both vDIBH and ABC-DIBH exert undeniable cardiac dose sparing compared to FB [251-255]. In a recent meta-analysis of all available 10 dosimetric studies, the mean cardiac dose was reduced by as much as 3.4 Gy[34]. However, as no real-life follow-up data regarding cardiac toxicity from these trials is available, the cardiac sparing effects must be estimated via retrospective studies and models. A large

retrospective study by Darby et al[29] showed that the risk of major cardiac event (myocardial infarction, coronary by-pass or cardiac death) increased linearly by 7,4% with every 1 Gy increase to the mean heart dose. In normal tissue complication probability (NTCP)-models, the cardiac mortality probability was estimated to reduce from 4.8% in FB to 0.1% with DIBH[250]. The effect on cardiac dose-reduction is similar with both DIBH-methods, yet the vDIBH is preferred by the patients and radiographers[256].

A) B)

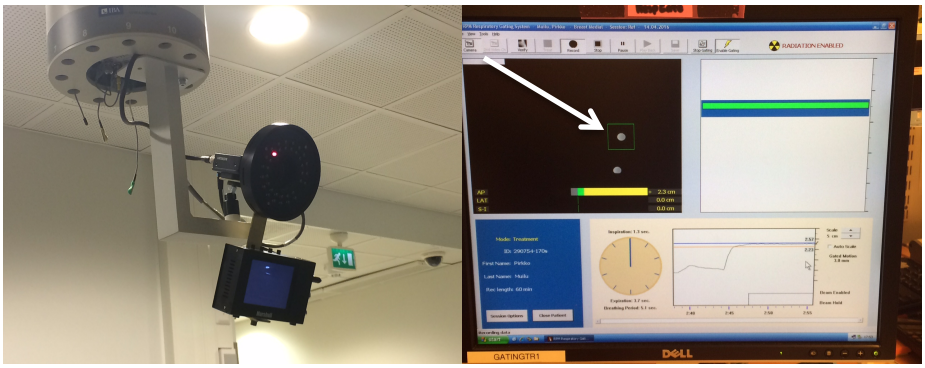

Figure 5. A) An in-room infrared camera tracks the movement of a marker block. B) The view from the monitor showing the marker block dots (arrow) and breath hold level. In this picture the breath hold level (green bar) is inside the desired window (blue bar) and the beam is enabled. Radiotherapists monitor throughout the treatment the movement of the marker block both during the normal breathing cycle and the breath hold.

2.2.4.3 The effect of RT technique

In FB, the cardiac dose can be decreased by IMRT or VMAT-techniques without compromising target coverage[220,257,258]. In DIBH, the tangential field-in-field IMRT excludes the heart from the beam completely in the majority of patients receiving breast-only RT. However, in patients with larger breasts or lymph node irradiation, VMAT or more complex IMRT might improve the cardiac sparing in DIBH[258,259]. Proton RT could be even more cardioprotective[216], but the availability of the proton units in Europe is currently limited.

2.2.5 Reproducibility of treatment

Inter- and intra-fraction variability in breast cancer treatments should be evaluated locally in RT institutions. Inter-fraction variability consists of position uncertainties or even errors in the patient set-up during immobilization or couch shifts. Intra-fraction errors occur after the set-up and are caused by breath movements or involuntary movements (coughing, muscle spams). In breast cancer RT, the reported inter-fraction set-up uncertainties in FB are usually within 5 mm, but larger errors have been observed especially during the first fractions[205,260,261]. Likewise, with DIBH, the reported inter-fraction set-up errors usually stay under 5 mm, but occasional large errors have been observed and thus daily image guidance is preferred with DIBH techniques throughout the treatment[262,263]. This is essential to maintain the desired cardiac-sparing benefit.

2.2.6 Normal tissue tolerance of RT

In external beam RT, different photon beams must usually enter the planned target volume by passing trough adjacent healthy tissue. Depending on the method used (3D, IMRT, VMAT), the dose outside the PTV is either higher in smaller volumes ("shower") or lower in larger volumes ("bath") as presented in Figure 6. The sum of the beams is the desired dose for the PTV, but in every fraction non-cancerous tissue is predisposed to DNA-damages via ionization and reactive radiation induced processes. Whether the subsequent tissue damage is repairable or permanent, grave or subclinical depends of the radiosensitivity of the cells themselves and on the interactions with the parenchyma and supporting cells. In addition, cell proliferation capability, stem cell recruitment and tissue remodeling

are essential in the repair processes[264]. Different tissues react differently to radiation - some with acute manifestations of radiation effects but with a good capacity of tissue repair and re-growth (such as mucosa) and some presenting mainly with late-emerging side effects, which can be unrepairable (such as nerves). In addition to different radio-sensitivity properties, also the dose-volume effects are critical in the tolerance of RT. This dose-effect knowledge is largely derived from historical reports and trials on various radiation fractionation schemes, in which the side effects were reported, as well as from studies utilizing animal models. The first attempts to summarize the available data and knowledge regarding normal tissue tolerance were made in 1991 by a group led by Bob Emami[265]. Further work is continuously ongoing to characterize the dose-volume tolerance and maximum dose of different organs. The QUANTEC (Quantitative Analysis of Normal Tissue Effects in the Clinic) guidelines and reviews are joint efforts to systemically summarize the current knowledge of normal tissue RT tolerance based on the literature[266,267].

A) B)

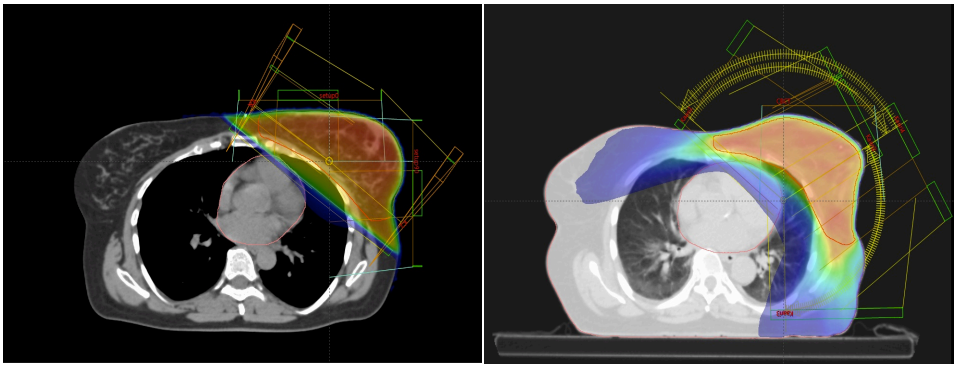

Figure 6. Left-sided breast cancer radiotherapy using A) tangential fields and B) VMAT-technique. In VMAT, 5 Gy doses shown in the picture B are scattered to a larger area outside the PTV (red line) than with tangential technique (A).

2.2.6.1 Lung

In adjuvant breast cancer RT, especially the ipsilateral lung is at risk for radiation-induced processes. The lung is regarded as an OAR and is contoured to the planning CT to calculate the incidental lung dose. RT-induced toxicities can present either as clinical symptoms, such as dyspnea, cough and shortness of breath, or as subclinical findings, such as radiological abnormalities or decreased pulmonary function test results. Clinical radiation pneumonitis, which is an inflammation of the lung tissue presenting as dry cough and dyspnea with typical radiological lesions, occurs in 1-5% patients after breast RT[268]. The risk of pneumonitis can be estimated using NTCP models, of which the Lyman-Kutcher-Burman (LKB)-model is the most widely used[269,270]. Generally accepted guidelines recommend limiting the percent volume of the ipsilateral lung receiving 20 Gy to <20% in breast only treatments and to <30% when the lymph node regions are irradiated[270-273]. Another option is to use the mean lung dose-constraint[274], which should not exceed 20 Gy[270], or more conservatively 15 Gy[275]. Within these limits, the estimated risk of clinical pneumonitis remains below 5%[270]. Subclinical changes in pulmonary functions can be measured in all patients 0.5-2 years after treatment, with gradual improvements later[276]. In another study, further reductions in pulmonary functions were observed at 10 years after RT[277].

Other pneumonitis risk factors in addition to higher radiation doses have been identified. Older age (defined as 60 or older) increases the risk of pneumonitis with an OR ranging from 1.66[278]-1.7[279]. Likewise, prior or concurrent chemotherapy exhibits a similar odds ratio (OR 1.6)[278] and existing lung co-morbidities, such as chronic obstructive pulmonary disease, display the highest risk with an OR of 2.27[278]. In contrast, prior or concurrent smoking appears to reduce the risk of radiation pneumonitis (OR 0.62-0.69)[278].

Symptomatic pneumonitis in treated with oral corticosteroids for 1-3 months[280]. Symptoms usually resolve within days, but improvements in the radiological findings occur slowly. In addition, pneumonitis represents only the early phase of an on-going process that eventually leads to lung fibrosis in the affected area [272,280-282].

Lung fibrosis represents the late stage of radiation induced lung injury and, once present, is irreversible. Different drugs or nutritional supplies have been tested to stop the evolving fibrotic process after RT. So far, the best candidates for clinical

use are angiotensin converting enzyme inhibitors (ACEI) and angiotensin II receptor blockers (ARB) [283-286]. RT-induced inflammation in tissues induces the production of transforming growth factor beeta (TGFβ), which is a major inducer of fibrinogenesis[287]. The renin-angiotensin system (RAS) influences this production chain. Blockage of RAS with ACEI or ARB reduces the excess fibrinogenesis[285]. Other, more early phase and largely rodent-based trials have tested various agents such as angiotensin (1-7) [288], soy [289], lignan[290], curcumin[291] and even blueberry anthocyanins[292] and have observed positive effects. On the contrary, the concurrent use of tamoxifen during RT doubles the risk of pulmonary fibrosis[293]. This finding is consistent with those of other trials[279,293,294].

In addition to the ipsilateral lung dose, the contralateral lung dose should be evaluated. This is especially important when using arc-techniques (VMAT) or IMRT, as the lung-volume receiving smaller doses is often enlarged[225,295]. No definitive dose restrictions have been presented in the literature.

2.2.6.2 Skin and breast tissue

Radiation dermatitis is early-phase radiation-induced skin toxicity. The severity of it can range from mild erythema to painful moist desquamation. The majority (75-100%) of patients receiving adjuvant breast RT experience some grade of dermatitis[296]. The toxicity is dependent on the dermal dose[297]. With modern RT technique, the doses to PTV are more homogeneous and maximal doses are often limited to 107% of the prescribed dose. In addition, after breast conserving surgery, the skin is usually subtracted from the PTV in the beam-planning phase and thereby the dose to the skin is reduced[298]. Furthermore, hypofractionation has been shown to decrease the risk of severe dermatitis[238,299,300]. After mastectomy, the skin in the thoracic wall is included in the target volume[220,301].

The treatment and prevention comprises topical daily application of moisturizers from the start of the RT[302]. Various different lotions and additional substances have been tested, but no product seems to be significantly better than the other[303-305]. If desquamation and breast pain develop, treatment can be intensified using painkillers, natrium chloride (saltwater)-dressings or dry wound dressings such as Mepitel or Duoderm[302]. Acute side effects resolve with time, but may later lead to increased skin fibrosis. Clinically observable, but mostly grade I-II skin or whole breast fibrosis occurs in approximately 10%-27% of patients[306-308]. An addition of

boost (e.g. increased local dose to the tumor bed) significantly increases the risk of severe breast fibrosis[208].

2.2.6.3 Thyroid gland

The thyroid gland is rather sensitive to radiation. Acute symptoms are rare, but late-toxicity occurs in the form of hypothyreosis. Half of the patients will develop hypothyreosis after 45 Gy dose to the gland[309]. In breast RT with nodal treatment, the ipsilateral thyroid gland is difficult to spare without compromising the target coverage. It is estimated, that the incidence of hypothyreosis among breast cancer survivors at 35-120 months after RT is 18% compared with the 6% of matched controls[310]. In NTCP models combined with retrospective dose-distribution data the risk is even higher: if the mean dose to thyroid gland exceeds 26 Gy, as it does in >40% of breast cancer N+ patients, the lifetime risk of hypothyreosis is significant[311-313].

2.2.6.4 Plexus brachialis and spinal cord

Long-term irreversible plexus brachialis toxicity is rare in modern 3D breast RT (<1%)[314]. The plexus brachialis begins at the caudal vertebrae (C5-C8 and Th1), runs along the thoracic wall and behind the clavicle through the axilla to the upper limb. Damage to this nerve induces paresthesia, pain and motoric dysfunction in the arm. The plexus brachialis is generally inside the treatment field in N+ cases. Absolutely safe doses to the plexus are difficult to define. The incidence of plexopathy with 60 Gy breast treatment was much higher than the 50 Gy regimen[314]. The Danish guidelines set a maximum dose to 54 Gy[191], while Emami estimated that the risk of plexopathy was 5% if 62 Gy was administered to one-third of the structure[265]. In addition to the dose-volume ratio, other factors seem to contribute to the risk such as more extensive lymph node surgery[315,316]. Furthermore, retrospective data reveals the significantly increased risk of plexopathy when larger (>3 Gy) daily fractions were used[317]. With modest hypofractionation (≤3 Gy/fraction), the plexopathy rate is similar to that associated with conventional 2 Gy fractionation at 10 years[238].

Regarding the spinal cord, myelopathy is the most feared complication of RT. In breast RT, the spinal cord is always defined as an OAR. The risk of myelopathy is

<1% at 54 Gy and <10% at 61 Gy[318]. Commonly used restriction for spinal cord in breast adjuvant RT is a maximal point dose of 50 Gy.

2.2.6.5 Radiation induced secondary malignancies

As ionizing radiation induces DNA damage in all tissues involved, RT is associated with a risk of secondary malignancies later in life. The standardized incidence ratio (SIR) of secondary malignancies in general after breast RT is 1.2[319]. The SIR of soft tissue sarcomas- especially angiosarcomas- in the treated breast is 2.34[319], yet the absolute numbers for in-field sarcomas are low (12.4 per 100000 person years) [320]. The other organs at risk are lung and contralateral breast. Smoking increases the secondary lung cancer risk 30-fold[321] and young age is a risk factor for secondary breast cancer[322]. In addition, the chosen beam technique can affect the risk – the tangential 3D is regarded safer than IMRT/VMAT[323,324].

2.3 Cardiotoxicity of breast cancer RT

The increased risk of cardiac mortality and morbidity among breast cancer survivors- especially in left-sided breast cancer patients- has been clearly shown in large trials, as summarized in Table 2.

Author, year, reference	Study population N	Design	End point	Result	Remarks
Gyenes G et al, 1998[325]	960	Follow up 20 years BC treated 1971-1976 RT vs. no RT Effect of cardiac dose	Myocardial infarct Cardiac death	In high-cardiac dose group: RR 1.3 AMI RR 2.0 CVD death RR 2.5 Death to ischemic heart disease	Difference started at 4-5 years
Carr et al 2005[326]	1859	Follow-up 22.5 years 1936-1965 CHD after peptid ulcer RT RT vs. no-RT controls	Incidence of CHD death	RR 1.44 AMI Risk of CHD increases with dose (mean >2.5 Gy)	Cardiac doses 1.6-3.9 Gy Risk became apparent after >10 years of follow-up
Darby S et al 2005[28]	308 861	Follow-up 3-30 years BC treated 1973-2001 Left vs. right	Cardiac mortality	Period 1973-1982: RR 1.2(<10 y.) RR 1.58 (>15 y.) Period 1983-1992: RR 1.04 (<10 y.) RR 1.27 (>10 y.) Period 1993-2001: no risk <10 y.	Smoking increased the risk of MI Chemotherapy increased the risk of CHF
Hooning MJ et al 2007[31]	4414	Follow-up 18 years BC treated 1970-1986 RT vs. no RT	Risk of CV disease	SIR 1.3 for CVD (whole group) With IMN-RT: HR 2.55 AMI RR 1.72-2.66 CHF RR 3.17 VD	Smoking increased the risk of MI Chemotherapy increased the risk of CHF
Shimitzu et al 2010[327]	86 611	50 years follow-up of Hiroshima and Nagasaki atomic bomb survivors. Total body dose 0-3 Gy	Excess risk of stroke or heart disease	Excess stroke risk 9%/Gy Excess heart disease risk 14%/Gy	No cardiac risk increase found with doses <0.5 Gy
Bouillon K et al 2011[27]	4456	28 year follow up BC treated 1954-1984 RT vs. no RT Left vs. right	CV mortality	RT vs. no-RT: HR 1.76 cardiac mortality HR 1.33 vascular mortality Left vs. right: HR 1.56 Cardiac mortality	
McGale et al 2011[328]	34 825	BC treated 1976-2006 Left vs. right	CV mortality CV morbidity	No difference IR 1.2 AMI IR 1.25 Angina IR 1.54 VD	Incidence as high in patients treated 1990´s as in 1976-89 Prior CVD increased the risk
Darby S et al 2013[29]	2168	Population based case-control study (963 patients with events, 1205 controls)	Mean heart dose and dose-dependent risk of major coronary events	Mean heart dose 4.6 Gy Risk increases with 7.4%/Gy to heart Prior CV risk factors have additive effect.	Increased risk over time
Henson K et	558 871	BC treated 1973-2008	CV mortality in different	Treated 1973-82:	Increased risk over time

al 2013[329]		Left vs. right	time periods and follow-up	RR 1.19 (<10 y.) RR 1.9 (at +20 y.)	
Rutter CE et al 2014[330]	344 831	6.4 years follow-up BC treated 1998-2006 Left vs. right sided	OS	No difference	Insufficient follow-up time
Wright et al 2015[331]	66 687	15 years follow-up BC treated 1990-1999 Left vs. right sided	Incidence of cardiac related mortality OS	No difference in cardiac mortality between left- and right-sided patients	

Table 2. Summary of the major studies regarding the radiation-induced cardiac morbidity and mortality. Abbreviations: BC breast cancer, RT radiotherapy, AMI acute myocardial infarction, CVD cardiovascular disease, CHD chronic heart disease, RR risk ratio, CHF chronic heart failure, OS overall survival, SIR standardized incidence ratio, IMN-RT internal mammary node radiotherapy, VD valvular disease, CV cardiovascular, MI myocardial infarction, IR incidence ratio, HR hazard ratio.

In these trials (Table 2.), the risk of cardiotoxicity is consistently shown to be time-dependent and the difference between groups starts to emerge after 10 years of follow-up. In addition, the risk is dose-dependent increasing with higher cardiac radiation doses. The risk of acute myocardial infarction (AMI) between RT versus no-RT ranges from 1.3 to 1.44 and the risk of cardiac mortality from 1.76 to 2.0 in patients treated in the 1970´s with more than 20 years follow-up[325,326]. The risk in left-sided patients vs. right-sided patients was more pronounced in patients treated during 1970-1982 than in those during 1983-1992, a finding reflecting the effect of better radiation planning, better techniques and thereby reduction in the cardiac doses[28]. However, specific data regarding cardiac doses became available only after the use of CT-based RT-planning. In a review by Taylor et al involving studies published in 2003-2013, the mean cardiac dose was 5.4 Gy (range, <0.1-28.6 Gy) [332]. In the trials performed by Rutter and Wright, which involved patients treated with the more modern CT-based RT-planning after 1990, no excess cardiac mortality or overall mortality was observed in left- vs. right-sided patients[330,331]. However, long-term cardiac toxicities have not vanished even in the modern DIBH-era, as 1) no safe radiation threshold for the heart has been established and 2) a complete cardiac avoidance is not possible for all patients.

2.3.1 Radiation-induced processes in heart

In fractionated breast RT, the radiation-induced injuries to different cell structures initiate various processes (2.2.1). The formed reactive oxygen species (ROS) and nitrogen oxygen species (NOS) activate the inflammatory and profibrinogenic processes in tissues[287].

Injury to endothelial cells induces vasodilatation (activated by NOS) and increases vessel permeability within minutes, resulting in an influx of macrophages and T-cells (enhanced by ROS) into the irradiated area[333]. This vascular swelling, further intimal hyperplasia and luminal narrowing added to leukocyte infiltration and thrombin release may lead to total clotting of the microvasculature[334,335]. The subsequent development of hypoxia in the adjacent myocytes may promote cell death. In larger vessels, the same process enhances local atherosclerosis[287]. Endothelial dysfunction and activation of several proinflammatory genes in irradiated arteries have been observed from months to years after RT[336].

Among other profibrinogenic cytokines, ROS releases active TGFβ from the extracellular matrix[287,337,338]. TGFβ is the best-characterized activator of radiation-induced fibrosis. In heart, the fibroblasts mature into myofibroblasts and collagen formation begins both in the endothelium and in the extracellular matrix between the myocytes. This radiation induced fibrosis has been demonstrated in animal models after radiation as well as in human skin biopsies and autopsies[333,339,340]. By therapeutically inhibiting TGFβ -formation or signalling, fibrinogenesis can be alleviated[341-343]. Additionally, RT and the ROS/NOS activate the RAS resulting in increasing levels of angiotensin II in tissues[344,345]. These increased concentrations of angiotensin II induce the fibrinogenesis either via upstream induction of TGFβ[346] or directly via a smad-pathway[347]. Commonly used hypertension medications targeting the RAS, namely ACEIs and ARBs, have been observed to reduce the RT induced fibrosis[344,348]. In addition, angiotensin (1-7), a heptapeptide hormone, is able to prevent the angiotensin II induced fibrinogenesis[349,350].

Human myocytes can tolerate RT rather well, as these cells are at non-mitotic stage in adults. However, some functional changes have been observed in myocyte cell culture after radiation: the single action potential was shortened after 7 days of radiation[351]and changes in the calcium handling were observed[352].

2.3.2 The detection of subclinical cardiac changes

The RT-induced changes described above can lead to detectable, yet subclinical changes in cardiac functions early after RT. These changes can be monitored using serum markers, electrocardiogram (ECG), echocardiography, MRI and SPECT- or PET-imaging.

2.3.2.1 Serum markers

Cardiac myocyte damage can be measured by assessing serum troponin T (TnT), troponin I (TnI), or, more recently, high sensitivity cardiac troponin T (hscTnT) [353] levels. HscTnT has a lower detection limit than TnT or TnI[353] and is a well-established biomarker of acute myocardial infarction (AMI) [353,354]. In addition, elevated hscTnT levels can be detected in chronic heart failure[355] and LV hypertrophy[356,357]. Elevated troponin levels without cardiac symptoms have been observed in breast cancer patients after anthracycline-based chemotherapy, even when using less sensitive methods[358-360] as well as after a combined trastuzumab-chemotherapy treatment[361]. Thoracic RT (breast or lung) has been shown to increase troponin levels[362], although the majority of patients in this study received also prior chemotherapy.

B-type natriuretic peptide (BNP) is a polypeptide secreted by the ventricles of the heart in response to excessive stretching of the myocytes[363] or hypoxia[364,365]. The biological effects of BNP lead to increased diuresis, natriuresis and strong vascular actions. Specifically, it transfers fluid and protein from the plasma to the interstitial compartments[363,366]. BNP or simultaneously secreted biologically inactive N-terminal probrain natriuretic peptide (NT-proBNP) can be reliably measured in plasma/serum-samples using immunoassays. They are used in the clinic to detect heart failure (acute or chronic)[367,368] and the severity of ischemic conditions (both mechanical and metabolical)[364,365,369]. In breast cancer patients, elevated NT-proBNP-levels have been detected after chemotherapy[370-372]. In a study by De Iuliis et al[370], significant ($p<0.01$) increases in NT-proBNP concentration were measured at 3 months, 6 months and 1 year after chemotherapeutic treatment, even before the EF decreased. In addition, a significant correlation between increases in NT-

proBNP levels and 1-year mortality was observed. Moreover, after left-sided breast RT, the increases in BNP values were correlated with cardiac radiation doses[369,373,374]. Palumbo et al[374] observed that the BNP levels increased compared with the baseline value at 1 month and 6 months after RT. The magnitudes of these increases were correlated with the cardiac volumes receiving 20 Gy, 25 Gy, 30 Gy and 40 Gy as well as the mean heart dose. D´Errico et al[373] observed similar results after adjuvant RT: the ratio of BNP at 12 months/BNP at baseline increased significantly ($p<0.01$). Furthermore, the BNP levels at 12 months correlated with higher cardiac radiation doses to 50% of the volume of the heart ($p=0.03$) and ventricle ($p=0.04$).

Cardiac biomarkers may be beneficial in selecting patients with the highest risk of long-term cardiac toxicity after breast cancer adjuvant treatments as well as in recognizing the concurrent risk factors. Biomarkers may also guide the administration of drugs to treat or prevent cardiotoxicity either during or immediately after the treatments[371,375].

2.3.2.2 Electrocardiogram

RT to the thoracic area has been shown to induce ECG-changes[376-379]. Such changes have been observed at the end of RT[376] as well as at 1-4 months after RT[377,378] in roughly one-third of the patients[376-378]. T-wave abnormalities are the most common findings[376,377]; namely, T-wave depression or inversion. The mechanism of this is not fully known. In a study with a longer follow-up period demonstrated, that the observed RT-induced T-wave changes had reversed in 60% of patients at one year after RT[376]. In addition, a fragmented QRS-complex has been reported at one year after completion of RT in 37% of patients with a correlation to mean cardiac radiation doses (OR 6.48 for doses over 2.2 Gy) [379].

As RT induced fibrinogenesis matures and collagen content between the myocytes increases during the months and years, it would be logical to observe conduction abnormalities at the later phase. However, no significant increase in severe arrhythmias has been observed or reported[33,380]. On the other hand, case reports describing severe conduction abnormalities requiring a pacemaker 10-20 years after thoracic RT have been published[381-383].

2.3.2.3 Single photon emission computed tomography

Single photon emission computed tomography (SPECT) is used to detect changes in myocardial perfusion either during physical exercise (ischemia) or at rest (scar tissue). Therefore, SPECT is a functional image modality and is regarded reliable in the diagnosis of an ischemic heart disease and in the assessment of myocardial function and viability[384]. However, smaller subendocardial infarcts can be missed with SPECT-imaging[385]. SPECT uses radionuclide tracers that behave like microspheres with a longer-term distribution, allowing the tracer injection to be decoupled from the actual imaging process with CT[384].

After thoracic RT, subclinical changes in myocardial perfusion by SPECT have been observed. Gyenes et al[386] detected new fixed (both under stress and at rest) SPECT perfusion defects in 6/12 patients at 13 months after breast RT. In addition, these defects were located in the anterior parts of LV corresponding to areas receiving higher radiation doses. Similar results were observed by Lind et al[241], who performed SPECT (only at rest) at 6, 12 and 18 months after left-sided breast cancer RT. In their study, perfusion defects at 6 months were found in the region supplied by left anterior descending artery (LAD). The defects associated independently with the percentage of irradiated LV (<0.01), hormonal therapy (p=0.005) and baseline hypercholesterolemia (p=0.006). The SPECT results did not change significantly between 6 and 12 or 18 months post-RT. In addition, Eftekhari et el[387] observed new defects in anterolateral and apical walls in stress-SPECT at 6 months after RT in left-sided breast cancer patients (n=35) compared to right-sided patients (n=36)(p<0.05). These observed changes reversed in SPECT performed at rest. On the contrary, in a trial involving 32 node-positive left-sided breast cancer patients with a mean cardiac dose of 2.8 Gy (range 1.1-6.1 Gy) significant changes in stress-test SPECT were not detected (p=0.38) at 1 year after RT[388].

The incidence of new perfusion defects increased with time from 27%, 29%, 38% and 42% in a time-scale of 6, 12, 18 and 24 months after RT in a study by Marks et al[389]. Perfusion defects occurred significantly more often in patients with >5% of LV inside the RT fields. These RT-induced perfusion defects appear to be long-term, as Prosnitz et al observed defects in SPECT-scans repeated at 3 and 6 years after left-sided breast cancer RT[390].

In addition, SPECT or other nuclear imaging could be useful in detecting early, subclinical chemotherapy- or targeted therapy-induced cardiac defects[391]. Small trials are published regarding SPECT detected cardiotoxicity of taxanes[392] and anthracyclines[393,394].

2.3.2.4 Positron emission tomography

Positron emission tomography (PET) is a nuclear imaging technique. It uses radioactive analogues of normal substrates (such as glucose or water) that are used by metabolically active cells. The radioactive component allows the dynamic imaging using a PET-scanner. The acquired images are often fused with either CT (PET-CT) or MRI (PET-MRI) to allow anatomical evaluation of metabolical changes. In heart, myocardial perfusion imaging with PET allows global and regional measurements of perfusion, blood flow as well as function at stress and rest.[395-397]

PET can be used to detect RT-induced cardiac changes. 18F-fluorodeoxyglucose (FDG) is the most commonly used radioactive analogue and reflects the glucose intake of active cells. Unal et al[398] performed FDG-PET-CT in 38 patients at 4 months after thoracic RT. In this study, 28/38 patients were found to have significantly ($p<0.01$) higher regional myocardial FDG uptake in the irradiated cardiac segments than in the non-irradiated segments. Similar results were reported in a case report by Zöphel et al[399], in which the areas of increased up-take in PET correlated with the areas receiving higher radiation doses. As FDG-PET characterizes inflammation in cardiac tissue[400], the observed subacute changes most likely reflect the inflammation induced processes. Interestingly, increased vascular PET-activity in aorta or vena cava has been shown to reflect the risk of future cardiovascular events in cancer patients (73% of the study population had received treatment for cancer, and 18% had received thoracic RT) [401].

In addition to FDG, other substances have been tested in cardiac PET-scanning. These include 13N-ammonia, 15O-water, 82Rb and 18F-flurpiridaz-radiotracers[397]. Cardiac PET-CT/MRI is becoming an essential part of cardiac diagnostics - especially in inflammatory diseases such as cardiac sarcoidosis, arteritis, coronary artery disease- as the availability of PET-scans increases in clinics[396,397,400].

2.3.2.5 Echocardiography

Cardiac echocardiography is the most widely used imaging technique in cardiology. It allows both structural and functional evaluation of heart in real-time and at low-cost. 2D-, 3D- and Doppler-imaging among other newer technologies can accurately measure chamber sizes, wall thicknesses, ventricular functions both in systole and diastole, valvular anatomy as well as blood-flow velocities and hemodynamics. Furthermore, advances in technology have made it possible to store echocardiography data digitally and analyze it retrospectively. In addition, echocardiography is non-invasive and non-ionizing and therefore safe to the patient, but pendent of the experience of the performer.[402-404]

Left ventricular diastolic function

At systole, kinetic energy is stored at the myocyte level (actin-myocyte interaction) as well as at the structural level by LV rotational movement. In early diastole this energy is discharged, resulting in an active restoration of LV diastolic volume and a negative pressure inside the LV. This negative pressure opens the mitral valve and induces passive blood flow from the left atrium (LA). This early part of filling accounts of 80% of the final end-diastolic volume under normal conditions and is measured and described in echocardiography as the ventricular filling velocity E (Figure 7). The latter part (ventricular filling velocity A) of the filling is caused by atrial contraction, which forces additional blood into the LV in patients with sinus rhythm. The E/A ratio can be used in diagnostics, since it is reduced (E/A <1) in diastolic dysfunction.[402,405,406]

Diastolic filling can be impaired for a number of reasons. The first part of the LV filling can be reduced or prolonged by numerous factors such as systolic heart failure or dyssynchrony, LV hypertrophy or simply by aging. The atrial filling can be impaired by non-compliant stiff myocardium or by increased LV filling pressure. Rarely, mitral valve stenosis can be the limiting factor in LV filling. A number of echocardiographic parameters in addition to the E/A ratio describe LV diastolic function: the velocity of mitral annular movement e´(relaxation), isovolumetric relaxation time IVRT (LA pressure) and deceleration time, dt, among the basic measurements (Figure 7). The combined information will allow the grading of diastolic dysfunction (I-IV).[402,405,406]

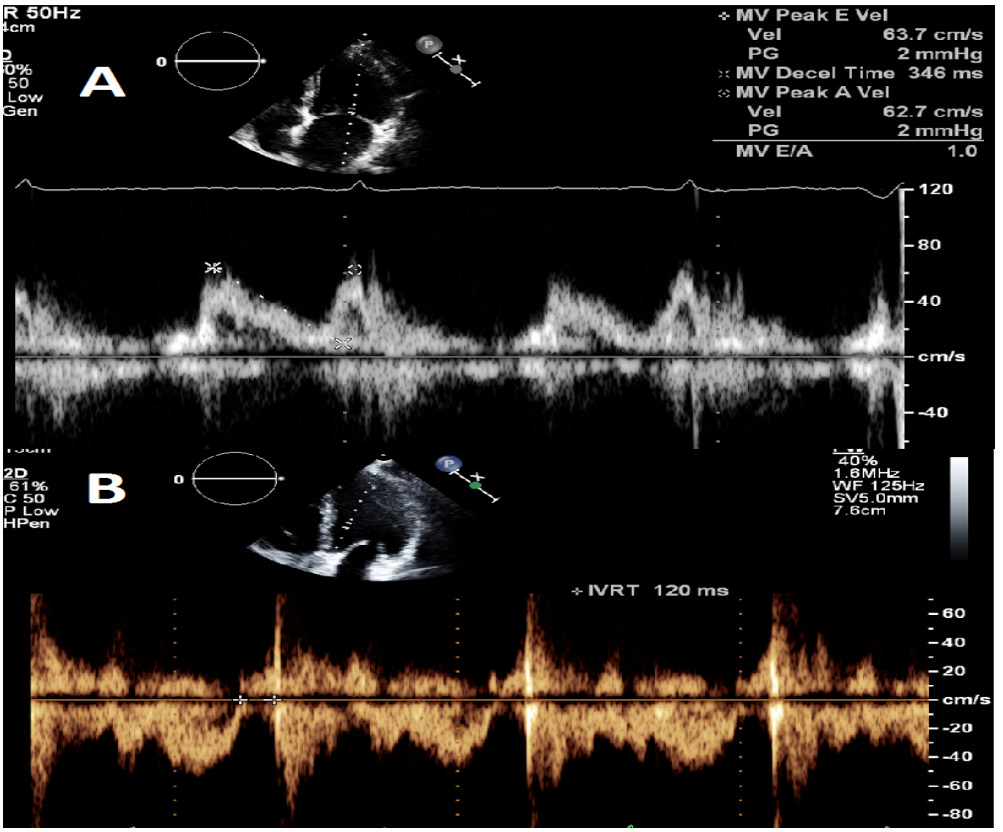

Figure 7. Measurements of left ventricular diastole in echocardiography. Mitral inflow measurements are displayed in figure 7A. The measurements are taken at the mitral valve opening level. The first part of the mitral inflow (E-wave) and its length (dt, declaration time) are directly related to left ventricles active relaxation properties. The A-wave is created by active atrial contraction in patients with sinus rhythm. In figure 7B measurement of isovolumetric relaxation time (IVRT) is displayed. The measurement is taken at the midpoint between mitral valve and left ventricular outflow track from closing signal of aortic flow to opening signal of the mitral valve inflow. All diastolic measurements are taken at the end-expirium and with pulsed Doppler.

Subclinical diastolic changes in echocardiography after thoracic RT have been evaluated in a few studies. Dogan et al[407] found a decrease ($p<0.01$) in E and the E/A ratio and increases ($0<0.01$) in dt and IVRT after thoracic RT in 40 patients. Likewise, Cao et al[408] observed negative diastolic changes in patients (with or without concurrent trastuzumab) measured immediately, at 3 months and 6 months after RT. On the contrary, Erven et al[240] did not detect any significant changes in E/A or dt after RT in patients with previous adjuvant chemotherapy. In addition to conventional parameters, strain rate imaging can be used to evaluate diastolic changes. Lo et al[409] observed decreases ($p<0.05$) in the longitudinal diastolic strain rate at 6 weeks after left-sided breast RT; however, the circumferential strain rate remained unchanged.

Left ventricular systolic function

After breast RT, subclinical changes in echocardiography have been observed with more thorough and sophisticated echocardiographic methods than the traditional measurement of LV´s EF (LVEF). Strain or strain rate imaging (SRI) is a sensitive method that can be used to describe LV´s systolic function. It can measure both global or regional strain as well as changes in the longitudinal or circumferential contraction[410,411]. Erven et al[240] performed sequential echocardiographs with SRI for 75 women (51 left-sided and 24 right-sided) before RT, immediately after RT, and at 8 and 14 months after RT. All patients had received adjuvant chemotherapy. A decrease in SRI was observed at all measured time-points after RT in the left-sided group ($p<0.01$), but not in the right-sided group. Conventional echocardiographic parameters did no change significantly. Lo et al[409] observed similar results with strain imaging among 40 women after left-sided breast RT without prior chemotherapy. In this study, the global longitudinal strain (GLS) and radial strain reduced significantly ($p<0.05$) from baseline whether measured during RT (after 3-4 weeks) or at 6 weeks after RT while the LVEF remained unchanged. Heggemann with his colleagues[412] followed 49 left-sided breast cancer patients with both echocardiography and MRI at 6, 12 and 24 months after RT. Longitudinal strain was reduced in apical-anterior, apical-inferior and mid-posterior segments at 24 months compared to baseline corresponding to LV volumes receiving higher RT doses.

The major component of RV systole is RV's longitudinal contraction. It can be measured as total displacement of the tricuspid annulus from end-diastole to end-systole (TAPSE, tricuspid annular plane systolic excursion). TAPSE is considered as simple, reproducible parameter of RV´s systolic function in transthoracic echocardiography[413,414]. Reduction in TAPSE is a negative prognostic factor in other cardiac diseases, such as heart failure –even in patients with preserved LVEF[415,416], pulmonary hypertension[417] and pulmonary embolism[418,419].

Heggemann et al observed a decrease in TAPSE with echocardiography at 24 months after RT in the entire cohort of 49 left-sided breast cancer patients (-3.1 mm) and this finding was similar to those observed with MRI[412]. There are no other studies examining the effect of RT on RV functions using echocardiography.

2.3.2.6 Magnetic resonance imaging

MRI provides good morphologic views of the heart, pericardium and great vessels. Differently weighted images allow visualization of oedema[420]. Using contrast agents (most often gadolinium-based agents), one can gather information of myocardial perfusion. Even very small areas of myocardial damage can be depicted[421,422]. In addition, information regarding changes in both the myocardium and the interstitium such as inflammation and fibrosis[423,424] as well as changes in functional parameters similar to those in echocardiography can be obtained[425].

Heggemann et al[412] performed MRI after breast RT and observed, that LVEF at 6 months was significantly reduced from baseline in patients without prior chemotherapy ($p=0.005$). The decrease was, however, transient and normalized later in the follow-up. In patients who had received prior chemotherapy, no changes in LVEF were observed during RT. In basic parameters, both mitral annular plane excursion (MAPSE, LV function) and TAPSE (RV function) were reduced over the entire study population.

The European Association of Cardiovascular Imaging (EACVI) and the American Society of Echocardiography (ASE) expert consensus statement suggest

using MRI to detect radiation induced heart disease (RIHD) second-line after echocardiography. The role of MRI is strongest in detecting cardiac fibrosis, yet the role in pure radiation-induced fibrosis in still unestablished[426]. In studies addressing this issue[427], most patients had received prior chemotherapy, which in itself has been shown to induce myocardial changes[428-430].

2.3.3 Clinical manifestation

The clinical manifestations of RIHD appear 5-20 years after RT (Table 2). The first manifestation can be sudden and fatal, such as AMI, or slowly progressing, such as valvular diseases or cardiac failure. Radiation induced long-term cardiac morbidities have substantial effect to the quality of life and appear earlier in life than in women without cardiac RT.

2.3.3.1 Ischemic heart disease

The risk of excess cardiac mortality due to ischemic heart disease is perhaps the best-defined parameter in RIHD, as data regarding cause of death are documented in many countries and can be combined with cancer registry data. Large population based trials and longitudinal studies have been derived, as presented in Table 2. Sarah Darby et al[28] published in 2005 a large US Surveillance Epidemiology and End Results (SEER) based data study involving more than 300 000 women with early breast cancer. The mortality ratios were higher among left-sided vs. right-sided patients: 1.43 for AMI and 1.60 for other forms of ischemic heart disease after 10 years or more after treatment. Henson et al[329] did an even larger analysis in 2013, going through SEER data of more than 500 000 women. In this trial, the risk ratio for cardiac death (all causes) in left-sided vs. right-sided patients increased steadily during years. The highest RR, 1.90, was observed in patients treated in 1973-1982, who had undergone more than 20 years of follow-up. A smaller study involving 4456 patients who were treated in Institut Gustave Roussy from 1954-1984 showed an overall risk of cardiac mortality 1.76 (RT vs. no-RT) and 1.56 (left vs. right) [27]. In addition, the results of this trial indicated an increased cardiovascular risk with time ($p=0.05$).

The risk of ischemic cardiac death has been shown to be dose-dependent: the more dose to the heart, the greater the risk. This relationship has been demonstrated not only in trials comparing left-sided and right-sided patients, but also in a trial comparing inner-quadrant tumors to lateral tumors in left breast[431] as well as in a study analyzing 1859 patients, who had received RT to treat peptid ulcer[326].

The radiation effect on ischemic cardiovascular morbidity -AMI, coronary artery disease, coronary revascularization and chronic ischemic heart disease- is of similar magnitude than on cardiac mortality. The overall risk is for AMI is around 1.2-1.5 (Table 2). The dose-volume-effect for cardiac morbidity and mortality is best described in a study by Darby et al in 2013[29]. They estimated the mean heart dose and the mean LAD dose from RT charts of 2168 women (963 with major coronary event and 1205 controls) as well as obtained individual patient information regarding concurrent diseases. A striking correlation with mean heart dose and the risk of major coronary event was observed; with every increase of 1 Gy, the risk increased by 7.4%. Furthermore, an additive negative effect was observed, if the patient had pre-existing cardiovascular risk factors (defined as a history of other circulatory diseases, diabetes, chronic obstructive pulmonary disease, smoking, obesity and regular analgesic use). No safe mean heart dose threshold dose was identified. Similar findings regarding the additive effect of concurrent smoking have been observed in other trials[28,31]. Hypertension was found to increase the risk of coronary artery disease in a study of 961 patients as follows: right-sided, no hypertension, HR = 1.0 (reference); right-sided, with hypertension, HR = 7.2 (95% CI, 2.9 to 17.9); left-sided, no hypertension, HR = 4.6 (95% CI, 2.0 to 10.4); and left-sided, with hypertension, HR = 11.4 (95% CI, 5.0 to 26.2) [30].

Radiation-induced stenoses in coronary arteries are most often located in the anterior arteries, mainly the LAD, corresponding to the areas receiving the highest cardiac doses during left-sided breast RT. In a study of Correa et al[432], 70% of the stress test abnormalities noted in left-sided breast cancer patients were within the LAD territory. In 62% of patients, who underwent cardiac catheterization, the stenoses were located solely in the LAD. A Swedish registry based study analyzed the coronary angiography data of 199 breast cancer patients[433]. For previously irradiated left-sided patients, the OR for grade 3 to 5 stenosis in the mid-LAD+ distal diagonal artery was 4.38 and the OR for grade 4 to 5 stenosis was 7.22 compared with right-sided patients.

2.3.3.2 Valvular dysfunction

Exposure of heart to RT is associated with a risk of later valve damage. The pathogenetic mechanisms underlying this damage are not clearly understood, as the cardiac valves do not omit vascular structures. However, increased fibrinogenesis and calcification in valves is observed after RT, within an interval of 10-20 years. Thickening and fibrosis of valves leads to restricted motion and either valvular stenosis or regurgitation.[434]

After breast RT, McGale et al[328] observed that the incidence ratio of valvular dysfunction between left- and right-sided patients was 1.54 (95% confidence interval 1.11-2.13, $p<0.01$) over a mean follow-up time of 18 years. Similarly, in Hodgkin lymphoma (HL) patients receiving mediastinal RT, the risk was observed to be dose-dependent[434-436]. In another study with HL patients, the incidence of moderate or severe valvular dysfunction was 1% at 10 years, 4 % at 15 years and 6% at 20 years after RT with estimated mediastinal doses of 33-37 Gy[437]-doses substantially higher than those used for breast RT. Prior anthracycline use and smoking are shown to further increase the risk of valvular disease compared with RT alone in HL patients[438]. Echocardiography is the method of choice to screen valvular changes in the follow-up of patients after thoracic RT[426,434]. Valvular operations can be performed for radiation-induced valvular disease either surgically[439] or percutaneously[440]with a similar complication rate than with patients with other valvular diseases.

2.3.3.3 Pericarditis

Radiation-induced pericarditis can manifest during the acute phase (inflammation) or at the later phase (fibrosis). The latter is more serious, as it may lead to severe constriction and HF. The overall incidence of pericarditis in breast cancer patients was not increased in the study of McGale et al[328] as it was observed in 96/37309 patients, who were not treated with RT and in 96/34825 patients with RT. Acute pericarditis was slightly more common after RT in left-sided (n=28) than in right-sided (n=12, p=0.03) patients, yet the absolute numbers are low. The risk of pericarditis increases when doses >27-30 Gy are administered to the whole pericardium and is low (<5%) when doses are <13 Gy according to QUANTEC

estimations[32]. Therefore, with modern 3D RT-planning, the risk of severe pericarditis after breast RT is low[332].

2.3.3.4 Cardiac dysfunction

Cardiac dysfunction can be separated into two entities: diastolic dysfunction and systolic dysfunction, which can occur separately or simultaneously.

Diastolic dysfunction

The term "diastolic function" usually denotes to the ability of the LV to fill sufficiently to produce the required stroke volume within normal pressure limits of LA and pulmonary capillaries. If LV filling is impaired during early diastole, subsequently the pressure in LA must increase to ensure sufficient filling volumes into LV. The physiological changes associated with LV diastolic dysfunction include impaired LV wall relaxation, reduced diastolic compliance and elevated LV filling pressure (mainly a compensatory mechanism). Furthermore, increased LA pressure causes pulmonary venous congestion, which can manifest as dyspnea in heart failure (HF) patients. Approximately half of the patients with clinical symptoms of HF are found to have normal, preserved LV systolic function (defined as an LVEF>50%), but major changes in LV diastolic parameters are observed. Hence, the term "heart failure with preserved ejection fraction" (HFpEF) has been introduced in cardiology, an entity in which diastolic dysfunction plays a major role but is not solely responsible for. Alternative diagnosis, such as COPD, CAD and valvular disease, should be ruled out and a thorough echocardiographic evaluation should be performed before settling into the diagnosis of HFpEF. [406,426,441]

Unlike LVEF for systolic function, there is no single measurement that characterizes diastolic function, but a combination of different indices can be used to determine whether diastolic function is normal or impaired. Basic echocardiographic measurements can be used to grade the diastolic dysfunction as follows[441,442]:

1. Grade I (mild dysfunction): Abnormal relaxation of LV, usually normal filling pressures. E/A <0.8, dt>200 ms

2. Grade II (moderate dysfunction): E/A 0.8-1.5, E/e´ 9-12 cm/s

3. Grade III (severe dysfunction): Abnormal relaxation and elevated filling pressures. E/A >2, dt<160 ms and E/e´>13 cm/s.

In addition, more sophisticated methods in echocardiography can be used to evaluate diastolic function, such as strain rate imaging, speckel tracking to detect torsional twisting and stress test echocardiography, as summarized by Maragiannis et al[441].

Grade I diastolic dysfunction is usually asymptomatic. The first clinical signs often appear during physical exercise and present as reduced performance status and fatigue. Furthermore, an extra load to heart such as atrial fibrillation may enhance these symptoms. In grade III dysfunction, dyspnea and reduced performance status manifest during normal, daily activities and a diagnosis of chronic heart failure (HF/HFpEF) can be made. Known risk factors for HFpEF include hypertension, diabetes, female gender and age.[443,444]

The mechanisms underlying diastolic dysfunction have been sought. Naturally, factors that modify the relaxation and stiffness of LV wall are a major cause. Comorbidities may induce a systemic pro-inflammatory state causing endothelial inflammation[445]. Inflammation leads to oxidative stress (ROS/NOS-induced), which increases diastolic dysfunction by inducing fibrosis and myocardial hypertrophy (by decreasing low protein kinase G activity) and altering intracellular calcium-handling[445-447]. Increased fibrosis in LV wall is associated with diastolic dysfunction[448,449].

Treatment options to treat early grade diastolic dysfunction concentrate to the risk factors. Hypertension and diabetes should be treated optimally. Dietary changes are introduced and physical activity is encouraged in obese patients to induce weight loss. If symptomatic HFpEF has evolved, medications (diuretics, ACEI, ARB, beetablockers) can mitigate the symptoms, but most of these agents have failed to decrease the mortality or hospital admissions due to HF[443]. Only one beetablocker, nebivolol, has been shown to improve these end-points in a randomized trial[450]. New drugs for treating HFpEF are eagerly awaited[443].

As the concept of HFpEF has emerged only within the last 10 years in the field of cardiology, there are no long-term data regarding the incidence of severe diastolic dysfunction in breast cancer survivors after RT. As presented in section 2.3.2, subclinical diastolic changes have been observed in patients after RT.

Systolic dysfunction

Cardiac systolic function most often refers to the LV´s systolic capability. If the LVEF is threatened by decreased myocardial contractibility, compensatory mechanisms are initiated to sustain the EF. These responses include increases in heart rate, the Frank-Sterling mechanism, catecholamine release, an activation of RAS and atrial natriuretic peptide release. Unfortunately, these mechanisms often only exacerbate the vicious cycle of cardiovascular stress in the long-term and eventually lead to HF.[451]

Echocardiography is the recommended imaging modality to detect systolic dysfunction or HF due to its availability, portability, safety and low-cost. Most often, an LVEF cutoff of <40% is used to determine systolic dysfunction. A normal LVEF (e.g. the percentage of blood volume pumped out of the total LV blood volume during systole) ranges from 50-70, while the range 40-50 is often called a borderline. In addition to providing the LVEF, a good echocardiography report must state the LV dimensions as well as an estimation of wall motion abnormalities to evaluate the LV systolic function.[402,404]

The clinical symptoms of HF include shortness of breath, coughing and fatigue. These originate from the increased pulmonary pressure and congestion. Additive workload to RV due to LV HF may also lead to right-sided HF, which is characterized by peripheral oedema (commonly known as pitting oedema). The clinical severity of HF is often graded using the New York Heart Association (NYHA)-scale, which was developed in 1928[452]:

NYHA	Symptoms
I	Cardiac disease, but no symptoms and no limitation in ordinary physical activity
II	Mild symptoms (mild shortness of breath and/or angina) and slight limitation during ordinary activity
III	Marked limitation in activity due to symptoms, even during less-than-ordinary activity, e.g. walking short distances (20–100 m). Comfortable only at rest.
IV	Severe limitations. Experiences symptoms even while at rest.

Chronic HF can be caused by ischemic heart disease, cardiomyopathies or in rare cases inflammatory heart diseases– basically every cardiac disease, if chronic or untreated, can eventually lead to HF. The long-term incidence of HF is increased in breast cancer survivors (HR 1.95, 95% CI 1.27-3.01) [453]. Furthermore, the risk of HF is associated with increased cardiac doses (RT to the internal mammary lymph nodes) as demonstrated in the study by Hooning et al[31].

2.3.4 Confounding factors to cardiotoxicity

2.3.4.1 Obesity

Obesity is a known risk factor for breast cancer recurrence and mortality as well as for overall mortality. A meta-analysis by Chan et al[454], which included 213 075 breast cancer survivors, analyzed the effect of body mass index (BMI, kg/m^2) on breast cancer mortality and overall mortality. BMI was categorized as underweight (<18.5), normal (18.5-<25), overweight (25-<30) and obese (>30). The relative risks (RRs) of total mortality were 1.41 (95% CI 1.29-1.53) for obese, 1.07 (1.02-1.12) for overweight and 1.10 (0.92-1.31) for underweight. The risk was highest among obese premenopausal patients, whose RR was 1.75 (1.26-2.41). In addition, each 5 kg/m^2 increase in BMI before, <12 months after and >12 months after the breast cancer diagnosis increased the risk of overall mortality by 17%, 11% and 8%, and breast cancer mortality by 18%, 14% and 29%, respectively.

The biological mechanisms underlying this additive risk are not clearly understood, but modified host factors such as abnormal production of leptin, adiponectin, steroid hormones and insulin as well as ROS-induced chronic inflammation, are thought to be involved[455]. Regarding the heart, being overweight or obese is associated with LVH independent of blood pressure levels[456]. One proposed mechanism is, that obesity induces pathological cardiac hypertrophy via the hypoxia-inducible factor (HIF) pathway by increasing the production of several proinflammatory cytokines and by inducing the overexpression of key cardiomyopathy-associated genes in adipose tissue[457]. Furthermore, this chronic inflammation is thought to enhance coronary artery disease in obese patients[458].

A BMI >30 was found to be a risk factor (RR 1.57, p=0.002) for major coronary events after breast cancer adjuvant RT in the study by Darby et al[29] whereas a BMI <30 was not found to increase the risk. The other large trials (Table 2) have not systematically evaluated the effects of BMI on RIHD.

2.3.4.2 Hypertension

It is widely known that hypertension causes different cardiac complications, such as left ventricular hypertrophy (LVH) as a result of increases in cardiac workload, and increases the risks of ischemic heart disease and HF[459-461]. In addition, hypertension has been found to be a moderate risk factor for breast cancer incidence[462,463].

As hypertension is a common CVD among postmenopausal women, many studies have addressed the safety of hypertensive medications with regard to breast cancer incidence, breast cancer recurrence rate or breast cancer mortality. Some studies have noted an increased risk of breast cancer recurrence[464] or second primary breast cancer in patients using ACEIs[462]. On the contrary, the use of ACEIs has been shown to decrease the risk of breast cancer recurrence in others[465-467]. In a large British study involving almost 380 000 ACEI/ARB-users, no evidence of an increased overall cancer risk was found[468]. A slight increase in breast cancer incidence was observed with the use of ACEI/ARB (HR 1.11(1.01-1.21)), but longer treatment durations did not seem to be associated with a higher risk. In the same study, the use of ACEI/ARB was associated with a protective

effect on lung cancer incidence. According to the authors, non-causal mechanisms cannot be ruled out and the beneficial effects of ACE/ARB on cardiovascular morbidity and mortality substantially outweigh the possible breast cancer risk. [468]

Harris et al observed that the incidence of ischemic heart disease was increased after breast RT in patients with prior hypertension [30]. In addition, hypertension is found to be an independent risk factor for higher coronary artery calcium score at 12 years after left-sided breast RT (p=0.022) [469].

2.3.4.3 Smoking

Smoking in general is known to induce carcinogenesis and cancer due to various mutagenic substances. Accumulating evidence indicates that the risk of breast cancer among smokers is increased. A meta-analysis[470] demonstrated that among current active smokers the summary RR was 1.13 (CI 1.09-1.17) in different prospective studies (n=27) and 1.08 (0.97-1.20) in retrospective studies (n=22). In patients with a history of former smoking, the RR was 1.10 (1.09-1.12) in 27 prospective studies and 1.08 (1.02-1.14) in 44 retrospective studies. In addition, smoking is attributed to an increased risk of breast cancer mortality. In two different trials the RR was 1.3 (CI 1.2-1.5) [471] and 1.75 (CI 1.13-2.60) [472].

Smoking increases the risk of cardiovascular mortality and morbidity. For the peripheral artery disease, the pooled OR was 2.71 (CI 2.28-3.21) [473]. For acute coronary events, the polled OR was 1.98 (1.75-2.25) [474], for stroke 1.58 (1.40-1.78) [474] and for cardiovascular mortality 2.07 (1.82-2.36) [474] (p<0.01 in all). The effect of smoking on the risk of coronary heart disease is even higher in women than in men: the pooled adjusted female-to-male relative RR of smokers compared to non-smokers was 1.25 (1.12-1.39, p<0.01) [475]. Even passive smoking has been shown to increase the risk of coronary heart disease with a RR of 1.25 (1.17-1.32, p<0.01) [476]. In addition, an increase in cardiovascular risk was observed in every meta-analysis with increasing tobacco consumption and additional smoking years[473-476].

The "2014 Surgeon General's Report: The Health Consequences of Smoking—50 Years of Progress" summarized also the known risks of smoking during active cancer treatment[477]. These data were reviewed by Warren et al in Lancet Oncology 2014[478] with the following conclusions regarding the concurrent smoking during curative-intent RT: smoking increases the risk of cancer mortality in head and neck

cancer with an HR of 2.19-2.51 and in prostate cancer with an HR of 2.86-4.27. Concurrent smoking during RT increases the risk of secondary cancers: after breast cancer RT with concurrent smoking the RR of lung cancer is 8.96. Furthermore, the long-term pelvic toxicity is increased with concurrent smoking.

The decreased effect of RT to tumors in current smokers is thought to evolve from the increased blood carboxyhemoglobin-concentration, which decreases the tumor oxygen utilization[479,480]. Likewise, the incidence of radiation pneumonitis after breast RT is found to be less in patients with current smoking (OR 0.6, $p<0.01$) [481]. However, the effect of smoking to long-term cardiovascular complications after breast RT is well established and especially the risk of MI increases[28,31].

2.3.4.4 Hypercholesterolemia

High serum cholesterol levels, especially high low-density lipoprotein (LDL)-cholesterol levels, are regarded as cardiovascular risk factors. Increased levels of LDL are associated with an increased risk of coronary heart disease, while higher levels of high-density lipoprotein (HDL) cholesterol appear to be cardioprotective. Statins are widely used to lower the risk of CVD due to hypercholesterolemia in both primary and secondary prevention. [482-484]

The effect of hypercholesterolemia and statin use on cancer incidence has been searched. Thus far, not even in large meta-analysis, the statin use has not been shown to affect cancer incidence, prevention or prognosis. [485-488]

The combination of hypercholesterolemia and cardiac irradiation induces an inflammatory response, microvascular and endocardial damage, and accelerates the development of coronary atherosclerosis[489,490]. In a study by Lind[241], hypercholesterolemia was associated with LV perfusion defects in SPECT at 6 months after RT

2.3.4.5 Diabetes

Diabetes mellitus, as well as obesity, is a known risk factor for breast cancer incidence. A large 2013 meta-analysis demonstrated that the overall HR for breast cancer incidence was 1.23 (95% CI 1.12-1.34) in patients with diabetes [491]. In another meta-analysis, the breast cancer risk was related to type II diabetes in postmenopausal women, but not to type I or premenopausal diabetes[492]. In this study, the risk of breast cancer was 27% higher among women with type II diabetes and after adjusting for BMI, the corresponding risk was 16% higher.

Diabetes not only increases the breast cancer incidence, but also worsens the outcome. In the meta-analysis by De Bruijn et al[491] the RR for breast cancer mortality was 1.38 (1.2-1.58) in diabetic patients. In addition, the risk of overall mortality was twice as high in women with a glycohemoglobin (HbA1C)>7.0% than in women with an HbA1C <6.5% (HR 2.35; 95% CI 1.56-3.54) [493].

Cardiovascular morbidity and mortality is increased among patients with diabetes. In a meta-analysis, the adjusted HR was 2.00 (95% CI 1.83-2.19) for coronary heart disease, 2.27 (1.95-2.65) for ischemic stroke, 1.56 (1.19-2.05) for hemorrhagic stroke and 1.84 (1.59-2.13) for unclassified stroke[494]. The HR of vascular mortality among diabetic patients was 2.32 (2.11-2.56) [495]. The effect of different glucose-lowering drugs on cardiovascular outcome are under research[496].

The effects of radiation under hyperglycemic conditions have not been studied widely. In a cell model, hyperglycemic retinal microvascular cells expressed more DNA damage after radiation than normoglycemic cells[497]. Hyperglycemia during chemoradiotherapy of glioblastoma patients is observed to impair the prognosis, but the mechanisms are not known[498]. Furthermore, after whole brain RT, the long-term negative changes in the brain white matter were increased in patients with poor glycemic control ($p<0.01$) [499]. Darby et al[29] explored the risk of major cardiovascular incidents after breast RT and determined that the RR for such events was as high as 3.23 ($p<0.01$) in patients with known diabetes.

2.3.4.6 Hormonal status

Premenopausal women are known to have less CVD than men of the same age, and the risk of CVD increases after menopause[500]. This protective effect in premenopausal women has been largely attributed to estrogen. With high hopes, two large randomized trials - Women´s Health Initiative (WHI)- were launched involving postmenopausal women to evaluate the effect of estrogen±progesterone replacement therapy on CVD incidence and CV mortality[501,502]. These studies accrued 10 739 women receiving estrogen only or placebo and 16608 women receiving combination therapy or placebo. Both studies were terminated early due to observed risk factors: the HR for coronary heart disease was 0.91 (0.75-1.12) among patients receiving estrogen only and 1.29 (1.02-1.63) among patients receiving estrogen+progesterone combination therapy. Moreover, the HRs of breast cancer were 0.77 (0.59-1.01) and 1.26 (1.00-1.59), for stroke 1.39 (1.10-1.77) and 1.41 (1.07-1.85) and for pulmonary embolism 1.34 (0.87-2.06) and 2.13 (1.39-3.25), respectively. These results, even after more than 10 years, remain controversial, especially if there is a subgroup of patients (mainly perimenopausal) that would benefit from hormone replacement therapy (HRT) in the prevention of CVD[503,504]. In a Danish study, the HRT use started early at perimenopause decreased the risk of the indicated primary endpoints (death, admission to hospital for HF or MI) with a HR 0.48 (0.26-0.87, p=0.015) [505]. However, the current guidelines recommend using HRT only to treat menopause-induced symptoms, not to prevent CVD[506].

Left ventricular diastolic dysfunction (LVDD) is coupled to postmenopausal hormonal status. The mechanism behind this phenomenon is thought to be the activation of RAS due to reduced estrogen levels. Angiotensin II levels increase in tissues, enhancing cardiac hypertrophy, diastolic dysfunction and cardiac fibrosis[507,508]. Estrogen receptor β (ERβ) stimulation is shown to block the effects of angiotensin II[507-509]. As a consequence of the increased tissue angiotensin II levels and low estrogen levels, the NOS system produces ROS, which further contributes to negative changes in cardiac functions[510]. Diastolic function can be improved in postmenopausal patients with both tibolone (a steroidal hormone) and HRT[511]. In a study by Cao et al, LVDD was observed in 20.7% of pre-menopausal patients and 42.4% of postmenopausal patient (HR 4.96) [408].

The function of RV is also prone to changes in estrogen levels. In a mouse model of pulmonary hypertension, the RV contractibility was improved with

estrogen[512]. The expression of ERβ in tissues is upregulated after RT and it is thought to represent a protective tissue response to radiation injury[513]. Likewise, ERβ promotes healing after vascular inflammation and ischemia[514,515].

2.3.4.7 Tamoxifen use

Although tamoxifen is an ER antagonist, it omits cardioprotective effects due to selective estrogen receptor modulation (SERM). Tamoxifen use decreases the incidence of coronary heart disease (CHD) (HR 0.83; 95% CI 0.70-1.00) and cardiac mortality (HR 0.72; 0.53-0.97), and within the years of active use the benefit is even more pronounced [516,517]. Similar results regarding the protective effect on CHD were observed in at trial involving prolonged tamoxifen use (10 years vs. 5 years), with a HR 0.76 (0.60-0.95, p=0.02) favoring the longer tamoxifen use[518].

The actual mechanisms behind this benefit are not thoroughly known. In mouse models, tamoxifen has prevented cardiac hypertrophy, omitted protective effects after myocardial infarction as well as altered myocyte contractibility[519-521].

The concurrent use of tamoxifen during breast RT is shown to increase the rate of lung fibrosis[279,293,294]. One explanation to this is the increase of TGFβ by tamoxifen[522]. No specific data is available regarding the risk of long-term cardiac toxicity between concurrent or sequential tamoxifen uses, although in a study by Lind et al, the use of tamoxifen during RT was associated with SPECT changes[241]. Regarding the local control and breast cancer survival rate, both approaches appear to be similar[523].

2.3.4.8 The use of aromatase inhibitors

As AIs further decrease serum estrogen levels in postmenopausal women, negative cardiovascular effects were feared to occur in the follow-up. Data from studies involving rodents indicate, that aromatase plays an important protective role in cerebrovascular function especially in female mice[524]. In the recent EBCTCG meta-analysis comparing the effect of AI, tamoxifen or the sequential use of both on breast cancer recurrence, breast cancer mortality and overall mortality in patient level-details, did not observe any significant differences in heart-related or vascular-

related mortality between the groups[112]. However, this EBCTCG meta-analysis did not incorporate details regarding long-term cardiac morbidity. Another meta-analysis by Amir et al, analyzed the toxicity of endocrine treatment and an OR of 1.26 (95% CI 1.1-1.43, p<0.01) was observed for CHD in patients receiving AI vs. tamoxifen[525]. In a retrospective study of angiography patients using AI or tamoxifen, AI use increased the risk of coronary artery disease with an HR 3.23 (95% CI 1.26-8.25) [526]. Likewise, a follow-up study by Obi et al involving 2542 breast cancer survivors at 69 months after diagnosis demonstrated that AI use was associated with an increased risk of CVD (HR 1.42; CI 1.09-1.84) [527].

The concurrent use of AI during RT is observed to have synergistic effect in cell models[528,529]. In clinical trials, sequential and concurrent use are equally effective with respect to preventing breast cancer recurrence and mortality[530,531]. In a study by Bourgier et al[530], a 6-year analysis of the concurrent vs. sequential AI use was published. This analysis did not identify any differences in cardiac outcome or cardiac symptoms. However, the study was small in size, (75+75 patients at the beginning of the study, only 34+36 assessed at 6 years), included both left- and right-sided patients and the only method of cardiac evaluation was CT (measuring the cardiac surface area). Furthermore, the follow-up time was insufficient.

2.3.4.9 Chemotherapy

In adjuvant breast cancer chemotherapy, the most commonly used regimens include taxanes, anthracyclines, 5-fluorouracil and cyclophosphamide. All of them are cardiotoxic with slightly different mechanisms.

Taxanes

Taxanes (docetaxel and paclitaxel) bind to the intracellular tubulin, causing microtubule bundling and interference with mitosis. In addition, taxanes induce apoptosis and impede angiogenesis.[532]

The cardiotoxicity of taxanes is less well documented than that of anthracyclines. In trials, asymptomatic sinus bradycardia is rather frequent (30%)[533], but more severe cardiac disturbances (ischemia or conduction abnormalities) have been detected in only 5% of patients[534]. Shimoyama et al[535] evaluated cardiac functions

in a small study involving 10 patients receiving docetaxel-treatment. They observed subclinical changes in the LV diastolic functions (E/A ratio, deceleration time) one day after the infusion ($p<0.05$) In breast cancer patients, taxanes are usually administered sequentially with anthracyclines[116,117], which hampers the analysis of taxane-induced cardiotoxicity in larger breast cancer trials.

Anthracyclines

The target of anthracyclines is topoisomerase II, which is present in both cancerous tissue (α) and heart (β). This binding initiates various detrimental processes involving multiple pathways. The biological mechanism of anthracycline-induced cardiac damage is thought to involve ROS generation leading to oxidative stress.[536,537]

The clinical presentations of anthracycline-induced cardiotoxicity are cardiomyopathy and progressive HF. The decrease in LVEF can appear early (within first year) or later. The incidence of HF depends of the total cumulative anthracycline dose and prior CV risk factors.[536,538-540]

In recent breast cancer trials the incidence of HF or cardiomyopathy was 1.2% (95% CI 1.0%-1.5%) after anthracycline-based chemotherapy at 1 year and 4.3% (3.5%-5.0%) at 5 years. The incidence was similar with other chemotherapy regimens, but increased with anthracycline+trastuzumab: 6.2% (4.1%-8.2%) at 1 year post-treatment and 20.1% (14%-25.6%) at 5 years[541]. The use of RT in this trial was well balanced, but no distinction between left- or right-sided patients was made.

Subclinical changes in cardiac function can be detected in much larger proportion of breast cancer patients (>30%) after adjuvant anthracycline treatment. These changes, which are mainly LV-function-related, can be measured with echocardiography, strain rate imaging, SPECT and cardiac MRI[393,429,430,542-544]. In addition, cardiac biomarkers (troponin, BNP) can be used in the detection of myocardial damage, hypoxia and HF after anthracycline-based chemotherapy[360,370,372,544-546]. These strategies can be utilized in the follow-up of breast cancer survivors, as suggested by recent guidelines[547].

5-fluorouracil and derivatives

5-fluorouracil (5-FU) is an antimetabolite drug that exerts anticancer effects by inhibiting thymidylate synthase (essential for DNA replication and synthase) and incorporating its metabolites into cell RNA and DNA (to disrupt RNA functions) [548]. 5-FU is infused intravenously, while capecitabine and S1 are oral 5-FU prodrugs that are metabolized to 5-FU in tissues[549].

5-FU is known to induce a wide range of cardiac toxicities, as reviewed by Sorrentino et al[550]. Some form of cardiac toxicity is reported to occur in 1.5-18% of patients. Coronary vasospasm is the most common side effect and usually emerges during or immediately after infusion. It can sometimes lead to significant myocyte damage even in patients without a previous history of CAD. Furthermore, 5-FU can induce endothelial damage, autoimmune mediated myocardial damage, direct myocardial toxicity and thrombogenic effects[550,551]. The pathophysiology behind these is largely unknown and multifactorial routes are suggested[551].

Cyclophosphamide

Cyclophosphamide belongs to the nitrogen-mustard group of drugs and is widely used in many cancers. It is an inactive prodrug, and has to undergo various enzymatic processes. Eventually, the metabolites form intra- and inter-strand DNA cross-links and DNA-protein crosslinks leading to inhibition of DNA replication and apoptosis[552]. Mitochondrial swelling, cardiomyopathy and increased lipid depositions are observed in cyclophosphamide-damaged heart in rodents[553,554]

Cardiotoxicity of cyclophosphamide is dose-dependent: with very high doses (>1.55 g/m^2) severe cardiotoxicity occurred in 25% of patients[555]. Subclinical echocardiographic changes and reversible ECG changes were observed in breast cancer patients receiving high-dose cyclophosphamide[556].

2.3.4.10 Trastuzumab

Trastuzumab, the monoclonal antibody of Her2-receptor, exerts cardiotoxic effects. The exact mechanisms are not known in detail, but existing evidence suggests that interactions with the Her2b-receptors in cardiomyocytes induce apoptosis, decrease myocyte viability and increase the ROS production[557,558]. In

healthy male volunteers, one dose of trastuzumab induced an immediate but transient extracellular volume increase (either as a primary or as a secondary response) [559].

The clinical presentation of the trastuzumab-induced cardiotoxity is CHF. The decrease in LVEF, if detected early, is usually reversible after discontinuation of the treatment. The incidence of cardiotoxicity is well documented, as echocardiography has been used in many early breast cancer trials involving trastuzumab. In a meta-analysis of more than 29 000 breast cancer patients, severe cardiotoxicity occurred in 2.9% of early breast cancer patients treated with trastuzumab, taxanes and anthracyclines compared with 0.92% in patients treated with only trastuzumab and taxanes[560]. In addition, cardiotoxicity was higher in smokers (5.3%) and in patients over 60 years of age (4.91%), with BMIs >25 (6.5%), dyslipidemia (3.9%), diabetes (6.2%), hypertension (5.5%) or prior CVD (19.1%). In a 2012 Cochrane database analysis, trastuzumab significantly increased the risk of CHF: RR 5.11 (90% CI 3.0-8.72, $p<0.01$), and the risk of LVEF decline: RR 1.83 (90% CI 1.36-2.47, $p<0.01$) [561].

In a study by Cao et al[562], the effect of trastuzumab to cardiac functions during left-sided breast cancer RT were evaluated. Trastuzumab use was the only significant risk factor for LVEF decrease (mean $2.9\pm4.8\%$ vs. $0.5\pm4.9\%$, $p<0.01$). In addition, in the group with LVEF declines, the mean heart RT dose and low-dose volume of cardiac structures were significantly higher than patients without LVEF decline. The same group has also analyzed cardiac diastolic function during concurrent trastuzumab and RT regimen[408]. In this study, among patients receiving concurrent trastuzumab, 11/29 (38%) left-sided and 8/25 (32%) right-sided breast cancer patients developed LVDD. In patients receiving left-sided RT alone, 12/61 (20%) developed LVDD. In addition, a higher dose-volume of the heart contributed significantly to the risk of LVDD treated concurrently with trastuzumab.

2.3.5 Suggested cardioprotective factors

2.3.5.1 Cardiac dose reduction

As higher radiation doses to heart are consistently shown to increase the risk of cardiac morbidity and mortality[27,29,31,240,328,408], the cardiac-sparing techniques (DIBH[251-254], prone-positioning[242]) are the most effective way to reduce long-term cardiotoxicities. In addition, attention should be paid to the reproducibility of treatment and image guidance protocols, as cardiac position variability relative to surface imaging occurs[563].

2.3.5.2 Angiotensin converting enzymes/angiotensin II receptor blockers

As discussed previously, RT induces activation of RAS and increases in angiotensin II levels, which in turn enhance fibrinogenesis[344-347]. Trials have shown a benefit of ACEI use in prevention of radiation pneumonitis and lung fibrosis[283,284,286,564] as well as radiation nephropathy[565]. In a study by van de Veen et al[286], the use of captopril (ACEI) in rodents during heart and lung radiation decreased the cardiac damage: the cardiac and perivascular fibrosis was less prominent with ACEI use, as was the LV end diastolic pressure. Likewise, in rodents, the cardiotoxicity induced by anthracycline and trastuzumab was partially attenuated by the prophylactic administration of RAS antagonists[566].

The first randomized trial to test the effects of ARBs (candesartan) and beta-blockers (metoprolol) on cardiac health in early breast cancer patients was reported in 2016[567]. In this Norwegian PRADA-trial, the use of candesartan concurrent with adjuvant chemotherapy (CEF±taxanes/trastuzumab) prevented the declines in LVEF measured immediately after chemotherapy ($p=0.026$). Unfortunately, this study did not report left- or right-sided patients separately and therefore the possible prophylactic effects of ARBs on RT-induced cardiotoxicity (RT was used in 65% of patients) could not be estimated.

2.3.5.3 Thyroid hormone

The role of thyroid hormone in cardiovascular diseases, especially in cardiac remodeling and recovery after AMI, has been under investigation in the recent years[568]. In animal models, thyroid hormone supplementation has been beneficial after AMI by improving cardiac functions and by reducing the myocyte damage and fibrosis[569-572]. Angiotensin I and II receptor expression levels as well as angiotensin mRNA and protein levels are elevated in RV of hypothyroid rat hearts[573]. Furthermore, in diabetic rats thyroid hormone levels were decreased in cardiac tissues despite normal thyroid hormone levels in serum and cardiac dysfunction was present[574]. In this study, low-dose triiodothyroid hormone (T3) reversed cardiac remodeling and dysfunction.

In humans, the changes in plasma T3 levels are correlated with cardiac recovery after AMI[575]. Among patients admitted to the hospital due to chest pain, a significant risk of future adverse cardiac events was observed in patients with low T3 and high hscTnT (HR 11.72; 95% CI 2.83-48.57, $p<0.01$) [576]. After pelvic RT, low T3 was associated with prolonged and more severe radiation enteritis[577]. In breast cancer patients, neoadjuvant chemotherapy has been shown to induce significant changes in thyroid function: the levels of free thyroxin (T4) decreased and the levels of thyroid stimulating hormone (TSH) increased during chemotherapy[578].

2.3.5.4 Angiotensin 1-7

Angiotensin 1-7 (Ang1-7) is an endogenous heptapeptide hormone of the RAS. It mediates biological responses by activating the mas-receptor, a G-protein coupled membrane receptor. Ang1-7 exerts cardioprotective effects in heart: it induces vasodilatation, reduces inflammation, hypertrophy, fibrosis and thrombosis formation – thereby counteracting the effects of angiotensin II[579]. In addition, its anticancer properties are studied[580].

In mice, administration of Ang1-7 reduced radiation-induced fibrosis in skeletal muscles. Both interstitial and perivascular fibrosis decreased ($p<0.01$) alongside with decreased TGFβ production and reduced muscular stiffening[581]. Ang1-7 also inhibits radiation-induced inflammation via MAP-kinase inhibition[582] and enhances the hematopoietic recovery after total body radiation[583].

2.3.5.5 Statins

Concomitant use of atorvastatin has been shown to ameliorate radiation induced cardiac fibrosis in a dose-dependent manner in animal models [584]. Another statin, pravastatin, has been shown to exert anti-inflammatory and anti-thrombotic properties in irradiated endothelial cells[585]. In skin, pravastatin reduced radiation-induced skin damage[586]. Similarly, simvastatin has reduced the radiation-induced tissue damage and the number of peripheral endothelial progenitor cells was markedly increased in simvastatin administered rats[587].

In humans, the use of statin ($p=0.04$) or statin+ACEI ($p=0.008$) during radical pelvic radiotherapy significantly reduced acute gastrointestinal symptom scores[588].

2.3.5.6 Acetylsalicylic acid

The preventative effects of acetylsalicylic acid (ASA) on CVD and cancer (namely colorectal cancer) are widely known; however, the potential gastrointestinal side effects of ASA complicate its use[589,590]. An average daily dose of 100 mg of coated ASA seems to confer favorable preventive effects on CV death and cancer[591].

In prostate cancer patients treated with curative intent RT, the use of ASA was associated with improved biochemical outcomes and a reduced incidence of distant metastases[592]. In rectal cancer patients treated with preoperative chemoradiotherapy, the concurrent use of ASA was associated with a higher rate of tumor down staging (67.6% vs. 43.6%, $p=0.01$) and better pathological responses (46% vs. 19%; $p<0.01$) than chemoradiotherapy alone [593]. In an observational study involving breast cancer patients, the post-diagnosis use of ASA was associated with a lower risk of all-cause mortality (HR=0.53, 95% CI 0.45-0.63, $p<0.01$) and breast cancer-specific mortality (HR=0.42, 0.31-0.55, $p<0.01$) [594], yet another study did not find any association[595]. Whether these observed results are based on differences in cancer biology among ASA-users vs. non-users or reflect a true preventive effect, needs to be confirmed in randomized trials.

3 Aims of the study

This study was designed to evaluate the acute effects of radiation and concomitant/prior medications on cardiac functions and biomarker levels and to enhance cardiac protection during adjuvant breast RT.

The specific aims were:

1. To evaluate, if modern 3D breast RT induces acute effects on cardiac diastolic or systolic function measured by echocardiography
2. To evaluate, if the possible changes are dependent on the radiation dose
3. To evaluate, if myocyte damage occurs during adjuvant RT and if it can be measured using serum hscTnT levels
4. To confirm, that modest hypofractionation does not induce more cardiotoxicity than conventional fractionation
5. To evaluate the effects of concurrent hormone (tamoxifen, AI) therapy on radiation-induced cardiac changes
6. To validate and improve the reproducibility of DIBH in our unit

4 Patients and methods

4.1 Prospective study

Altogether 127 eligible patients with an early stage breast cancer or DCIS were included in this prospective, observational single-center trial. Eleven of them withdrew the consent, leaving 116 patients for the final analysis. All patients had to receive adjuvant RT after breast resection or mastectomy. (Figure 8.)

Exclusion criteria included age >80 years, dialysis, recent AMI, chronic atrial fibrillation, symptomatic heart failure, pacemaker therapy and severe lung disease. The local ethical committee approved the study protocol (ETL R10160) and all patients signed informed consent before study enrollment.

Baseline information was gathered from all patients including age, BMI, smoking history, concomitant medications and diagnoses. Regarding the adjuvant treatment, the used chemotherapy regimens, cumulative anthracycline doses and the use of concurrent hormonal therapy with RT was documented. In this thesis, only left-sided patients (n=60) without adjuvant chemotherapy were analyzed.

All patients underwent 3D computed tomographic (CT) treatment planning (Philips Big Bore CT, Phillips Medical Systems, Madison, WI, USA; or Toshiba Aquilion LB, Toshiba Medical System, Tokyo, Japan) on a breast board in supine position with both arms above the head. Three millimeter-thick CT slices without intravenous contrast were obtained. In total, 107 patients were scanned under free breathing, whereas 9 last left-sided patients were scanned and treated under the voluntary deep-inspiration breath-hold technique as this method was implemented as clinical practice in our unit in April 2013. In this technique, the breathing cycle was monitored using the Varian RPM system (Varian Medical Systems, Palo Alto, CA, USA). Treatment contouring and planning were performed with the Eclipse v.10 system (Varian Medical Systems).

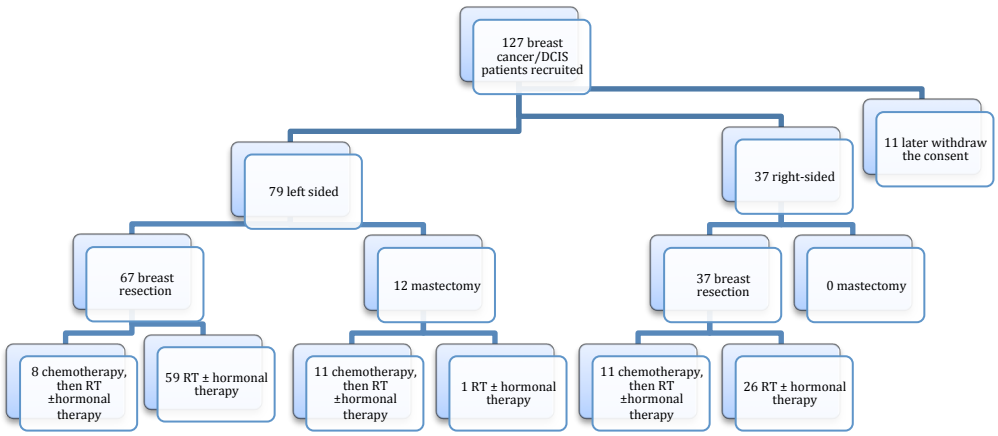

Figure 8. Patient flow chart

Planning target volume (PTV) covered the remaining breast tissue/thoracic wall as well as regional lymph node regions in node-positive patients with sufficient margins to account for inter- and intrafraction movements (5-8 mm in our unit).

The heart, RV, LV and LAD were contoured from the planning CT scans by the same physician (TS). Additionally, the anterior free wall of the RV was contoured with an estimated wall thickness of 4 mm derived from echocardiographic examinations. (Figure 9.)

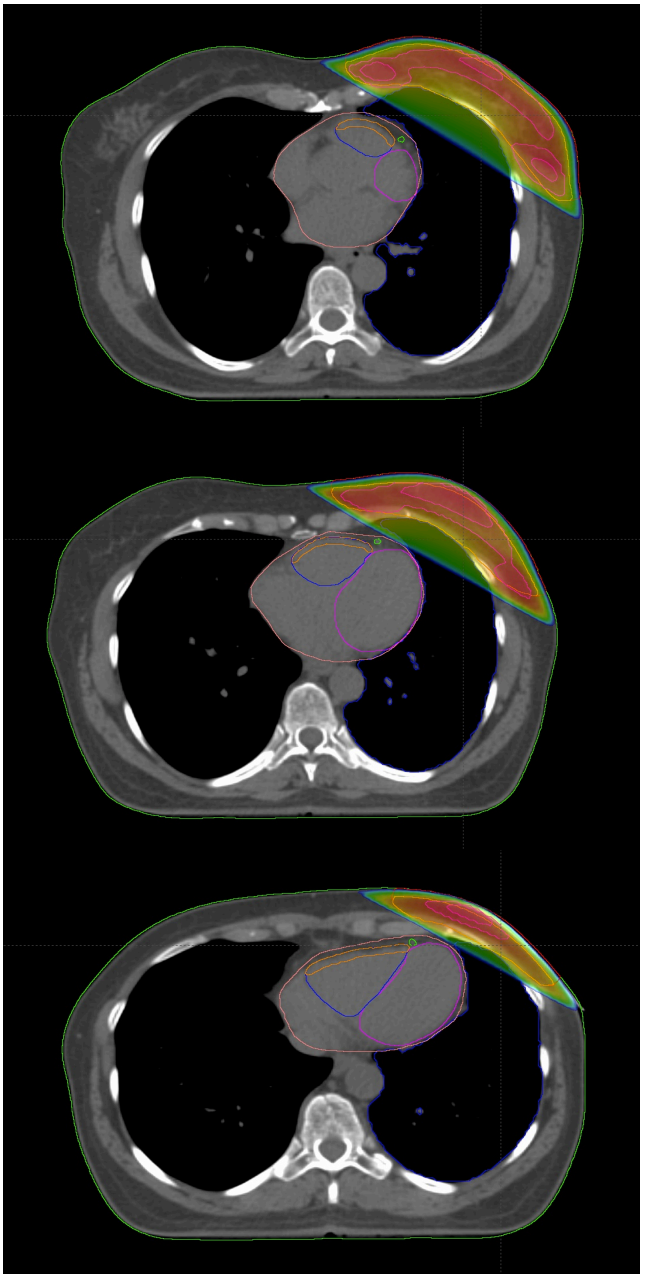

Figure 9. The contoured cardiac structures: heart (pink), left ventricle (magenta), right ventricle (blue), the free wall of the right ventricle (orange) and left anterior descending artery (green). Doses of 25 Gy to the left breast are shown in colour wash.

The radiation dose was either 50 Gy in 2 Gy fractions over five weeks with or without an additional boost (10-16 Gy, 5-8 fractions) to the tumor bed, or 42.56 Gy in 2.66 Gy fractions (hypofractionation) over 3.5 weeks according to then-current local guidelines; hypofractionation was recommended for grade I or II tumors with margins over 5 mm, age >50 years and tangential breast length <25 cm. After breast conserving surgery, tangential photon fields were used to treat the breast and additional 2-3 fields to cover the axillary and supraclavicular lymph node regions. After mastectomy, the breast wall was treated either with tangential photon fields or with electron fields combined with photon field to the lymph node regions. Doses were calculated using an Anisotropic Analytical Algorithm for photons and Generalized Gaussian Pencil Beam or Monte Carlo for electrons. Dose–volume histograms of various structures were generated for each patient. To account for the different dosing schedules, an α/β-ratio of 3 was used for the heart and lung to calculate 2 Gy equivalent doses.

HscTnT (ng/l) and BNP (ng/l) were analyzed from serum samples taken before RT, after two (hypofractionated RT) or three (conventional RT) weeks of treatment and at the last day of RT. As the lowest detection limit of hscTnT was 5 ng/l, the values below this (<5 ng/l) were estimated to be 4 (lowest detection limit (LOD)/$\sqrt{2}$) when calculating the percentage increase from the baseline value). A predefined increase of >30% from the baseline was considered to be clinically significant. Total cholesterol levels (mmol/l) were measured at baseline under fasting conditions. (Figure 10.)

A comprehensive echocardiography study and 12-lead ECG were performed at the baseline, after chemotherapy and at the completion of RT (Figure 10.). All echocardiography examinations were performed using the same ultrasound machine (Philips iE33 ultrasound system, Bothell, WA, USA) and a 1-5 MHz matrix-array X5-1 transducer by the same cardiologist. All images were acquired at rest. Subcostal imaging was performed in the supine position, while other imaging was performed with the patient in the lateral decubitus position with simultaneous superimposed ECG. Doppler recordings were acquired at the end of expiration during shallow breathing. Images were stored digitally for use with offline analysis software (Excelera, Philips, Koninklijke, Netherlands; Qlab, Philips, Bothell, USA). Echocardiographic measurements were performed in a standardized manner.

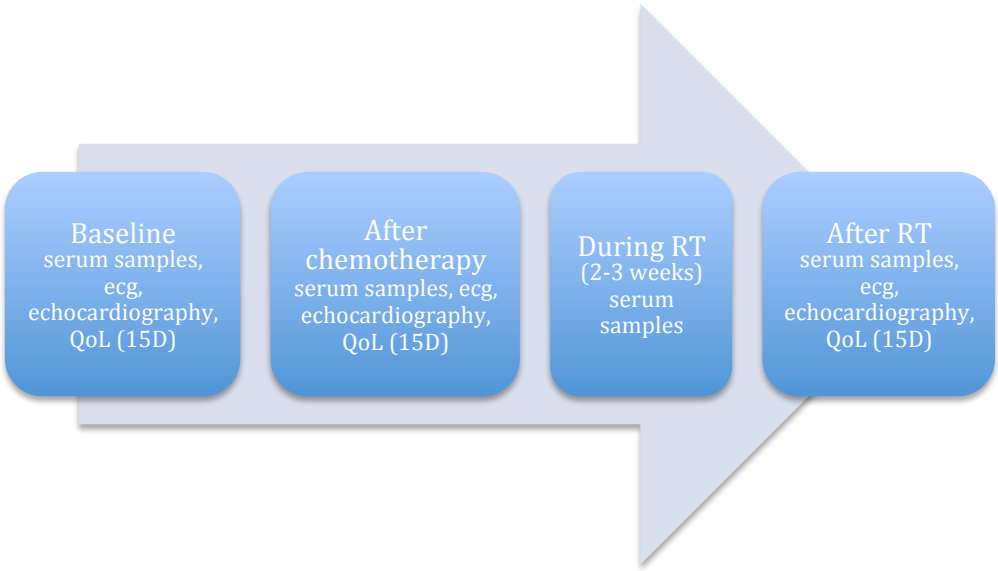

Figure 10. Study flow chart.

4.2 Retrospective study

In the retrospective study regarding the reproducibility of vDIBH (paper IV), we included 148 consecutive left-sided breast cancer patients receiving adjuvant RT with vDIBH following breast conserving surgery or mastectomy. Two different marker block positions were compared and the effect of breath hold level (BHL) correction was analysed.

A marker block with two infrared reflectors was placed on the abdominal wall between the lower sternum and umbilicus, approximately 4 cm from the xyphoid process in the first 63 patients, who were treated from 10/2013-3/2014. The second group of 85 patients, who were treated from 4/2014-10/2014, had the marker block placed on the bony sternum 4 cm cranial to the xyphoid process. The gating window was set to a maximum of ± 5 mm around the average breath-hold level and individually further narrowed. All patients had monitors to visually guide breathing levels.

In our vDIBH image guidance protocol, the treatment couch corrections are based on anterior and lateral setup images acquired using an on-board imaging system (OBI, Varian Medical Systems, Palo Alto, CA) at least in the first three fractions for all patients. The BHL is verified from the lateral image once a week thereafter. Tangential breast images are acquired daily. Anterior image is acquired once a week in patients with lymph nodes involvement to correct for potential patient rotation. Couch corrections are required, if position errors exceed 4 mm, and/or the BHL deviates more than 4 mm from the planned.

For these 148 patients, 1940 orthogonal setup images were matched online by experienced radiation therapists.

The reference BHL was recorded in the AP direction using the RPM system during the planning CT. The actual BHL was determined from the distance between the sternum and the middle part of the vertebra from the lateral on-board kV-images. If the measured BHL differed by more than 4 mm from that in the lateral reference image, the height of the BHL window in the RPM was adjusted.

Residual errors of the bony surrogates of the lymph node regions were determined offline. The potential error in distance of the heart surface inside the treatment field was retrospectively analysed from the tangential images. The effect of the BHL correction on the heart position inside the treatment field and the subsequent cardiac dose were analysed.

4.3 Statistical analysis

Data are expressed as the mean ± standard deviation (SD) for normally distributed continuous variables and as medians with inter quartile ranges (IQRs) for variables with non-normal distributions. The groups were compared using the t-test for independent samples or the Mann-Whitney U test. Friedman's analysis of variance was used for repeated measures of non-normal variables and the Wilcoxon signed rank test was used to compare the hscTnT changes. Categorical data are expressed as numbers (%) of subjects. The Chi-squared test was used for categorical variables

and the Fisher's exact test was used when appropriate. The related samples Cochran´s Q test was used to study the change in dichotomous variables measures more than twice. Area under curve (AUC) was calculated using the trapezium rule. All the tests were two-sided and p value <0.05 was considered statistically significant. Analysis was performed using IBM SPSS Statistics for Windows (version 21.0, Armonk, NY, USA, IBM Corp).

5 Summary of results

5.1 Paper I: Early Effects of Adjuvant Breast Cancer Radiotherapy on Right Ventricular Systolic and Diastolic Function

In this study, 49 patients with left-sided breast RT were included. The early radiation-induced changes in echocardiography were analyzed, with a special focus on RV systolic functions and LV diastolic functions.

The mean age of the population was 63 (range 49–79) years. The most common underlying diseases were hypertension (35%), hypercholesterolemia (16%), hypothyreosis (10%) and diabetes (4%).

RT caused changes in RV systolic function. TAPSE declined in 67% of the patients. The average reduction was 2.1±3.2 mm ($p<0.001$). A decrease of ≥4 mm was observed in 39% of the patients. No correlation between TAPSE and cardiac radiation dose, smoking, ECG changes or BMI was observed. Thyroxin use ($p=0.03$) and diuretic use ($p=0.03$) were associated with smaller TAPSE reduction. Likewise, the use of ACEIs or ARBs tended to protect against TAPSE decline ($p=0.06$). Other RV systolic parameters exhibited a tendency to decrease, although statistical significance was not reached. Peak systolic annular velocity (S') declined from 12.7±3.1 to 12.2±2.7 ($p=0.11$) and pulmonary flow (VTI) decreased from 16.6±3.1 to 15.9±2.3 ($p=0.07$).

RT had no significant effect on LV systolic or diastolic function. Structural changes were observed, as both the interventricular septum (10.0±1.2 mm vs. 10.3±1.3 mm, $p=0.02$) and the posterior wall (9.7±1.0 mm vs. 10.3±1.2 mm, $p=0.01$) were slightly thicker after RT than at baseline.

Conclusion: Left-sided breast RT induced early changes in RV systolic functions, especially TAPSE.

5.2 Paper II: The Concurrent Use of Aromatase Inhibitors and Radiotherapy Induces Echocardiographic Changes in Patients with Breast Cancer

This sub-study included 60 women who received left-sided adjuvant RT. Prior chemotherapy was not allowed. 22 patients received AI concurrently with RT and 38 received RT alone. The effects of AI+RT vs. RT alone on echocardiographic changes, ECG changes and cardiac biomarkers were evaluated. The groups were otherwise well balanced with respect to their baseline parameters, but AI users were more obese (mean BMI 29.0 vs. 26.2 kg/m^2). Cardiac radiation doses did not differ between groups: the mean heart dose was 3.1 Gy in both groups.

The most prominent RT-induced reduction in cardiac functions was observed in TAPSE. Among AI users (n=22), this measurement of RV systolic function was reduced from baseline by 3.0 mm (95% CI=1.9-4.1 mm, p<0.001). In the non-AI users (n=38), TAPSE was reduced from baseline by 1.4 mm (95% CI=0.3-2.4 mm, p=0.013). The decrease in TAPSE was significantly greater among AI users compared with non-AI users (p=0.04).

RT induced no significant changes in LV systolic function in either group. LV diastolic measurements changed among the AI users during RT. At the end of RT, E-wave decreased from 75±16.5 to 69.2±13.5 cm/s (p=0.006), further accompanied by an increase in IVRT from 101±25 to 111±23 ms (p=0.12). In non-AI users, the changes observed in LV diastole were non-significant.

Circulating serum estradiol levels were evaluated for all patients and measured at the completion of RT. Estradiol levels were significantly (p=0.004) reduced in AI users (median=18 pmol/l, IQR=15-47 pmol/l) compared with non-AI-users (median=39, 32-57), as anticipated.

No significant differences were observed in cardiac biomarkers or ECG-changes between AI-users and non-users.

Conclusion: The concurrent use of AI during RT for left-sided breast cancer led to a more pronounced early change in RV systolic function and LV diastolic function compared with RT alone.

5.3 Paper III: Troponin T-release associates with cardiac radiation doses during adjuvant left-sided breast cancer radiotherapy

This study included 60 patients with left-sided breast RT without prior chemotherapy. Dose-volume histograms for cardiac substructures (entire heart, LV, RV and LAD) were created and compared with patient hscTnT values. Two patients were excluded from the analysis due to missing serum samples.

The levels of hscTnT increased more than the predefined 30% from the baseline value in 12/58 (21%) patients constituting the group A. In these 12 patients, hscTnT was detectable in 3 (25%) before RT, in 9 (75%) at 2-3 weeks during RT and in 12 (100%) patients after RT. The median (IQR) values at these time points were 4 (4-4.5) ng/l, 5.5 (4.5-6) ng/l and 7 (7-9.5) ng/l, respectively. In hscTnT stable patients (n=46, group B), the median values did not change during RT.

The baseline variables did not significantly differ between groups A and B.

The radiation doses to cardiac structures were significantly higher in troponin-releasing group A than in group B (Figure 11). The mean heart dose was 4.0 Gy in group A vs. 2.8 Gy in group B (p=0.02), the mean LV dose was 6.7 Gy vs. 4.5 Gy (p=0.02) and the mean LAD dose 23.8 Gy vs. 17.5 Gy (p=0.07), respectively.

In group A, the increase in hscTnT was accompanied by minor changes in the echocardiographic measurements. The interventricular septum thickened (p=0.01), a finding accompanied by changes in LV diastolic function as the deceleration time increased (p=0.008) more among group A than in group B. In other LV diastolic parameters, the mitral E-peak decreased in both groups.

Conclusions: The elevations in hscTnT levels suggest that adjuvant RT causes subclinical myocardial damage in patients with left-sided breast cancer. As these increases in hscTnT are associated with increased cardiac radiation doses, all efforts should be made to keep the cardiac radiation doses to as low as possible.

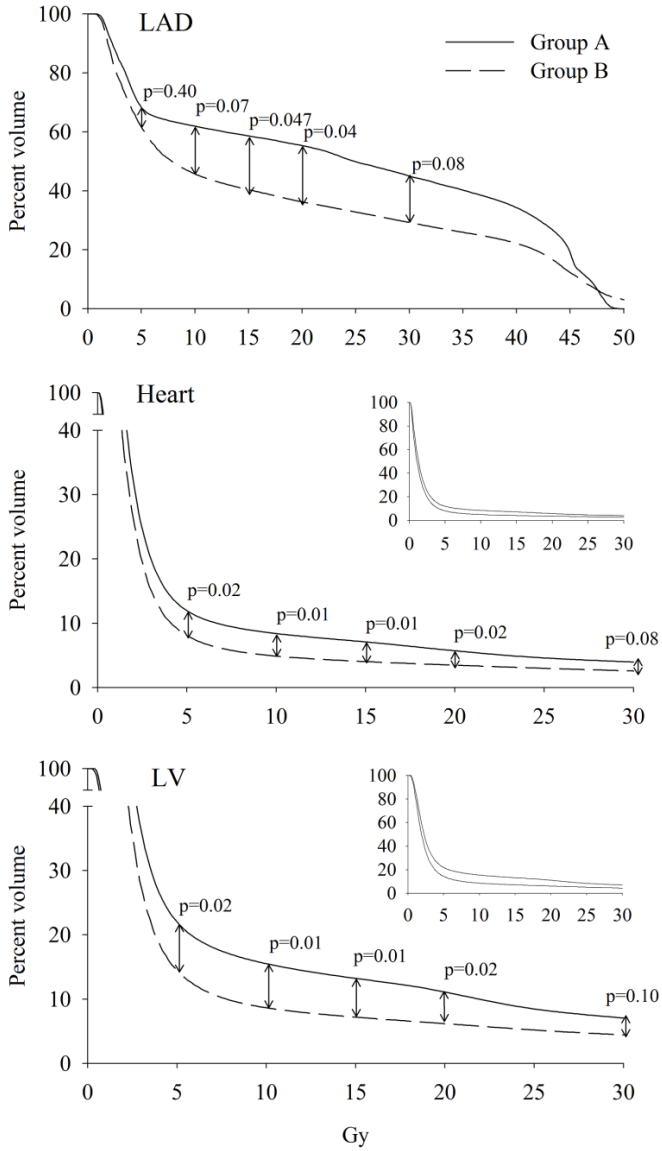

Figure 11. Dose-volume histograms (DVH) of the left anterior descending artery (LAD), heart and left ventricle (LV). The solid line represents the median cardiac values in the troponin-releasing group (group A) and the dashed line the troponin-stable group B. Gray (Gy) values are shown in the figures. The DVH´s among group A were significantly higher than those in group B.

5.4 Paper IV: Improving the reproducibility of voluntary deep inspiration breath hold technique during adjuvant left-sided breast cancer radiotherapy

In this retrospective study, 148 consecutive left-sided breast cancer patients receiving adjuvant RT with the vDIBH technique were included. Two different marker block positions were compared: the first was below the xiphoid process (abdominal wall, group A, n=63) and the second on the sternum (group S, n=85). In addition, we evaluated the effect of active breath hold level (BHL) correction on cardiac doses and on reproducibility during treatment.

Placement of the marker block on the sternum resulted in less random residual errors in the actual BHL than placement on the abdominal wall. The random error (σ) reduced from 2.3 mm to 1.8 mm ($p<0.05$) in the AP-direction and from 3.0 mm to 2.5 mm ($p<0.05$) in the SI-direction. The systematic error (Σ) decreased from 3.1 mm to 2.6 mm ($p=0.13$) in AP-direction and from 4.2 mm to 3.7 mm ($p=0.13$) in SI-direction with the use of sternal marker placement.

Among patients with lymph node region irradiation (n=25 in group A, n=30 in group S), the sternal marker block decreased significantly both the Σ and σ of the residual errors in the SI direction ($p<0.05$), but the errors in the LAT direction were similar with both marker positions.

The BHL correction was required for 26/63 (41%) patients in group A, and for 26/85 (31%) patients in group S ($p=0.18$) as the systematic error of the actual BHL in lateral images exceeded the predefined 4 mm tolerance. The effect of this correction not only improved the reproducibility (both σ and Σ decreased, $p<0.05$) but also translated into reduced cardiac radiation doses: the average mean cardiac dose in group A would have been 2.6 Gy if uncorrected and 2.1 Gy after the correction ($p<0.01$), and in group S 2.9 Gy and 2.3 Gy ($p<0.05$), respectively.

Conclusions: In this study, we showed that a sternal position of the marker block is superior to a lower marker block position on the abdominal wall. Furthermore, we observed changes in the BHL during treatment compared to the reference image. The BHL correction based on the lateral kV images significantly improved

the reproducibility of the vDIBH and decreased cardiac doses. Image guidance is mandatory to ensure optimal treatment results.

6 Discussion

In this study, we prospectively evaluated early RT-induced cardiac changes using serum biomarkers, echocardiography and ECG. In addition, we aimed to determine whether the cardiac doses correlated with the observed changes and if other confounding factors were present. Furthermore, to enhance the cardiac radioprotection, we analyzed methods of optimizing vDIBH-reproducibility.

6.1 Patients

In papers I-III, only left-sided breast cancer patients who had not received prior chemotherapy were analyzed. We wanted to exclude the effects of prior chemotherapy on cardiac functions and focus only on the early cardiac effects induced by breast RT. To increase study homogeneity, right-sided patients were excluded from the analysis. In addition, right-sided patients would not have served as a true non-irradiated comparative group, as radiation to cardiac structures - mainly to right atrium - occurs. These 60 left-sided patients (49 in paper I, 60 in papers II and III) represent a real-life breast cancer patient population with a mean age of 63±6 years. One-third of these patients had concomitant hypertension, a number equivalent to the general prevalence of this disease among middle-aged Finnish women[596]. The mean BMI was 27±4, falling to a category of moderate overweight (BMI 25-30), similar to the BMI of middle-aged Finnish women in general[597]. Patients with a recent AMI or known HF were excluded in accordance with study inclusion criteria. In papers II and III, the groups were well balanced regarding baseline characteristics.

In paper IV, the study population consisted of 148 consecutive left-sided breast cancer patients treated with vDIBH in our institution. As this was a retrospective study focusing on the reproducibility of the daily treatment, no other patient characteristics were collected except age and RT data.

6.2 Radiotherapy

The RT contouring and beam planning were done according to local guidelines in all patients in this single-institution study. Both conventional fractionation (2 Gy) and modest hypofractionation (2,66 Gy) were used according to our institutional indications, which at that time allowed hypofractionation for the breast-only treatment in postmenopausal patients. As one of our study objectives was to confirm the cardiac safety of the hypofractionated protocol, the two fractionation groups were compared at all stages of the study and no differences in measured parameters (not reported) were observed between the hypofractionation and the conventional fractionation groups. The heart was contoured in a standardized manner according to the cardiac atlas published by Feng et al[598].

The use of vDIBH in left-sided breast cancer patients was launched at our institution in April 2013. The last two of the 60 patients in papers II and III were treated with vDIBH, whereas the first 58 were treated during FB. In paper I, all patients were treated during FB. The cardiac dose was calculated from the planning CT, which was obtained during the FB cycle in the above 58 patients and during vDIBH in the remaining 2 patients. Therefore, the cardiac dose is an estimate based on the planning CT and the actual daily cardiac dose may vary substantially depending on the set-up and the breathing phase or the BHL during the beam-on time. In a study using daily CBCT in breast cancer RT with FB, the average respiratory amplitude of the heart was ~4 mm in the LAT/AP direction and ~9 mm in the SI direction. Moreover, the off-line analyzed set-up variabilities of $\sum$ were 2.4/3.7/2.2 mm and those of σ were 2.9/4.1/2.7 mm in LAT/SI/AP-directions, respectively[599]. In this study, the mean heart dose was increased from the planning dose (1,9 Gy) to the actual online dose (2,3 Gy). In our patients in papers II and III, the mean heart dose calculated from the planning CT was 3.1±1.5 Gy. That is similar to those presented in a review article by Taylor et al[332], in which the mean cardiac dose in trials published between 2003-2013 was 4.2 Gy in patients who did not receive internal mammary chain irradiation.

Even though vDIBH is superior to FB in reducing cardiac doses[251,252], it does not eradicate the daily set-up variability and errors in the BHL. With vDIBH, the average heart position shifts relative to the bony anatomy were 2±3 mm in the SI-,

1±2 mm in the LAT- and 1±3 mm in the AP-direction in a study by McIntosh et al[600]. We observed similar errors in paper IV among patients with good BHL reproducibility: the mean signed displacement of the heart in the anterior direction was 2.0 mm in group S and 2.9 mm in group A. In patients requiring BHL correction, the displacement after the correction was reduced to 0.8 mm (group S) and 2.0 mm (group A). This led to a significant reduction in the actual cardiac dose.

The set-up errors in the bony structures were improved with the positioning of the marker block on the sternum instead of the soft abdominal wall in paper IV. This is the first study to evaluate these two marker block positions in RPM and its effect on the daily reproducibility. This study changed the vDIBH practices of our institution and the marker block is now placed on the sternum in all patients thereafter.

6.3 Cardiac serum markers

Myocardial injury caused by various clinical conditions can be measured with hscTnT (see 2.3.2.1). In paper III, we observed hscTnT release in 21% of left-sided breast cancer patients without prior chemotherapy – indicating that this treatment induced negative effects on myocardial cells. D`Errico et al[369] did not observe any increases in TnI after adjuvant breast RT, but the values were measured 5-22 months after the end of RT and therefore they do not represent the acute radiation induced myocardial damage. Nellessen et al[362] measured TnI weekly during and after thoracic RT (including 5 breast cancer patients) and observed TnI increases during the treatment. The mean pre-RT value of TnI was 7 ng/l and post-RT 14 ng/l, while in our hscTnT-positive group the median pre-RT hscTnT value was <5 ng/l and post-RT 7 ng/l. Unlike our patients, most of the patients in the Nellessen´s study had received prior chemotherapy. Furthermore, we showed that troponin release was dependent on the RT dose, as cardiac radiation doses were significantly higher among our troponin-releasing patients (Figure 11). This finding is consistent with those of larger studies evaluating the dose-volume effects on cardiac risk[29,32,326,431].

Our study population was too small to analyze the effects of other possible confounding factors on hscTnT release and the baseline characteristics were statistically similar in the hscTnT releasing and non-releasing groups in paper III. However, thyroid hormone supplementation was present only in patients without troponin release (0 vs. 8 patients, p=0.19) whereas hypertension medication was slightly more often used in patients with troponin release (50% vs. 30%, p=0.31).

There was a minor increase in BNP values during and after the RT; however, due to wide variations in baseline values and the limited number of patients enrolled in this study, the absolute increase was not statistically significant. However, in other trials BNP values were analyzed as BNP ratios (BNP at some time point/baseline) [369,373,374] and increasing BNP ratios were correlated with high radiation doses in small volumes of heart and LV[369,373]. In addition, higher BNP ratios were correlated with increased cardiac volumes receiving 20, 25, 30 and 45 Gy[374]. Regarding the 60 patients reported in paper III, 15 had BNP ratios $\geq$1.3 at the end of RT and the cardiac volumes receiving 45 Gy in those patients were significantly ($p<0.05$) larger than those in the rest of the group (not reported). Neither the differences in the mean heart dose nor the differences in the volumes receiving lower doses were not significantly different. Furthermore, the prevalence of hypertension was higher (60%) in those 15 patients than in the remainder of the group (27%) ($p<0.05$).

6.4 Echocardiography

Echocardiography was performed in a standardized manner by the same experienced cardiologist under similar conditions throughout the study. Data were stored digitally to enable subsequent analysis and inter-observer variation tests.

In paper I, we showed that left-sided adjuvant RT induced adverse effects on RV´s systolic functions measured with TAPSE ($p<0.01$) as well as caused minor thickening to the ventricular walls. Similar subclinical decreases in TAPSE after breast RT were observed by Heggemann et al[412]. The changes in LV systolic and

diastolic functions were not statistically significant in this 49 patient population in paper I. The mean RV dose was 3.03±2.03 Gy and the mean anterior RV wall dose was 6.09±4.74 Gy – both high enough to cause radiation induced inflammation and fibrosis in tissues[287,333,601]. The interval between the baseline echocardiographic examination and the examination after RT was 41±11 days and therefore these observed early phase cardiac changes might represent both inflammatory processes and the early fibrotic changes[287].

In paper II, 60 left-sided patients underwent similar echocardiographic evaluations before and after RT. These patients were divided into two groups according to AI use. The changes in TAPSE were more pronounced in AI-users than in non-users. In addition, changes in diastolic LV function were observed in AI-users as the mitral inflow E-wave decreased from 75±16.5 to 69.2±13.5 cm/s ($p=0.006$). Furthermore, the interventricular septum thickened in both groups. Similar subclinical changes in diastolic functions after thoracic RT have been reported by others[407,408]. Follow-up echocardiography at three years will confirm whether these observed changes return to baseline level or worsen in time. To improve the knowledge of adjuvant breast radiation induced changes in echocardiography, results from 3D echocardiography, speckle tracking and strain imaging in our patients will be published shortly.

6.5 Observed confounding factors

In paper II, the effects of concurrent AI treatment to cardiac functions were analyzed. As stated previously (2.3.4.8), concurrent use of AI potentiates the effects of radiation in cell models[528,529] as well as increases the long-term risk of CHD and CVD[525,527]. In rodents, the start of letrozole immediately after single 12 Gy thoracic RT increased the cardiac fibrosis compared with anastrozole or exemestan use ($p<0.05$) [602]. The echocardiography changes were more pronounced in our patients using concurrent AI, which in all but one patient was letrozole. Low serum estrogen levels may cause these negative changes by reducing ERβ activation and thereby inhibiting its healing effects[513-515]. However, the concurrent use of AI did not significantly influence hscTnT release, as demonstrated in paper III. The effects of concurrent AI to cardiac functions measured with speckel tracking and strain rate imaging will be reported in the near future.

Thyroid hormone use appeared to be beneficial with respect to preventing TAPSE-decreases, as shown in paper I. In paper III, thyroid hormone use seemed to protect the heart from radiation induced myocyte damage based on the hscTnT levels reported therein; however this was not a statistically significant observation. Additional studies are needed to confirm the benefits of T3/T4 use after AMI, as well as its role in radiation protection.

The use of ACEI/ARB tended to protect against TAPSE decline ($p=0.06$) in paper I. In paper II analyzing the effect of AIs on cardiac functions, the number of patients using ACEIs/ARBs in each group was too small to evaluate the effects of these agents on cardiac function – particularly because hypertension itself is a known risk factor for RIHD[29]. Whether these drugs are beneficial in preventing later cardiac fibrosis, will be analyzed in follow-up studies involving our patients.

6.6 Limitations

The study was non-randomized and observational and therefore the study groups in papers II and III were not perfectly balanced according to their baseline factors. In addition, the number of patients enrolled in the study was limited and subgroup analyses of all possible confounding factors to cardiotoxicity could not be performed.

6.7 Future studies

Additional analyses of the entire study group will report the combined effects of adjuvant chemotherapy on RT-induced cardiac effects. RT induced LV and RV strain rate functions will be analyzed. Different serum markers of inflammation and fibrosis will be analyzed. The three-year follow-up data will be mature at the end of 2016. In addition, a follow-up study of our patients will be performed using echocardiography and serum markers at 6 and 9 years post-RT.

6.8 Recommendations for cardiac protection and follow-up during and after breast cancer radiotherapy

The trials presented in Table 2 (page 49) encourage the use of cardiac sparing RT techniques in the adjuvant left-sided breast cancer RT to reduce the later cardiac toxicity. Absolutely safe cardiac doses have not been presented. In the retrospective study by Darby et al[29], the risk of later cardiac morbidity and mortality increases linearly after 0 Gy. In our unit, we have successfully implemented the use of vDIBH in clinical practice and have so far (April 2016) treated >600 patients with this method. As presented in paper IV, new techniques must be validated and can be further improved during time. Substantial reductions in cardiac doses have been achieved in whom vDIBH has been used compared to previous FB technique since the launch in April 2013.

The European Association of Cardiovascular Imaging and the American Society of Echocardiography have formulated joint guidelines regarding the cardiac follow-up after thoracic radiotherapy[426] and cancer treatment in general[547]. Baseline echocardiography is recommended for all patients prior receiving thoracic RT. Yearly clinical controls are proposed after RT to evaluate new cardiac signs or symptoms, such as heart murmurs, chest pain or increased dyspnea. To asymptomatic patients, a screening echocardiography is recommended after 5 (high risk) or 10 years after RT. High risk features include both technical aspects (dose >30 Gy, anterior or left-sided RT, lack of cardiac shielding), other treatment related aspects (prior or concurrent chemotherapy) as well as patient derived aspects (younger age, cardiovascular risk factors or pre-existing CVD). In our study population in papers II and III, all patients would fall to the high risk-category based on the left-sided treatment and the total prescribed dose. In addition, >30% of them had other cardiac risk factors. There are no national guidelines regarding the cardiac follow-up after RT thus far. It is our hope that our studies will facilitate the creation of such guidelines in the future.

7 Summary and conclusions

In these studies (I-III), the early cardiac changes induced by adjuvant breast cancer radiotherapy were evaluated. Only left-sided patients without prior chemotherapy were included in these analyses to exclude the effects of chemotherapy induced cardiotoxicity. The effects of other concomitant medications on radiation-induced cardiotoxicity were analyzed. In paper IV, practical methods of improving the daily reproducibility of vDIBH radiation treatment were evaluated.

The main findings were the following:

1. Adjuvant left-sided breast RT induces subclinical changes in cardiac function that can be observed with echocardiography at the end of treatment. These early changes were observed in RV´s systolic function and LV´s diastolic functions as well as in structural measurements.

2. Concurrent aromatase inhibitor use had an impact on cardiac functions in patients who received left-sided breast cancer RT. More pronounced early changes in RV´s systolic function and LV´s diastolic function were observed with echocardiography in these patients than in patients treated with RT alone.

3. Serum hscTnT levels increased >30% from baseline in 12 out of 58 (21%) patients. The cardiac radiation doses in these 12 patients were significantly higher than in patients with stable hscTnT values, proposing a dose-volume relationship to observed minor myocyte damage.

4. The daily reproducibility of vDIBH was improved by placing the RPM marker block on the sternum instead of the abdominal wall.

5. The actual treatment BHL varied from the reference scan BHL. Active BHL correction based on lateral kV images significantly improved the reproducibility of the vDIBH and decreased cardiac doses.

6. Image guidance is mandatory to ensure optimal treatment results in vDIBH.

8 Acknowledgements

This study was carried out at the Department of Oncology in Tampere University Hospital and the Heart Center Co, Tampere University Hospital between 2011-2014. Funding for this thesis was received from Pirkanmaa Hospital District Science Center, Elna Kaarina Savolainen and Seppo Nieminen funds; from Finnish cultural foundation, Pirkanmaa Regional Fund; from Finnish Foundation of Cardiovascular Research and from the Finnish Medical Foundation.

I wish to thank my esteemed supervisor Professor Pirkko-Liisa Kellokumpu-Lehtinen, MD, PhD for the encouragement, guidance and expertise throughout the process of this thesis and for all the interesting sidetracks it has led us during the past years. This has been a great learning experience and I am grateful for sharing the passion to this topic.

I am indebted to my co-author and a master of echocardiology, Suvi Tuohinen, MD for sharing the pains and joys during this study and co-writing of articles. I hope to continue our good collaboration in the future. Furthermore, I am thankful for Pekka Raatikainen, MD, PhD and Vesa Virtanen, MD, PhD for being part of my steering group and for your expertise and valuable comments. Your open-minded attitude to the original idea and the enhancement of cardio-oncology is most appreciated.

Improving the daily verification and image guidance and thereby treatment quality and safety has been one of the main priorities in our department of radiotherapy during the past few years. I am happy to work in such a community, where technical improvements are eagerly but consciously implanted to the benefit on patients. I thank my co-authors, physicists Mika Kapanen, PhD, Eeva Boman, PhD, Seppo Peltola, PhD and Simo Hyödynmaa, PhD and radiotherapists Marko Laaksomaa and Mikko Haltamo for their enormous amount of work within this field.

Sincere thank you to Helena Hänninen, MD, PhD and Kauko Saarilahti, MD, PhD for taking the time to supervise this thesis and improving it with comments.

For the original idea to research this topic of radiation-induced cardiotoxicity, I need to thank my colleague Tapio Tulijoki, MD. He gave me the Quantec-article regarding cardiac side effects over one coffee break – now a few years back. This was the start of this whole cascade. My other dear fellow colleagues and friends in radiotherapy have had to endure my tossing of ideas and hyping of findings regarding (but not limited to!) this topic since then. Therefore: a big thank you to Sirpa-Liisa Lahtela, Petri Reinikainen, Hanna Aula, Tuija Tuunanen, Krista Sillanpää among others for listening patiently. Special thanks to Hanna Mäenpää for volunteering to read and comment my thesis and to the head of radiotherapy Tuija Wigren for allowing to organize my work so that all of this could be made. It is a joy and pleasure to work with you. In addition, I am grateful for the support from the whole radiotherapy personnel, nurses and radiotherapists for their assistance during the study and for everyday companionship.

In practical issues I got valuable help from study nurses Emmi Vettenranta and Kirsi Maria Rintala as well as from Taina Ahlgren. I also wish to thank the study nurses from the department of cardiology, Virpi Räsänen and Hanna Näppilä for their co-operation throughout the years.

I am indebted to all the great women participating in this study.

Apart from work, I wish to thank my parents Tellervo and Pertti for their steady support and encouragement regarding not only this thesis, but also life in general. Combining work, research and motherhood wouldn´t have been possible this way without you. I also thank my in-laws Liisa and Eero for your kindness and support.

Many other people should be thanked for making the ordinary days easier and the special days more joyous. Friends, thank you for being there.

And finally, my family. A bustling crowd of hands and legs, noise, laughter – and yet, a quite moment and a cuddle. Naatan, Nooa and Miska: you are my pride and joy – no achievement is superior to having you. And my dear husband Henrik: you can still make me smile.

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Supplementary table 1. The 7th AJCC edition of breast cancer TNM.

Primary tumor (T)	
TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
Tis	Carcinoma in situ
Tis (DCIS)	Ductal carcinoma in situ
Tis (LCIS)	Lobular carcinoma in situ
Tis (Paget)	Paget disease of the nipple NOT associated with invasive carcinoma and/or carcinoma in situ (DCIS and/or LCIS) in the underlying breast parenchyma. Carcinomas in the breast parenchyma associated with Paget disease are categorized based on the size and characteristics of the parenchymal disease, although the presence of Paget disease should still be noted
T1	Tumor ≤ 20 mm in greatest dimension
T1mi	Tumor ≤ 1 mm in greatest dimension
T1a	Tumor > 1 mm but ≤ 5 mm in greatest dimension
T1b	Tumor > 5 mm but ≤ 10 mm in greatest dimension
T1c	Tumor > 10 mm but ≤ 20 mm in greatest dimension
T2	Tumor > 20 mm but ≤ 50 mm in greatest dimension
T3	Tumor > 50 mm in greatest dimension
T4	Tumor of any size with direct extension to the chest wall and/or to the skin (ulceration or skin nodules)
T4a	Extension to chest wall, not including only pectoralis muscle adherence/invasion
T4b	Ulceration and/or ipsilateral satellite nodules and/or edema (including peau d'orange) of the skin, which do not meet the criteria for inflammatory carcinoma
T4c	Both T4a and T4b
T4d	Inflammatory carcinoma

Regional lymph nodes (N)	
Clinical	
NX	Regional lymph nodes cannot be assessed (eg, previously removed)
N0	No regional lymph node metastasis
N1	Metastasis to movable ipsilateral level I, II axillary lymph node(s)
N2	Metastases in ipsilateral level I, II axillary lymph nodes that are clinically fixed or matted or in clinically detected* ipsilateral internal mammary nodes in the *absence* of clinically evident axillary lymph node metastasis
N2a	Metastases in ipsilateral level I, II axillary lymph nodes fixed to one another (matted) or to other structures
N2b	Metastases only in clinically detected* ipsilateral internal mammary nodes and in the *absence* of clinically evident level I, II axillary lymph node metastases
N3	Metastases in ipsilateral infraclavicular (level III axillary) lymph node(s), with or without level I, II axillary node involvement, or in clinically detected *ipsilateral internal mammary lymph node(s) and in the *presence* of clinically evident level I, II axillary lymph node metastasis; or metastasis in ipsilateral supraclavicular lymph node(s), with or without axillary or internal mammary lymph node involvement
N3a	Metastasis in ipsilateral infraclavicular lymph node(s)
N3b	Metastasis in ipsilateral internal mammary lymph node(s) and axillary lymph node(s)
N3c	Metastasis in ipsilateral supraclavicular lymph node(s)

*"Clinically detected" is defined as detected by imaging studies (excluding lymphoscintigraphy) or by clinical examination and having characteristics highly suspicious for malignancy or a presumed pathologic macrometastasis on the basis of fine-needle aspiration (FNA) biopsy with cytologic examination.

Pathologic (pN)*	
pNX	Regional lymph nodes cannot be assessed (for example, previously removed, or not removed for pathologic study)
pN0	No regional lymph node metastasis identified histologically. *Note:* Isolated tumor cell clusters (ITCs) are defined as small clusters of cells ≤ 0.2 mm, or single tumor cells, or a cluster of < 200 cells in a single histologic cross-section; ITCs may be detected by routine histology or by immunohistochemical (IHC) methods; nodes containing only ITCs are excluded from the total positive node count for purposes of N classification but should be included in the total number of nodes evaluated
pN0(i-)	No regional lymph node metastases histologically, negative IHC

pN0(i+)	Malignant cells in regional lymph node(s) ≤ 0.2 mm (detected by hematoxylin-eosin [H&E] stain or IHC, including ITC)
pN0(mol-)	No regional lymph node metastases histologically, negative molecular findings (reverse transcriptase polymerase chain reaction [RT-PCR])
pN0(mol+)	Positive molecular findings (RT-PCR) but no regional lymph node metastases detected by histology or IHC
pN1	Micrometastases; or metastases in 1-3 axillary lymph nodes and/or in internal mammary nodes, with metastases detected by sentinel lymph node biopsy but not clinically detected†
pN1mi	Micrometastases (> 0.2 mm and/or > 200 cells, but none > 2.0 mm)
pN1a	Metastases in 1-3 axillary lymph nodes (at least 1 metastasis > 2.0 mm)
pN1b	Metastases in internal mammary nodes, with micrometastases or macrometastases detected by sentinel lymph node biopsy but not clinically detected†
pN1c	Metastases in 1-3 axillary lymph nodes and in internal mammary lymph nodes, with micrometastases or macrometastases detected by sentinel lymph node biopsy but not clinically detected†
pN2	Metastases in 4-9 axillary lymph nodes or in clinically detected‡ internal mammary lymph nodes in the absence of axillary lymph node metastases
pN2a	Metastases in 4-9 axillary lymph nodes (at least 1 tumor deposit > 2.0 mm)
pN2b	Metastases in clinically detected‡ internal mammary lymph nodes in the absence of axillary lymph node metastases
pN3	Metastases in ≥ 10 axillary lymph nodes; or in infraclavicular (level III axillary) lymph nodes; or in clinically detected‡ ipsilateral internal mammary lymph nodes in the presence of ≥ 1 positive level I, II axillary lymph nodes; or in > 3 axillary lymph nodes and in internal mammary lymph nodes, with micrometastases or macrometastases detected by sentinel lymph node biopsy but not clinically detected†; or in ipsilateral supraclavicular lymph nodes
pN3a	Metastases in ≥ 10 axillary lymph nodes (at least 1 tumor deposit > 2.0 mm); or metastases to the infraclavicular (level III axillary lymph) nodes
pN3b	Metastases in clinically detected‡ ipsilateral internal mammary lymph nodes in the presence of ≥ 1 positive axillary lymph nodes; or in > 3 axillary lymph nodes and in internal mammary lymph nodes, with micrometastases or macrometastases detected by sentinel lymph node biopsy but not clinically detected†
pN3c	Metastases in ipsilateral supraclavicular lymph nodes

*Classification is based on axillary lymph node dissection, with or without sentinel lymph node biopsy. Classification based solely on sentinel lymph node biopsy without subsequent axillary lymph node dissection is designated (sn) for "sentinel node"—for example, pN0 (sn).

† "Not clinically detected" is defined as not detected by imaging studies (excluding lymphoscintigraphy) or not detected by clinical examination.

‡ "Clinically detected" is defined as detected by imaging studies (excluding lymphoscintigraphy) or by clinical examination and having characteristics highly suspicious for malignancy or a presumed pathologic macrometastasis on the basis of FNA biopsy with cytologic examination.

Distant metastasis (M)

M0	No clinical or radiographic evidence of distant metastasis
cM0(i+)	No clinical or radiographic evidence of distant metastases, but deposits of molecularly or microscopically detected tumor cells in circulating blood, bone marrow, or other nonregional nodal tissue that are no larger than 0.2 mm in a patient without symptoms or signs of metastases
M1	Distant detectable metastases as determined by classic clinical and radiographic means and/or histologically proven > 0.2 mm

Stage	T	N	M
0	Tis	N0	M0
IA	T1	N0	M0
IB	T0	N1mi	M0
	T1	N1mi	M0
IIA	T0	N1	M0

	T1	N1	M0
	T2	N0	M0
IIB	T2	N1	M0
	T3	N0	M0
IIIA	T0	N2	M0
	T1	N2	M0
	T2	N2	M0
	T3	N1	M0
	T3	N2	M0
IIIB	T4	N0	M0
	T4	N1	M0
	T4	N2	M0
IIIC	Any T	N3	M0
IV	Any T	Any N	M1

10 Original communications

Early Effects of Adjuvant Breast Cancer Radiotherapy on Right Ventricular Systolic and Diastolic Function

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Abstract. *Aim: Reduced right ventricular (RV) systolic function correlates with poor prognosis in several heart diseases. The aim of this prospective single-Center study was to investigate whether conformal three-dimensional (3D) breast cancer radiotherapy impairs RV function. Patients and Methods: Forty-nine patients with early-stage left-sided breast cancer underwent comprehensive two-dimensional (2D) echocardiography before and after radiotherapy. RV function was evaluated with tricuspid annular plane systolic excursion (TAPSE), pulsed tissue Doppler peak velocity at the lateral RV wall (S') and RV and venous flow analysis. Results: Radiotherapy reduced TAPSE from 24.5±4.0 mm to 22.4±3.9 mm (p<0.001), S' from 12.7±3.1 m/s to 12.2±2.7 m/s (p=0.11) and pulmonary flow velocity time integral (VTI) from 16.6±3.1 cm to 15.9±2.3 cm (p=0.07), respectively. These changes were unrelated to changes in LV function. Conclusion: Modern radiotherapy reduced RV systolic function. As a readily-available and sensitive measurement, TAPSE is as a practical tool for detection of radiotherapy-induced cardiac changes.*

Breast cancer is the most common cancer in women (1). Improved diagnostics and adjuvant therapies have increased breast cancer survival rates (2). On the other hand, cardiac exposure from adjuvant radiotherapy (RT) has been shown to cause adverse cardiovascular effects. The late sequel includes left ventricular (LV) dysfunction, valvular heart disease and coronary artery disease (3-6). It is important for cardiologists and oncologists to recognize these adverse effects and find means to limit late co-morbidities.

Several investigators have demonstrated that the reduction of right ventricular (RV) systolic performance correlates with poor prognosis across a broad spectrum of diseases (7-9). Despite the important prognostic role of RV function, no prior study has systematically evaluated the effects of breast cancer RT on RV function. The aim of this prospective single-Center study was to investigate whether modern conformal three-dimensional (3D) breast cancer RT impairs RV systolic and diastolic function in the early phase.

Patients and Methods

Patient selection. Forty-nine eligible female patients with an early left-sided breast cancer who received postoperative adjuvant conformal RT without concomitant chemotherapy were included in this single-Center, prospective study. The study was conducted from July 2011 to February 2013. The exclusion criteria were age under 18 years or over 80 years, other malignancy, pregnancy or breast feeding, acute myocardial infarction within 6 months, symptomatic heart failure (NYHA 3-4), dialysis, permanent anti-coagulation and severe psychiatric disorder. To optimize echocardiography image quality, patients with atrial fibrillation, left bundle branch block (LBBB), pacemaker therapy and severe lung disease were excluded. The institutional board of ethics approved the protocol and all participants signed informed consent before enrollment in the study.

Radiotherapy. All patients underwent 3D computer tomography (CT)-based treatment planning (Philips Big Bore CT; Philips Medical Systems, Madison, WI, USA) in a supine position on a

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Key Words: Radiotherapy, breast cancer, right ventricle, TAPSE.

Table I. *Radiation doses to the different cardiac structures (N=49)**

	Mean±SD Gy†	Max±SD Gy‡
Whole heart	3.27±1.53	46.17±9.09
Left ventricle	5.41±3.03	44.44±9.52
LAD	20.35±10.63	41.87±13.15
Right ventricle	3.03±2.03	32.06±15.57
Free wall of right ventricle	6.09±4.74	31.91±15.62
Ipsilateral pulmonary dose	8.03±2.01	50.65±5.14

Gy, Grey; LAD, region of the heart perfused by left anterior descending coronary artery; SD, standard deviation. *The radiation doses are derived from three-dimensional (3D) computed tomography (CT) planning pictures by manual tracing. †The average dose to the appointed volume. ‡The maximum point dose to the appointed volume.

breast board with 3 mm thick slices. The breath-hold technique was not used. Treatment planning and contouring were performed with an Eclipse v.10 system (Varian Medical Systems, Palo Alto, CA, USA). Heart contouring was performed by the same oncologist (TS). Treatment doses were either 50 Gy in 2 Gy fractions (standard) or 42.56 Gy in 2.66 Gy fractions (hypofractionated) according to the local guidelines. An additional boost of 16 Gy in 2 Gy fractions to the tumor bed was used if clinically indicated. Doses were calculated using the anisotropic analytical algorithm (AAA) (Figure 1) and dose-volume histograms (DVHs) for different structures were generated for each patient (Table I). The average treatment time was 36±10 days (20-70 days).

Echocardiographic examinations. A comprehensive echocardiography and electrocardiography (ECG) were performed at baseline and at the end of RT (1.0±2.8 days from the last radiation dose). All examinations were performed with the same cardiac ultrasound machine (Philips iE33; Philips, Bothell, WA, USA) and a 1-5 MHz matrix-array X5-1 transducer by the same cardiologist (SST). The interval between the baseline and control studies was 41±11 days. All images were acquired at rest with a simultaneous superimposed ECG. Subcostal imaging was performed in a supine position and other imaging was performed with the patient in the left lateral decubitus position. Doppler recordings were acquired at the end expiration during shallow breathing. Raw data were stored digitally for offline analysis with the Qlab software (Philips). RV systolic performance was measured in an apical four chamber view. Care was taken to identify the true apex and optimize the depth and the sector width of the image. Tricuspid annular plane systolic excursion (TAPSE) was measured with the M-mode cursor placed between the junction of the tricuspid valve and the RV lateral free wall annulus as total displacement of the tricuspid annulus from end-diastole to end-systole. Pulsed tissue Doppler was acquired from a point 1-1.5 cm apical from lateral tricuspid annulus (Figure 2). A 12-lead ECG was recorded at each visit.

Statistical analysis. Means and standard deviations were given for normally-distributed variables and medians and ranges for continuous variables with skewed distributions. Differences between measurements were tested by the paired samples *t*-test or by the Wilcoxon signed-rank test. The Spearman correlation was used to test the linear associations between variables. The associations between TAPSE and other variables were analyzed by the independent samples Mann-Whitney U test (continuous variables) or by the Fisher's exact test (categorical variables). All tests were two-sided and p values <0.05 were considered statistically significant. Statistical analyses were performed using the IBM SPSS (IBM Corp. Released 2010. IBM SPSS Statistics for Windows, Version 19.0. Armonk, NY: IBM Corp.) statistical software package (http://www-01.ibm.com/software/analytics/spss/).

Table II. *Baseline characteristics of the study cohort (N=49).*

Variable	Mean±SD
Age (years)	63±6
Systolic blood pressure (mmHg)*	145±19
Diastolic blood pressure (mmHg)	80±12
Height (cm)	164±6
Weight (kg)	73±13
Body mass index (kg/m^2)	27±4
Body surface area (m^2)	1.80±0.17
	N (%)
Smoking	
Previous	5 (10%)
Current	8 (16%)
Prior diagnosis†	
Hypertonia	17 (35%)
Diabetes mellitus	2 (4%)
Hypercholesterolemia	8 (16%)
Hypothyreosis	5 (10%)
Atherosclerosis	2 (4%)
Significant valvular abnormality	2 (4%)
Medical treatment	
Beta blocker	6 (12%)
Calcium channel blocker	2 (4%)
ACE inhibitors/ARBs	10 (20%)
Diuretics	5 (10%)
Thyroxin	5 (10%)
Nitrates	1 (2%)
Aspirin	3 (6%)
Statin	7 (14%)
Oral diabetes medication	2 (4%)

ACE, Angiotensin-converting enzyme; ARB, angiotensin II blocker. *Measured at first visit. †Defined as medication requiring disease state. The values are presented either as the mean±SD (standard deviation) or the number of cases and percentage in the present study population.

Results

General characteristics. The baseline characteristics of the patients are presented in Table II. The mean age of the population was 63 (range=49–79) years. The most common underlying diseases included hypertension (35%), hypercholesterolemia (16%), hypothyreosis (10%) and diabetes (4%). Twenty-two percent of the patients had no other diseases.

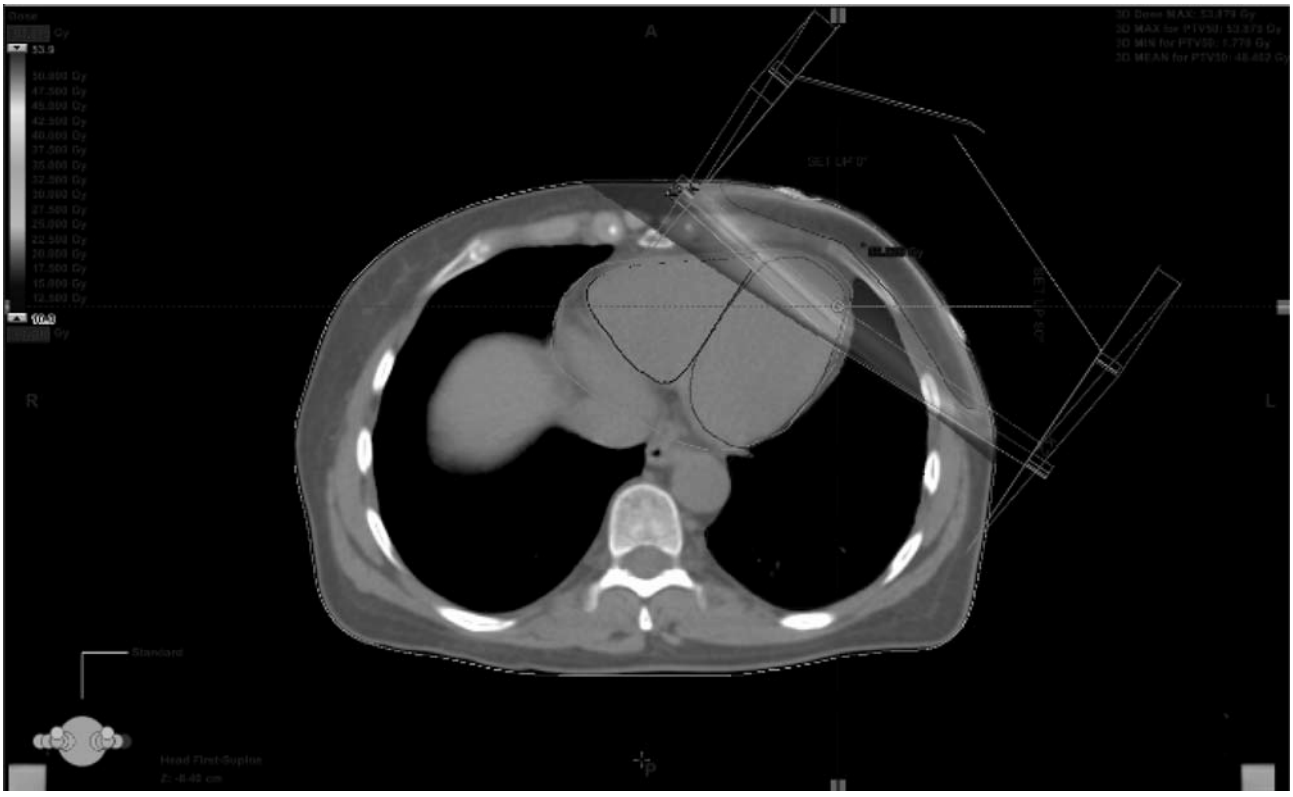

Figure 1. *Three-dimensional (3D) computed tomography (CT) treatment planning. Target volume is planned to cover remaining breast tissue. The dark area shows sites achieving more than 10 Grays with highest dose in the apex. Manually depicted heart contouring is also shown for the whole heart, left ventricle, right ventricle, right ventricle's free wall.*

RV echocardiographic measurements. RT caused significant changes in RV systolic function (Table III). TAPSE declined in 67% of the patients. The average reduction was 2.1±3.2 mm ($p<0.001$). A decrease of 4 mm or more was observed in 39% of the patients. There was no correlation between these changes and the cardiac or pulmonary radiation dose, smoking, ECG changes, body mass index (BMI) or underlying disease, other than hypothyreosis. The use of thyroxin ($p=0.03$) and diuretics ($p=0.03$) were associated with smaller TAPSE reduction. Likewise, the use of angiotensin-converting enzyme inhibitors (ACEIs) or angiotensin II receptor blockers (ARBs) tended to protect against TAPSE decline ($p=0.06$). The reduction in TAPSE was 1.0±4.0 mm and 2.4±3.0 mm ($p=0.22$) among patients using and not using ACEIs/ARBs, respectively.

In keeping with the reduction of TAPSE, the other RV systolic parameters showed a declining tendency, although statistical significance was not reached. S' declined from 12.7±3.1 to 12.2±2.7 ($p=0.11$) and pulmonary flow VTI decreased from 16.6±3.1 to 15.9±2.3 ($p=0.07$). Isovolumetric velocity (IVV) ($p=0.82$) and acceleration of the IVV did not change ($p=0.43$) (Table III). Neither TAPSE nor any other RV systolic parameter was related to LV systolic or diastolic changes.

The changes in the RV diastolic parameters were less obvious than the systolic parameters. The maximal diameter of the inferior *vena cava* was 15.7±4.1 mm at baseline and 14.8±3.5 mm after RT ($p=0.12$). Its respiratory variability remained unchanged. There were no significant changes in the tricuspid Ea and Ee' ratio, whereas the tricuspid inflow E wave showed a slight (48.5±8.5 cm/s *vs*. 45.6±8.4 cm/s) but not statistically significant reduction ($p=0.10$) (Table III).

Minor tricuspid and pulmonary regurgitation were observed in 94% and 69% of the patients, respectively. The regurgitation was hemodynamically insignificant in all patients and no patient had stenosis in the right-sided heart valves. There were no changes in tricuspid or pulmonary valve status between baseline and control examinations.

LV echocardiographic measurements. At baseline, LV dimensions and function were compatible with patient´s age and underlying disease profile in all participants. RT had no significant effect on LV systolic or diastolic function. However, both the interventricular septum (10.0±1.2 mm *vs*. 10.3±1.3 mm, $p=0.02$) and the posterior wall (9.7±1.0 mm *vs*. 10.3±1.2 mm, $p=0.01$) were slightly thicker after RT than at baseline (Table IV).

Table III. *Echocardiographic measurements of the right ventricle (N=49).*

	Baseline		Measurement after radiotherapy		*p*-Value
	Mean±SD	Median (range)	Mean±SD	Median (range)	
RV basal dimension (mm)	33.7±5.5	33.0 (24.0-44.0)	33.3±4.5	33.1 (22.1-44.4)	0.513
TAPSE (mm)	24.5±4.0	24 (16-33)	22.4±3.9	22 (16-33)	<0.001
S' (cm/s)	12.7±3.1	12.1 (8.9-22.2)	12.2±2.7	11.5 (8.9-20.7)	0.114
IVV (cm/s)	13.0±4.8	12.4 (4.9-29.3)	12.6±3.8	12.4 (4.6-21.9)	0.828
IVA (cm/s^2)	2.8±1.0	2.7 (1.0-5.1)	2.7±0.9	2.6 (1.3-5.1)	0.439
Pulmonary peak flow velocity (cm/s)	69.6±14.3	71.0 (46-101)	67.6±11.5	67.4 (47-92)	0.382
Pulmonary flow at (ms)	149.4±33.0	145 (71-239)	147.4±29.3	146 (85-204)	0.668
Pulmonary flow VTI (cm)	16.6±3.1	16.6 (10.9-24.4)	15.9±2.3	15.8 (11.5-22.4)	0.071
Tricuspid gradient (mmHg)*	21.6±5.8	22 (8-34; n=42)	21.2±5.0	22 (8-32; n=39)	0.430
Tricuspid inflow E velocity (cm/s)	48.5±8.7	48.3 (32.6-66.6)	45.6±8.4	45.2 (24.9-68.9)	0.096
Tricuspid inflow a velocity (cm/s)	38.3±8.0	37 (22.7-61.7)	37.5±8.0	37.6 (22.8-61.7)	0.547
Tricuspid inflow dt (ms)	236±73	235 (132-444)	257.5±65,8	252 (140-454)	0.212
Tricuspid Ea ratio	1.30±0.24	1.26 (0.88-1.85)	1.24±0.23	1.26 (0.75-1.81)	0.281
Tricuspid Ee' ratio	4.2±1.1	4.1 (2.5-7.3)	4.3±1.4	4.1 (2.1-8.0)	0.406
IVC maximal diameter (mm)	15.7±4.1	15.9 (1.6-25.9)	14.8±3.5	14.8 (8.8-22.5)	0.119
IVC respiration variability (%)†	62.8±15.2	60.2 (30.6-97.7)	63.8±15.5	62.8 (28.2-92.2)	0.670
Hepatic vein flow SD ratio‡	1.53±0.46	1.55 (0.9-2.8; n=36)	1.62±0.46	1.50 (0.7-3.9; n=35)	0.318

RV, Right ventricle; TAPSE, tricuspid annular plane systolic excursion; S' and IVV, systolic and isovolumetric velocity of pulsed tissue Doppler derived from the lateral basal RV free wall; IVA, acceleration of the IVV; at, acceleration time; VTI, velocity time integral; dt, declaration time; Ee' ratio, ratio between tricuspid inflow E-velocity and pulsed Doppler e' velocity derived from the RV basal free wall; IVC, inferior vena cava; Mean±SD, mean±standard deviation. *Measurable in 83%. †Inferior vena cava respiration variability tested with sniffing. ‡Hepatic vena flow SD ratio calculated as the ratio between the maximal velocities of systolic and diastolic components.

Six patients had moderate valvular abnormalities at the baseline echocardiography examination. Two of them had been diagnosed before (one with stenosis in aortic bioprosthesis and one with moderate mitral regurgitation) and 4 had a new diagnosis (one with moderate aortic stenosis, two with moderate mitral regurgitation and one with mild aortic regurgitation). There were no changes in aortic or mitral valve status between the baseline and control examinations.

ECG. Patients had sinus rhythm, narrow QRS complex and normal PQ and QT time in all ECG recordings. There were no signs of right atrial or ventricular abnormalities in any recordings. RT caused moderate T-wave alterations in 16 patients (33%). These changes did not correlate to the change in TAPSE.

Discussion

We demonstrated that RV systolic function was reduced in the early phase after modern conformal 3D left-sided breast cancer RT, despite a lack of changes in LV function. This novel finding indicates that measurement of RV function is a sensitive indicator of radiation-induced myocardial injury and an attractive tool for the follow-up of patients after RT.

RV function after RT. RV wall is thinner than the LV wall. Therefore, tissue swelling, reduced contractility and diastolic changes can be detected earlier in the RV than in the LV. In the current study, RT did not cause any significant changes in LV systolic or diastolic function, whereas the average decline in TAPSE was 2.1±3.2 mm and declined by 4 mm or more in 39% of the patients. The same tendency was found in the other RV systolic parameters. The changes in RV function were not accompanied by any significant changes in echocardiographic measures of pulmonary function and resistance. Moreover, there was no correlation between TAPSE decline and pulmonary radiation dose.

TAPSE and S' are measurements of RV longitudinal function, whereas IVV and VTI reflect rotational contractility and global RV function, respectively. Longitudinal contractility is the main determinant of RV systolic function (10, 11). The basal circumferential muscle fibers shared by the right and left ventricles initiate systolic contraction and cause rotational contraction (12). In elderly subjects and in diseases that overload the RV, the rotational contractility increases proportionally as the longitudinal contractility is reduced (14) and rotational contractility is increased (12-15). In our patients, the reductions in TAPSE, S' and pulmonary VTI were not accompanied by a compensatory increase in IVV. This indicates that TAPSE-alone may underestimate the RT-induced RV damage.

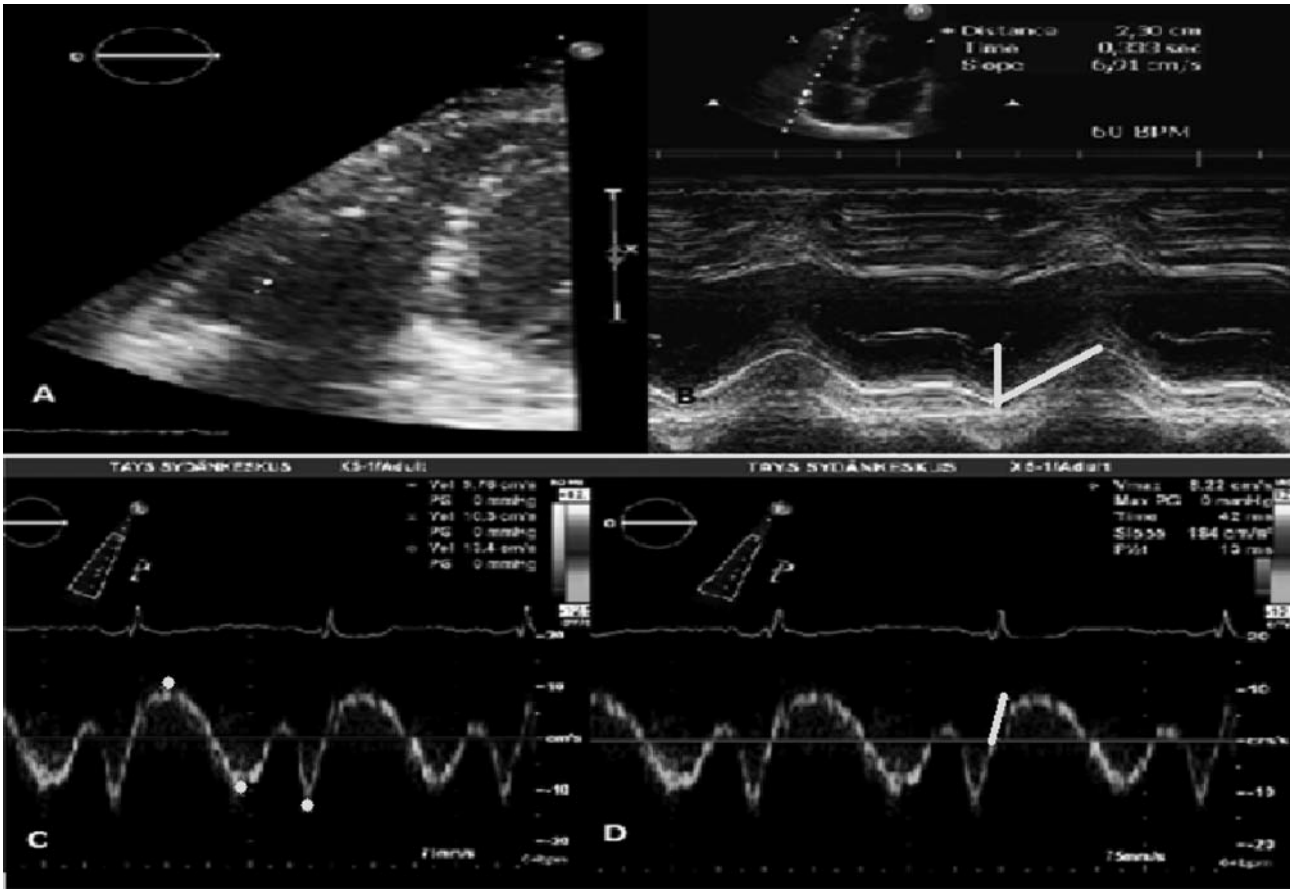

Figure 2. *Measurements of tricuspid annular plane systolic excursion (TAPSE) and pulsed tissue Doppler velocities at the lateral RV wall. A: Focused RV image with cursor placed in the junction of RV lateral wall and tricuspid annulus. B: TAPSE measurement with M-mode. C: Measurements of peak systolic velocity (S'), peak E' velocity and peak A' velocity, respectively. D: Measurements of IVV and IVA.*

Mechanism of the changes in RV function. The inflammatory reaction due to RT begins within hours (16, 17). The second or latent stage of radiation injury is characterized by reduced capillary density (17, 18). The complex fibrotic cascade is initiated as early as 2 weeks after the onset of RT (17, 19) and the earliest evidence of increased myocardial fibrosis has been observed within 40 days after a single radiation dose (16). Since our patients were exposed to RT for 4-5 weeks, all these mechanisms may contribute to the observed changes. Whether thyroxin or ACEI/ATR medication protects the RV from radiation-induced adverse effects remains to be established in larger clinical studies.

Limitations. Our study population was uniform in many ways, which reduced the confounding effects but also made extrapolation of the results to other groups difficult. Due to the small number of patients, a reliable multivariate analysis of related factors was not possible. Time-consuming magnetic resonance imaging and sophisticated 3D echocardiographic measurements were not used because the main idea was to determine whether the change in RV function could be measured with the tools we use in everyday practice.

Clinical implications of RV functional changes. The most important implication of this study was that the modern 3D conformal RT caused prominent changes in RV function in most of our patients. On the basis of the results of previous studies (3, 4), these changes may progress over time and clinically significant cardiac adverse events may emerge during long-term follow-up. These findings support the consensus statement recommending a comprehensive cardiac evaluation and a long-term follow-up of patients after breast cancer RT (20). According to our data, TAPSE is a more sensitive tool for the detection of the radiation-induced early myocardial deterioration than conventional LV measurements.

The result of this and prior studies imply that even small radiation doses may induce myocardial changes (21). In the

Table IV. *Echocardiographic measurements of the left ventricle.*

	Baseline		Measurement after radiotherapy		*p*-Value
	Mean±SD	Median (Range)	Mean±SD	Median (Range)	
LVEDD (mm)	45.4±4.1	45 (34.8-57.1)	44.7±4.1	45.2 (35.0-53.1)	0.084
LVESD (mm)	30.3±3.4	30.6 (18.9-40.0)	30.1±3.8	30.7 (19.0-36.7)	0.983
IVS (mm)	10.0±1.2	10.0 (8.0-12.8)	10.3±1.3	10.0 (8.0-13.0)	0.020
PW (mm)	9.7±1.0	10.0 (7.3-11.3)	10.3±1.2	10.0 (7.3-13.0)	0.011
LVEF (%)	62.2±5.0	62 (52-78)	61.5±5.2	62 (52-73)	0.473
Mitral inflow E (cm/s)	70±12	70 (47-102)	68±13	66 (39-109)	0.138
Mitral inflow a (cm/s)	77±19	74 (38-121)	75±14	77 (46-109)	0.128
Mitral inflow Ea-ratio	0.95±0.26	0.93 (0.56-1.78)	0.94±0.26	0.86 (0.59-1.67)	0.792
IVRT	104±26	103 (58-187)	109±21	106 (77-155)	0.283
Ee' ratio	9.0±2.6	8.7 (5.7-16.7)	8.8±2.1	8.5 (5.7-16.6)	0.554
LAVI (ml/m^2)	32.2±8.8	32.2 (16.7-56.3)	31.9±8.8	31.1 (13.7-53.2)	0.622
Pulmonary vein flow SD ratio	1.3±0.2	1.3 (0.8-1.8)	1.3±0.2	1.3 (0.7-1.8)	0.699

LVEDD, Left ventricular end diastolic diameter; LVESD, left ventricular end systolic diameter; IVS, interventricular septum; PW, posterior wall; LVEF, left ventricular ejection fraction; IVRT, isovolumetric relaxation time; Ee' ratio, ratio between mitral inflow E-velocity, and averaged pulsed Doppler e' velocity derived from septal, lateral, anterior and inferior walls; LAVI, left atrial volume indexed to the patient's body surface area; Pulmonary vein flow SD ratio, ratio between systolic and diastolic peak velocities; Mean±SD, mean±standard deviation.

present study, the RV systolic function was impaired, although the mean dose to the RV free wall in our study group was only 6.09±4.74 Gy. Hence, it is important to use techniques that reduce cardiac radiation exposure, *e.g.* respiratory gating and breath-hold techniques.

Conclusion

Right ventricular systolic function is impaired after breast cancer adjuvant RT. TAPSE is a sensitive and reliable marker of early myocardial injury and can be used as a practical tool to identify patients who would benefit the most from a longterm follow-up.

Acknowledgements

The Authors thank research nurses Virpi Räsänen, Hanna Näppilä and Emmi Vettenranta for their expert assistance during the study. This study was supported by the Pirkanmaa Hospital District, Seppo Nieminen Fund (grant 150613), by Paavo and Eila Salonen Legacy, by Ida Montin Fund and by Finnish Medical Foundation.

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Received January 9, 2015
Revised January 24, 2015
Accepted January 26, 2015

The Concurrent Use of Aromatase Inhibitors and Radiotherapy Induces Echocardiographic Changes in Patients with Breast Cancer

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Abstract. *Aim: Adjuvant radiotherapy (RT) for left-sided breast cancer has a negative impact on cardiac health. The concurrent use of aromatase inhibitors (AIs) during RT was found to increase the anticancer efficacy of radiation in pre-clinical models. We evaluated whether the acute effects of RT on cardiac functions are augmented by the concurrent use of AIs. Patients and Methods: Sixty patients with early-stage left-sided breast cancer underwent a 2D echocardiography, electrocardiogram and cardiac biomarker measurements before and after adjuvant breast RT. Data were analyzed in two groups according to AI use. Results: We observed a significant ($p<0.05$) decrease in right ventricular systolic function during RT in tricuspid annular plane systolic excursion (TAPSE). TAPSE decreased by 3.0 mm [95% confidence interval (CI)=1.9-4.1 mm] in the AI group and 1.4 mm (95% CI=0.3-2.4 mm) in the non-AI group. In addition, left ventricular diastolic function decreased among patients using AI, as the mitral inflow E-wave decreased 5.8 cm/s (95% CI=1.8-9.7 cm/s) ($p=0.006$). Conclusion: The concurrent use of AI during RT for left-sided breast cancer led to a more pronounced change on right ventricular systolic function and left ventricular diastolic functions compared to RT alone.*

Postoperative adjuvant radiotherapy (RT) for early-stage breast cancer has been shown to reduce the local recurrence rate and also the risk of death from breast cancer (1). Conventional RT of 50 Gy in 25 fractions, or hypofractionated regimens, such as 42.56 Gy in 16 fractions, are equally effective and well-tolerated (2). As patients with early-stage breast cancer have an excellent cancer-specific prognosis (1), it has become increasingly important to avoid the long-term adverse effects of cancer treatments.

Large retrospective trials have demonstrated that adjuvant RT to the left-sided breast particularly increases cardiovascular morbidity and cardiovascular death (1, 3). Patients with breast cancer with pre-existing cardiovascular risk factors, such as hypertension, coronary artery disease, diabetes, smoking or obesity, have an increased risk from RT compared to healthy women (3). RT induces inflammatory tissue responses that progress to fibrogenesis (4). Consequently, radiation increases the risk of long-term cardiac side-effects due to fibrotic alterations in exposed cardiac structures. These changes could later manifest as constrictive pericarditis, coronary artery disease, congestive heart failure or valvular dysfunction. These conditions may eventually lead to either diminished quality of life or premature death due to RT (3, 5).

Modern three-dimensional (3D) RT planning allows for improved delineation of cardiac structures and hence heart protection during RT planning. Furthermore, the introduction of deep-inspiration breath-hold techniques reduces the radiation dose to the heart (6). Yet no safe radiation dose threshold for the heart has been established (3), and direct radiation of cardiac structures should therefore be avoided. In animal models, doses as low as 0.2 and 2 Gy have generated cardiac dysfunction and fibrosis (7).

Adjuvant hormonal treatment for estrogen receptor (ER)-positive breast cancer functions by suppressing ER activation in breast cancer cells and other tissues. Aromatase inhibitors (AIs) (*e.g.* letrozole, anastrozole and exemestane) minimize the levels of circulating estrogen by suppressing estrogen

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Key Words: Breast cancer, radiotherapy, cardiotoxicity, aromatase inhinitors.

Table I. *Baseline characteristics of patients treated with radiotherapy for left-sided breast cancer.*

	All (n=60)	AI users (n=22)	AI non-users (n=38)	*p*-Value*
Age (years)	63±6	65±7	62±6	0.16
BMI (kg/m^2)	27.2±4.2	29.0±4.7	26.2±3.7	0.01
Hypertension	21 (35%)	9 (41%)	12 (32%)	0.47
ACE or ARB	14 (23%)	6 (27%)	8 (21%)	0.58
Beta blocker	8 (13%)	5 (23%)	3 (8%)	0.13
Calcium channel blocker	5 (8%)	3 (14%)	2 (5%)	0.26
CAD	2 (3%)	1 (5%)	1 (3%)	1.0
Statin use	13 (22%)	8 (36%)	5 (13%)	0.05
Diabetes	4 (7%)	3 (14%)	1 (3%)	0.14
ASA	5 (8%)	3 (14%)	2 (5%)	0.35
Hypothyreosis	8 (13%)	4 (18%)	4 (11%)	0.45
Current smoking	8 (13%)	4 (18%)	4 (11%)	0.45
Tamoxifen use	2 (3%)	0 (0%)	2 (5,3%)	0.53

BMI: Body mass index; ACE: angiotensin-converting enzyme; ARB: angiotensin II receptor blockers; CAD: coronary artery disease; statin use: hypercholesterolemia with statin use; diabetes: blood glucose lowering diabetic medication; ASA: daily low-dose acetylsalicylic acid; hypothyreosis: continuous thyroid hormone supplementation. *Difference between aromatase inhibitor users and non-users: Independent samples *t*-test for continuous variables; Chi-squared test for obesity, hypertension and ACE or ARB use; Fisher's exact test for other variables.

Table II. *Total radiation dose and dose to cardiac structures and ipsilateral (left) lung of patients treated for left-sided breast cancer. No statistical difference was observed in radiation doses between the aromatase inhibitor users versus non-users.*

	AI users (n=22)	AI non-users (n=38)	
Dose			
50/2 Gy*	13 (59%)	26 (68%)	
10 Gy boost	1 (5%)	0 (0%)	
16 Gy boost	2 (9%)	9 (24%)	
42.56/2.66 Gy†	9 (41%)	12 (32%)	
Structure	Mean±SD	Mean±SD	*p*-Value
Heart (volume, cm^3)	678±108	676±101	0.94
Mean dose (Gy)	3.1±1.4	3.1±1.7	0.86
Max dose (Gy)	45±9.4	45±10.7	0.99
V20 Gy (%)	3.6±2.6	5.8±9.5	0.29
LV (volume, cm^3)	172±33	170±34	0.87
Mean dose (Gy)	4.5±2.6	5.4±3.2	0.28
Max dose(Gy)	42.4±9.9	42.8±12.0	0.90
V20 Gy (%)	5.5±5.2	8.4±7.5	0.10
V10 Gy (%)	8.4±6.8	11.4±9.2	0.19
RV (volume, cm^3)	85±16	86±18	0.88
Mean dose (Gy)	2.7±1.5	3.1±2.3	0.48
Max dose (Gy)	30.4±15.6	30.3±17.3	0.97
V20 Gy (%)	1.7±3.0	2.8±4.6	0.24
V10 Gy (%)	3.6±5.6	5.3±7.1	0.33
RV free wall (volume, cm3)	16.2±2.5	16.9±3.2	0.38
Mean dose (Gy)	5.5±4.2	6.2±54	0.60
Max dose (Gy)	30.2±15.6	30.2±17.3	1.00
V20 Gy (%)	6.6±11.7	8.6±12.9	0.55
LAD (volume, cm^3)	0.8±0.3	0.7±0.5	0.51
Mean dose (Gy)	18.4±10.6	19.5±11.6	0.72
Max dose (Gy)	38.8±16.0	39.5±14.5	0.86
V40 Gy (%)	17.9±21.5	27.2±27.2	0.17
V20 Gy (%)	38.1±30.4	41.6±29	0.66
Ipsilateral lung			
Mean dose (Gy)	7.6±2.2	8.0±1.9	0.51
Max dose (Gy)	48.7±4.3	51.0±5.0	0.07
V30 Gy (%)	10.4±3.7	11.2±3.7	0.39

AI: Aromatase inhibitor; dose: planned dose to target volume; boost: additional radiation dose to tumor bed; mean dose: mean radiation dose to designated structure; max dose: maximal point dose to designated structure; SD: standard deviation of mean value; V# Gy: volume of designated structure receiving # Gy radiation dose; LV: left ventricle of heart; RV: right ventricle of heart; RV free wall: anterior 4 mm-thick free wall of right ventricle; LAD: left anterior descending artery; *50 Gy total dose in 2 Gy daily fractions, 5 days a week; †42.56 Gy total dose in 2.66 Gy daily fractions, 5 days a week.

synthesis from testosterone in adipose tissues and the adrenal cortex in post-menopausal women. Tamoxifen is primarily a direct antagonist of ER in breast cancer cells. Tamoxifen also induces cardioprotective effects, which may be related to its action as a selective ER modulator in the heart (8). In comparison to tamoxifen, letrozole was found to increase cardiac morbidity (9).

The concurrent use of AI and RT has an additive synergistic antitumor effect against breast cancer cell lines and in rodents (10, 11). In clinical settings, the combination is regarded as safe in terms of skin toxicity and fibrosis (12, 13). To the best of our knowledge, early cardiac toxicity resulting from the combination of AI and RT has not been prospectively evaluated. Hence, we evaluated the effects of the concurrent use of AI and RT on right and left ventricular function by comprehensive echocardiography and analysis of cardiac biomarkers in patients postoperatively undergoing adjuvant RT for left-sided breast cancer.

Patients and Methods

This single-Center, prospective, observational clinical study included 60 eligible female patients with operable early stage left-sided breast cancer or ductal carcinoma *in situ*. All patients received adjuvant conformal 3D RT after local breast tumor resection (n=59) or mastectomy (n=1) without axillary or supraclavicular lymph node RT. Patients treated with adjuvant chemotherapy were excluded. Other exclusion criteria were age over 80 years, dialysis, recent acute myocardial infarction, symptomatic heart failure, chronic atrial fibrillation, pacemaker therapy and severe lung disease. The local Ethical Committee approved the protocol (ETL R10160), and all participants signed informed consent before study enrollment. The study was conducted from June 2011 to May 2013.

Table III. *Mean baseline values and changes in echocardiographic measurements measured before and after radiotherapy for left-sided breast cancer in aromatase inhibitor (AI) users and non-users.*

	AI users				Non-AI users			
Parameter	N	Baseline mean±SD	Mean change after radiotherapy (95% CI)	*p*-Value	N	Baseline mean±SD	Mean change after radiotherapy (95% CI)	*p*-Value
LVEDD (mm)	22	45±3	0.18 (–0.79-1.1)	0.702	38	46±4	–0.74 (–1.74-0.25)	0.137
LVESD (mm)	22	30±3	0.86 (–0.22-1.94)	0.111	38	31±4	-0.61 (–1.65-0.44)	0.248
IVS (mm)	22	10.6±1.6	0.27 (–0.16-0.71)	0.208	38	9.8±1.2	0.21 (–0.09-0.51)	0.160
PW (mm)	22	10.5±1.4	0.45 (–0.09-1.00)	0.096	38	9.6±1.1	0.45 (0.04-0.86)	0.033
LAVI (ml)	22	31.5±8	1.55 (2.00-5.11)	0.374	38	33.4±8.7	–1.59 (–3.85-0.66)	0.161
RV (mm)	22	35±5	–0.02 (–1.41-1.36)	0.974	38	34±5	–0.17 (–0.81-0.48)	0.601
RV systolic								
TAPSE (mm)	22	24±4	–3.00 (–4.14-–1.86)	<0.001	38	24±4	–1.37 (–2.43-–0.30)	0.013
RV's (cm/s)	22	12.7±3.7	–0.02 (–1.41-1.36)	0.974	37	12.1±2.6	–0.17 (–0.81-0.48)	0.601
TF gradient (mmHg)	17	21±6	0.06 (–1.89-1.77)	0.947	29	22±6	–0.45 (–2.56-1.66)	0.666
RV diastolic								
HV s:d ratio	14	1.5±0.5	–0.25 (–0.58-0.08)	0.129	20	1.5±0.5	0.17 (–0.16-0.50)	0.297
RV Ee'ratio	21	4.3±1.3	0.24 (–0.47-0.96)	0.485	38	4.1±1.1	0.04 (–0.41-0.5)	0.851
LV systolic								
EF (%)	22	62±6	0.45 (–2.80-3.71)	0.774	38	62±5	–0.5 (–2.36-1.36)	0.590
LV diastolic								
Mitral E (cm/s)	22	75±16.5	–5.77 (–9.71-–1.83)	0.006	38	72.1±13.4	–2.72 (–6.27-0.84)	0.130
Dt (ms)	22	247±45	1.6 (–14.8-18.1)	0.838	38	226±37	12.8 (–1.0-26.5)	0.068
EA ratio	22	0.93±0.25	–0.03 (–0.11-0.05)	0.420	38	1.00±0.31	–0.01 (–0.06-0.05)	0.852
IVRT	22	101±25	10.1 (–2.9-23.1)	0.121	38	106±25	3.6 (–4.1-11.2)	0.355
Ee'ratio	22	10.1±3.1	–0.47 (–1.52-0.57)	0.359	38	8.9±2.5	–0.17 (–0.77-0.43)	0.568

N: Number of reliable paired measurements acquired; SD: standard deviation; LVEDD: left ventricular end diastolic diameter; LVESD: Left ventricular and systolic diameter.

Radiotherapy. All patients underwent 3D computed tomographic (CT) treatment planning (Philips Big Bore CT, Philips Medical Systems, Madison, WI, USA; or Toshiba Aquilion LB, Toshiba Medical System, Tokyo, Japan) on a breast board in supine position with both arms above the head. Three millimeter-thick CT slices without intravenous contrast were used. In total, 58 patients were scanned under free breathing, whereas the remaining two were scanned and treated under the voluntary deep-inspiration breath-hold technique as this method was implemented as clinical practice in our unit from April 2013. In this technique, the breathing cycle was monitored using the Varian RPM system (Varian Medical Systems, Palo Alto, CA, USA). Treatment contouring and planning were performed with the Eclipse v.10 system (Varian Medical Systems). Planning target volume (PTV) covered the remaining breast tissue in 59 patients and the chest wall in one patient (mastectomy) with sufficient margins to account for inter- and intrafraction movements (5-8 mm in our unit).

The heart, right ventricle (RV), left ventricle (LV) and left anterior descending artery (LAD) were contoured from the treatment planning CT scans as suggested by Feng *et al*. (14). Additionally, the anterior free wall of the RV was contoured with an estimated wall thickness of 4 mm derived from echocardiographic examinations. All cardiac structures were contoured by the same radiation oncologist (TKS).

The radiation dose was either 50 Gy in 2-Gy fractions over five weeks with or without an additional boost (10-16 Gy, 5-8 fractions) to the tumor bed, or 42.56 Gy in 2.66 Gy fractions (hypofractionation) over 3.5 weeks according to local guidelines (for hypofractionation: grade I or 2 tumors with margins over 5 mm, age >50 years and tangential breast length <25 cm). Tangential photon fields were used for 59 patients, and the chest wall of one mastectomy patient was treated with electron beams. Doses were calculated using an Anisotropic Analytical Algorithm for photons and Generalized Gaussian Pencil Beam for electrons. Dose-volume histograms of various structures were generated for each patient. To account for the different dosing schedules, an α/β-ratio of 3 was used for the heart and lung to calculate 2 Gy equivalent doses.

Aromatase inhibitors. Aromatase inhibitors were prescribed to postmenopausal patients if indicated by the breast cancer stage and biology. Local breast cancer treatment guidelines were used in this adjuvant hormonal therapy decision. Two different orally administered AIs were used in this study population. Letrozole (various manufacturers) was administered at a daily dose of 2.5 mg. Exemestane (various manufacturers) dose was 25 mg once daily. AI therapy was initiated at the beginning of RT.

Cardiac biomarker and estradiol analysis. Markers for cardiac myocyte injury, namely high sensitivity cardiac troponin T (detection limit 5 ng/l), and N-terminal pro-brain natriuretic peptide (ng/l, BNP) were analyzed in serum samples taken before, at the third week and end of RT. Total cholesterol levels (mmol/l) were measured at baseline and the end of RT under fasting conditions. Estradiol levels (pmol/l) were analyzed with liquid chromatography-tandem mass spectrometry (LC-MS/MS from the samples obtained at the completion of RT.

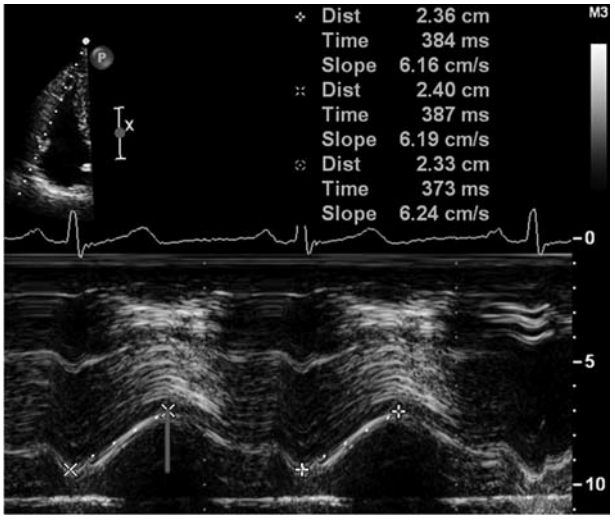

Figure 1. *Measurement of tricuspid annular plane systolic excursion (TAPSE). TAPSE was acquired with the M-Mode cursor placed between the junction of the tricuspid valve and the lateral free wall annulus of the right ventricle. TAPSE was measured as total displacement of the tricuspid annulus from end-diastole to end-systole (straight line).*

Echocardiographic examinations. A comprehensive echocardiography study and echocardiogram (ECG) were performed at baseline and completion of RT. All echocardiography examinations were performed with a commercially available ultrasound machine (Philips iE33 ultrasound system; Philips, Bothell, WA, USA) and a 1-5 MHz matrix-array X5-1 transducer by the same cardiologist (ST) certified by the European Association of Echocardiography for adult transthoracic echocardiography. All images were acquired at rest. Sub-costal imaging was performed in the supine position, whereas additional imaging was performed with the patient in the lateral decubitus position with simultaneous superimposed ECG. Doppler recordings were acquired at the end of expiration during shallow breathing. Images were stored digitally for offline analysis using analysis software (Excelera; Philips, Koninklijke, Netherlands, Qlab, Philips, Bothell, WA, USA). Echocardiographic measurements were performed in a standardized manner according to the European guidelines (15).

Statistical analysis. Data are presented as the percentages or means±standard deviations. Non-normal variables are described as medians (with inter-quartile range). The within-group changes from baseline to the end of RT were analyzed using the paired samples *t*-test. The Wilcoxon signed-ranks test was used for non-normal variables. The differences between groups at the end of RT were estimated using analysis of covariance (ANCOVA), where the baseline measurement was included as a covariate. The Mann-Whitney *U*-test for independent samples was used for non-normal variables. The Chi-squared test was used for categorical variables, and the Fisher's exact test was used if the Chi-squared test was not appropriate. All the tests are two-sided, and a *p*-value of less than 0.05 was considered statistically significant. Analysis was performed using IBM SPSS Statistics for Windows (version 21.0; IBM Corp., Armonk, NY, USA).

Results

General characteristics. The baseline characteristics of the patients divided into two groups according to the use of AI are presented in Table I. The body mass index was 2.8 kg/m^2 higher in the AI users compared with the non-users (p=0.01). Statin use was more common in the AI users (p=0.05). No statistically significant differences in other baseline characteristics were recorded.

Cardiac RT doses. Radiation doses to various cardiac structures and the lungs were not significantly different between the groups (Table II). The mean doses to the heart were 3.1±1.4 *vs*. 3.1±1.7 Gy (p=0.86), left ventricle 4.5±2.6 *vs*. 5.4±3.2 Gy (p=0.28), right ventricle 2.7±1.5 *vs*. 3.1±2.3 Gy (p=0.53) and left anterior descending coronary artery (LAD) 18.4±10.6 *vs*. 19.5±11.6 Gy (p=0.72) in AI users and non-users, respectively.

Cardiac biomarkers and estradiol analysis. Paired serum samples drawn at baseline and at the completion of RT were evaluable for 20/22 patients in the AI group and 36/38 in the non-AI group. The mean baseline cholesterol was 5.3±1.4 mmol/l and 5.7±0.9 mmol/l in the AI users and non-users, respectively. No significant changes in cholesterol levels were observed at the end of RT in either group: 5.4±1.4 mmol/l in the AI users and 5.7±1.0 mmol/l in the non-users. The baseline-adjusted difference between the AI users *vs*. the non-users was 0.2 mmol/l (95% CI=–0.1 to 0.5, p=0.28).

High sensitivity cardiac troponin T increased during RT by more than 30% from baseline in 4/20 (20%) patients in the AI group and 6/36 (17%) in the non-AI group (p=0.73). However, the absolute measurable troponin levels were low (<5 to 15 ng/l) in both groups.

BNP did not change significantly during RT; in the AI users, the median (IQR) BNP level was 100 (53-173) ng/l at baseline and 83 (54-147) at the end of RT (p=0.96). Among the non-AI-users, BNP values were 57 (37-102) ng/l and 72 (35-115) ng/l (p=0.36) at baseline and the end of RT, respectively. Changes from baseline were not significantly different between the AI users and non-AI users (p=0.57).

Circulating serum estradiol levels were evaluated for all patients and measured at the completion of RT *i.e*. 3-5 weeks after AI initiation. The estradiol level was significantly (p=0.004) reduced in the AI users (median=18 pmol/l, IQR=15-47 pmol/l) compared with the non-AI users (median=39 pmol/l, IQR=32-57 pmol/l).

Echocardiographic examinations. The most prominent RT-induced reduction in cardiac functions was observed in TAPSE (Figure 1). Among the AI users (n=22), this measurement of RV systolic function was reduced from a baseline value of 24±4 mm by 3.0 mm (95% CI=1.9-4.1 mm) (p<0.001) (Table II).

Table IV. *The baseline-adjusted means (95% confidence interval=CI) for basic echocardiographic measurements after radiotherapy for left-sided breast cancer in aromatase inhibitor users and non-users. The difference between users and non-users was estimated using analysis of covariance (ANCOVA), where the baseline measurement was included as a covariate.*

	AI users		AI non-users		Users *vs*. non-users		
	Mean	95% CI	Mean	95% CI	Mean	95% CI	*p*
Left heart measurements							
LVEDD (mm)	45.3	44.2-46.4	44.6	43.8-45.5	0.6	–0.8 to 2.0	0.37
LVESD (mm)	31.1	30.0-32.3	30.0	29.1-30.8	1.2	–0.3 to 2.6	0.12
IVS (mm)	10.4	10.0-10.9	10.3	10.0-10.6	0.2	–0.4 to 0.7	0.55
PW (mm)	10.6	10.0-11.1	10.3	9.9-10.7	0.2	–0.4 to 0.9	0.47
EF (%)	62.2	60.0-64.4	61.6	59.9-63.2	0.6	–2.1 to 3.3	0.65
LAVI (ml/m^2)	34.1	31.1-37.2	31.3	28.9-33.7	2.8	–1.1 to 6.7	0.16
Mitral inflow E (cm/s)	67.8	63.7-71.9	70.2	67.0-73.3	–2.3	–7.5 to 2.8	0.37
Dt (ms)	239	223-256	244	231-256	–4	–26 to 17	0.68
EA ratio	0.93	0.87-1.00	0.98	0.93-1.03	–0.04	–0.13 to 0.04	0.30
IVRT (ms)	112	103-121	109	102-115	4	–7 to 15	0.51
Ee' ratio	9.1	8.3-9.9	9.0	8.4-9.6	0.1	–1.0 to 1.1	0.89
Right heart measurements							
RV dimension (mm)	33.7	32.1-35.3	33.6	32.4-34.8	0.1	–1.9 to 2.1	0.94
TAPSE (mm)	21.2	20.0-22.4	22.8	21.9-23.7	–1.6	–3.1 to –0.1	0.036
RV's (cm/s)	12.4	11.4-13.4	12.1	11.3-12.8	0.3	–0.9 to 1.6	0.59
TR gradient (mmHg)	21.4	19.4-23.4	21.3	19.8-22.8	0.1	–2.3 to 2.6	0.91
RV Ee'ratio	4.47	3.93-5.01	4.29	3.89-4.70	0.18	–0.50 to 0.85	0.61

AI: Aromatase inhibitors; LVEDD= left ventricular end diastolic diameter; LVESD: left ventricular end systolic diameter; IVS: interventricular septum thickness; PW: thickness of left ventricle's posterior wall; EF: ejection fraction; LAVI: left atrial volume indexed; Mitral inflow E: first peak of diastole, active filling; Dt: deceleration time during diastole; EA ratio: ratio of diastolic peaks E and A; IVRT: isovolumic relaxation time; Ee'ratio: ratio of early transmitral flow velocity (E) to early diastolic velocity of the mitral valve annulus (e); RV: right ventricle; TAPSE: tricuspid annular plane systolic excursion; RV's: systolic tissue doppler measurement of right ventricle's free wall; TR gradient: tricuspid regurgitation maximal gradient; RV Ee'ratio: ratio of early tricuspid inflow to annular diastolic velocity.

In the non-AI users (n=38), TAPSE was reduced from a baseline value of 24±4 mm by 1.4 mm (95% CI=0.3-2.4 mm) (p=0.013). The decrease in TAPSE was significantly greater among the AI users compared with the non-AI users given that the mean baseline-adjusted difference after RT between AI-users and non-AI users was −1.6 mm (95% CI=−3.1 to −0.1, p=0.04) (Table IV).

RT induced no significant changes in LV systolic function in either group (Table III). In contrast, the LV diastolic measurements changed among the AI users. At the end of RT, the mitral inflow E-wave had decreased from 75±16.5 to 69.2±13.5 cm/s (mean decrease 5.77 cm/s, 95% CI=9.71-1.83 cm/s) (p=0.006) further accompanied by an increase in isovolumetric relaxation time from 101±25 to 111±23 ms (mean=10.1 ms, 95% CI=−2.9-23.1) (p=0.12). In the non-AI users, the changes observed were non-significant in mitral inflow E-wave (from 72.1±13.4 to 69.4±15.1 cm/s) and in isovolumetric relaxation time (from 106±25 to 109±22 ms) (Table III.).However, after RT, the baseline-adjusted differences between groups were non-significant for all LV diastolic variables (Table IV).

ECG. All patients displayed sinus rhythms in the ECG recordings, with no signs of atrial or ventricular abnormalities. RT caused moderate T-wave alterations in 6/22 (27%) patients in the AI group and 14/38 (37%) in the non-AI group (p=0.45).

Discussion

In this study, we demonstrated for the first time that concurrent use of AIs during left-sided adjuvant breast cancer RT impaired RV systolic and LV diastolic functions measured with echocardiography greater than RT alone. Our results represent alterations induced exclusively by RT alone or the combination of AI and RT, given that all our patients were chemotherapy naïve.

Effect on cardiac functions. TAPSE is a measurement of the RV's longitudinal contractibility and a marker of the RV's systolic function. Decreases in TAPSE correlate with poorer survival and worse prognosis in different cardiovascular diseases (16, 17). In our patients, the concurrent use of AIs

during RT induced a more pronounced decline in TAPSE than RT alone. Whether this observed change is clinically relevant and irreversible remains to be seen in further follow-up of these patients.

Minor functional changes in LV diastolic functions in both groups were observed and these changes were more pronounced in the AI group. Mitral inflow E-wave was significantly reduced in patients using AI and this effect was accompanied by a non-significant increase in isovolumetric relaxation time. These functional changes were not secondary to significant changes in LV hemodynamics as the left atrial volume indexed and the ratio of early transmitral velocity to early diastolic velocity of the mitral valve annulus remained unchanged during RT. No significant changes in LV systolic function were observed, best represented by the ejection fraction. It is noteworthy that diastolic dysfunction, in general, precedes systolic dysfunction and can also cause cardiac morbidity presented clinically as dyspnea on exertion and fatigue (18).

Minor myocyte damage was apparent in 17-20% of patients at the end of RT, as reflected by elevated troponin values. AI use did not statistically influence this finding in our patients. BNP secretion, which serves as a marker of elevated cardiac pressure or overload, did not significantly change during RT among our patients. However, marked variation in the baseline BNP levels, which interferes with the interpretation of changes and differences between groups, was observed.

Mechanisms of RT-induced cardiac changes. Given that the mean radiation doses to the hearts of our patients were relatively low (3.1 Gy), the radiation-induced damage to the myocardium is thought to be primarily attributed to changes in the microvasculature (19). Alterations in the micro-environment begin with initial inflammation and macrophage activation. These alterations progress to increases in various cytokines (*e.g.* transforming growth factor beta, tumor necrosis factor alpha, interleukin 6) that cause microvessel clotting within weeks. These effects eventually progress to decreased microvessel density, fibrinogen formation in vessel walls and ultimately increased fibrosis in cardiac walls and valves. This process is reviewed in detail by Stewart (19).

Estrogen, in general, protects premenopausal women from cardiac diseases. Although large randomized trials, such as the Women's Health Initiative (20), have failed to identify any benefit of estrogen replacement therapy after menopause, estrogen seems to benefit cardiovascular health in perimenopausal women (21). In patients with breast cancer, tamoxifen induces beneficial cardiac effects compared with letrozole (9). There are no data available concerning the effects of AI use alone on cardiac functions.

Estrogen levels were significantly reduced in our patients who were administered AI. Low levels of circulating estrogen potentially led to reduced repair of RT-induced damage in the heart *via* the ERβ-mediated pathway. ERβ is expressed at higher levels than ERα in vascular endothelium and the heart. Interestingly, ERβ receptor expression in tissues increases during RT, and this increase may regulate the healing processes after RT-induced damage (22). Similar increases in ERβ receptors in blood vessels have been documented after vascular injury (23). Furthermore, ERβ plays an important role in the renin-angiotensin system (RAS) in the development of fibrosis. ERβ stimulation decreases the activation of angiotensin II (ATII) receptors, thereby leading to diminished fibrinogenesis (24). These findings may partly explain the previously observed synergy between AI and RT (10, 11).

As alterations in RAS activity play a major role in radiation injury, angiotensin-converting enzyme (ACE) and ATII receptor blockers (ARB) were shown to prevent radiation-induced damage in other tissues, *e.g.* kidneys (25). In this study, only 6/22 patients in the AI group and 8/38 in the non-AI group were administered ACE/ARB as contiguous medication; thus, no conclusions regarding the potential benefits of these drugs can be drawn from this study.

Limitations. The two study groups were similar in terms of breast cancer-specific prognostic factors, age, menopause status, irradiated area and RT doses to the heart and lungs. In addition, the baseline echocardiographic measurements and cardiovascular risk factors did not differ between the groups. As this was an observational, non-randomized study, the groups were not equal in size. The women in the AI group were more obese than those in the non-AI group. Obesity may lead to alterations in RT response by other metabolic and hormonal mechanisms. A decrease in estrogen level itself might also cause alterations in cardiac functions. Furthermore, these results are primarily applicable to letrozole and cannot be extrapolated to patients treated with anastrozole or exemestane (only one patient). Two patients with concurrent tamoxifen use were included in the non-AI group, and no conclusions can be drawn about the possible benefits or detriments of this drug used concomitantly with RT.

Patients with right-sided breast cancer were not included as a reference group, as the cardiac radiation dose is not null: In our patients with right-sided breast cancer (n=26), the average maximum cardiac dose was 5.6±3.4 Gy and the mean cardiac dose 0.9±1.0 Gy (unpublished data).

Clinical implications. The concurrent use of AIs during adjuvant RT is common in many cancer Centers, based on previous results (12, 13). Sequential use appears to be as effective as concurrent use in terms of progression-free survival (26), however, long-term observational data are lacking. As patients with early-stage breast cancer have excellent cancer-specific prognosis, any possible detrimental

side-effects of cancer treatments must be balanced against potential benefits. RT-induced cardiac toxicity is a serious threat and all possible means must be used to reduce this risk. Newer RT techniques, such as the deep-inspiration breath-hold technique, significantly reduce the cardiac radiation dose. Nevertheless, the patient's baseline and other treatment-derived cardiac risk factors should be considered during treatment planning.

We observed that even relatively low cardiac RT doses induced measurable changes in cardiac functions, which can be detected in conventional 2D echocardiographic examinations at the end of RT. These changes were more pronounced in patients with concurrent use of AIs. A further follow-up of these patients will clarify whether these acute changes are reversible or progressive with time.

Conclusion

The concurrent use of AI during RT for left-sided breast cancer led to a more pronounced change in RV systolic function and LV diastolic functions compared to RT alone in chemotherapy-naive women. Whether these early changes in cardiac function impact the long-term prognosis of the patients remains to be established. Further follow-up of these patients and additional studies are warranted to confirm this finding.

Conflict of Interest

None to declare.

Acknowledgements

The Authors wish to thank Mrs. Tuija Poussa for the statistical analyses. The Authors also thank research nurses Virpi Räsänen, Hanna Näppilä and Emmi Vettenranta for their expert assistance during the study. Financial support was provided by the Pirkanmaa Hospital district Science center, Elna Kaarina Savolainen and Seppo Nieminen funds and Finnish cultural foundation, Pirkanmaa regional fund and Finnish Foundation for Cardiovascular Research.

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Received November 11, 2014
Revised November 30, 2014
Accepted December 4, 2014

Skyttä et al. Radiation Oncology (2015) 10:141
DOI 10.1186/s13014-015-0436-2

RADIATION ONCOLOGY

RESEARCH Open Access

Troponin T-release associates with cardiac radiation doses during adjuvant left-sided breast cancer radiotherapy

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Abstract

Background: Adjuvant radiotherapy (RT) for left-sided breast cancer increases cardiac morbidity and mortality. For the heart, no safe radiation threshold has been established. Troponin T is a sensitive marker of myocardial damage. Our aim was to evaluate the effect of left-sided breast cancer RT on serum high sensitivity troponin T (hscTnT) levels and its association with cardiac radiation doses and echocardiographic parameters.

Methods: A total of 58 patients with an early stage, left-sided breast cancer or ductal carcinoma in situ (DCIS) who received adjuvant breast RT without prior chemotherapy were included in this prospective, non-randomized study. Serum samples were taken before, during and after RT. An increase of hscTnT >30 % was predefined as significant. A comprehensive 2D echocardiograph and electrocardiogram (ECG) were performed before and after RT. Dose-volume histograms (DVHs) were generated for different cardiac structures.

Results: The hscTnT increased during RT from baseline in 12/58 patients (21 %). Patients with increased hscTnT values (group A, $N = 12$) had significantly higher radiation doses for the whole heart ($p = 0.02$) and left ventricle ($p = 0.03$) than patients without hscTnT increase (group B, $N = 46$). For the left anterior descending artery (LAD), differences between groups A and B were found in volumes receiving 15 Gy ($p = 0.03$) and 20 Gy ($p = 0.03$) Furthermore, after RT, the interventricular septum thickened ($p = 0.01$), and the deceleration time was prolonged ($p = 0.008$) more in group A than in group B.

Conclusions: The increase in hscTnT level during adjuvant RT was positively associated with the cardiac radiation doses for the whole heart and LV in chemotherapy-naive breast cancer patients. Whether these acute subclinical changes increase the risk of excessive long-term cardiovascular morbidity or mortality, will be addressed in the follow-up of our patients.

Keywords: Radiotherapy, Breast cancer, Cardiotoxicity, High sensitivity cardiac troponin T

Background

Postoperative radiotherapy (RT) for breast cancer is an essential part of adjuvant cancer treatment. RT reduces the risk of local recurrence by 50 % and the risk of breast cancer mortality by 16 % [1]. However, left-sided RT, especially, has been shown to induce excess cardiovascular mortality and morbidity [1–4]. In a retrospective study, Darby et al. showed that the risk of major cardiac events in patients with left-sided breast cancer increased by 7.4 % for each increase of 1 Gy of radiation to the heart. Furthermore, prior cardiovascular diseases, such as hypertension, may further increase the risk of radiation induced cardiac damage [4].

Radiation induced cardiac changes have been demonstrated with echocardiographic strain rate imaging [5], myocardial perfusion scintigraphy [6, 7] and single photon emission tomography [8]. In contrast, no changes in the left ventricle (LV) systolic function were detected in basic echocardiographic measurements [5, 7]. Majority of patients in these studies also received prior or concurrent chemotherapy [5–8], which is known to be associated with cardiotoxicity [9, 10]. High sensitivity cardiac troponin T

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(hscTnT) is able to detect minor myocardial damage during RT [5, 11]. The mechanism of this acute damage is thought to evolve from negative changes in the microvasculature during RT [12].

In this study, we included only chemotherapy-naive breast cancer patients to exclude chemotherapy-induced prior cardiotoxicity. The patients were studied prospectively using a cardiac biomarkers, comprehensive 2D echocardiography and 12-lead electrocardiogram (ECG) before and after adjuvant RT to detect subclinical cardiac changes. Furthermore, dose volume histograms (DVHs) for different cardiac structures were generated and correlated with serum markers and echocardiographic measurements.

Methods

Patient population

This single centre, prospective, observational clinical study included 60 eligible female patients with early stage left-sided breast cancer or DCIS. All patients were treated with adjuvant conformal 3D RT after breast conserving surgery ($n = 59$) or mastectomy ($n = 1$) without axillary or supraclavicular lymph node RT. Two patients were excluded from final analysis due to missing serum samples. Patients with prior adjuvant chemotherapy were excluded. Other exclusion criteria were age over 80 years, dialysis, recent acute myocardial infarction, symptomatic heart failure, chronic atrial fibrillation, pacemaker therapy and severe lung disease. The local ethical committee approved the protocol and all participants signed informed consent before study enrollment. The study was conducted from June 2011 to May 2013.

Radiation therapy

All patients had 3D computer tomography (CT) treatment planning with Philips Big Bore CT (Phillips Medical Systems, Madison, WI, USA) or Toshiba Aquilion LB (Toshiba Medical System, Tokyo, Japan) on a breast board in the supine position with their arms above their heads. CT slices (3 mm thickness) were used without intravenous contrast. Scanning was performed during free breathing in 56 patients, whereas 2 patients were scanned and treated using the voluntary deep inspiration breath hold (vDIBH) technique, which was implemented in our unit in April 2013 as part of clinical practice. In vDIBH, the breathing cycle was monitored with Varian real time position management (RPM) system (Varian Medical Systems, Palo Alto, CA, USA). Treatment contouring and planning were done with Eclipse v.10 system (Varian Medical Systems, Palo Alto, CA, USA). Planning target volume (PTV) covered the remaining breast tissue in 57 patients and the chest wall in one patient (mastectomy) with sufficient margins to account for the inter- and intrafraction movements (5–8 mm in our unit).

Treatment doses were either 50 Gray (Gy) in 2 Gy fractions with or without additional boost (10–16 Gy, 5–8 fractions) to tumor bed or 42.56 Gy in 2.66 Gy fractions (hypofractionation) over 3.5 weeks. Tangential photon fields were used in 57 patients and chest wall in one patient with mastectomy was treated with electron beams. Doses were calculated with anisotropic analytical algorithm (AAA v.10, Varian Medical Systems, Palo Alto, CA, USA) for photons and Electron Monte Carlo algorithm (eMC v.11, Varian Medical Systems, Palo Alto, CA, USA) for electrons. Dose volume histograms (DVH) of different structures were generated for each patient. To account for the different dosing schedules, an α/β-ratio of 3 was used for heart and lung to calculate 2 Gy equivalent doses.

The heart, LV and LAD were contoured from the treatment planning CT scans as suggested by Feng et al. [13]. All cardiac structures were contoured by the same radiation oncologist (TS).

Serum biomarker analysis

High sensitivity cardiac troponin T (hscTnT, ng/l) and B-type natriuretic peptide (BNP, ng/l) were analyzed from serum samples taken before, after two (hypofractionated RT) or three (conventional RT) weeks of treatment and at the last day of RT. As the lowest detection limit of hscTnT was 5 ng/l, the values below this (<5 ng/l) were estimated to be 4 (lowest detection limit (LOD)/$\sqrt{2}$) when calculating the percentage increase from the baseline value [14]. A predefined increase of >30 % from the baseline was considered to be clinically important according to the study protocol. The patients were divided into two groups based on their hscTnT change: group A with a hscTnT increase more than 30 % from baseline during RT and group B without a hscTnT increase.

Total cholesterol levels (mmol/l) were measured at baseline under fasting conditions.

Echocardiographic examinations

A comprehensive echocardiography study and 12-lead ECG were performed at the baseline and at the completion of RT. All echocardiography examinations were performed using the same ultrasound machine (Philips iE33 ultrasound system, Bothell, WA, USA) and a 1–5 MHz matrix-array X5-1 transducer by the same cardiologist certified by European Association of Echocardiography for adult transthoracic echocardiography. All images were acquired at rest. Subcostal imaging was performed in the supine position, while other imaging was performed with the patient in the lateral decubitus position with simultaneous superimposed ECG. Doppler recordings were acquired at the end of expiration during shallow breathing. Images were stored digitally for use with offline analysis software (Excelera, Philips, Koninklijke, Netherlands; Qlab, Philips, Bothell, USA). Echocardiographic measurements

were performed in a standardized manner according to the European guidelines [15].

Statistical analysis

Data are expressed as means ± standard deviation (SD) for normally distributed continuous variables and as medians with inter quartile range (IQR) for variables with non-normal distributions. The study groups were compared using the *t*-test for independent samples or the Mann–Whitney *U* test. Friedman's analysis of variance was used for repeated measures of non-normal variables and the Wilcoxon signed ranks test was used to compare the hscTnT before RT vs. hscTnT after RT. Categorical data are expressed as numbers (%) of subjects. The Chi-squared test was used for categorical variables and the Fisher's exact test was used when appropriate. The related samples Cochran's Q test was used to study the change in dichotomous variables measures more than twice. Area under curve (AUC) was calculated using the trapezium rule in order to summarize the information from DVH. All the tests were two-sided and a p value <0.05 was considered statistically significant. Analysis was performed using IBM SPSS Statistics for Windows (version 21.0, Armonk, NY, USA, IBM Corp.).

Results

Baseline characteristics of the patients are presented in Table 1. The baseline variables (e.g. age, body mass index, cholesterol and hormonal use) were similar between the two groups A and B based on the troponin increase. However, in the group A patients tended to have more hypertension ($p = 0.31$) and less thyroid hormone supplementation ($p = 0.19$).

High sensitivity cardiac troponin T

In the whole study population, hscTnT was detectable (≥5 ng/l) in 25 (43 %) patients before RT, in 28 (48 %) at 3 weeks and in 30 (52 %) patients after RT. The serum samples after RT were taken at the last day of RT (median 0, range −1 – +3 day).

Median (IQR) hscTnT for the whole study population was 4 (4–6) ng/l before RT, 4 (4–6) ng/l at 3 weeks and 5 (4–7) ng/l after RT. The difference in hscTnT level before RT and after RT was not significant for the whole population ($p = 0.08$). However, hscTnT increased over 30 % from the baseline value in 12/58 (21 %) patients constituting the group A. In these 12 patients, hscTnT was detectable in 3 (25 %) before RT, in 9 (75 %) at 2–3 weeks and in 12 (100 %) patients after RT. The median (IQR) values at these time points were 4 (4–4.5)ng/l, 5.5 (4.5-6)ng/l and 7 (7–9.5)ng/l, respectively (Fig. 1). In the hscTnT stable group B ($N = 46$,) hscTnT was detectable in 22 (48 %) at the beginning, 19 (41 %) at 2–3 weeks and 18 (39 %) after RT with median (IQR) values of 4(4–7), 4(4–6) and 4(4–6) ng/l, respectively.

B-type natriuretic peptide

In the whole study population BNP levels were 58 (37–124), 69 (42–135) and 74 (34–126) mmol/l before RT, at 2–3 weeks and after RT, respectively ($p = 0.72$). The BNP change between the first and the last time point was −5 (−26 to 5) mmol/l in the group A vs. 5 (−14 to 22) mmol/l in the group B ($p = 0.33$).

Cardiac doses

The DVH curves for the both groups are presented in Fig. 2. The AUC for heart and left ventricle was significantly higher in the hscTnT-positive group A compared with group B ($p < 0.05$, Table 2). The same trend was seen for the LAD ($p = 0.08$). In addition to AUC, some relevant dose-volume parameters (volume of structure receiving 5 Gy radiation dose = V5 and similarly V10, V15, V20 and V30) were separately tested (Fig. 2 and

Table 1 Patient baseline characteristics. Results are presented as mean ± SD for normally distributed continuous variables, median (inter-quartile range) for non-normal variables and n (%) for categorical variables

	All ($N = 58$)	Group A ($N = 12$)	Group B ($N = 46$)	*p*-value*
Age	63 ± 6	65 ± 6	63 ± 7	0.37
BMI, kg/m^2	27 ± 4	27 ± 5	27 ± 4	0.76
Hypertension	20 (34 %)	6 (50 %)	14 (30 %)	0.31
ACE or ARB	14 (24 %)	4 (33 %)	10 (22 %)	0.46
Beta-blocker	7 (17 %)	3 (25 %)	4 (9 %)	0.15
Calcium channel blocker	4 (7 %)	2 (17 %)	2 (4 %)	0.19
CAD	1 (2 %)	0 (0 %)	1 (2 %)	1.00
Statin use	13 (22 %)	3 (25 %)	10 (22 %)	1.00
Diabetes	4 (7 %)	1 (8 %)	3 (7 %)	1.00
Hypothyreosis	8 (14 %)	0 (0 %)	8 (17 %)	0.19
Current smoking	8 (14 %)	2 (17 %)	6 (13 %)	0.66
Hormonal therapy	23 (40 %)	4 (33 %)	19 (41 %)	1.00
AI	22 (38 %)	5 (42 %)	17(37 %)	0.75
Tamoxifen	2 (3 %)	0 (0 %)	2 (4 %)	1.00
Troponin T, ng/l	4 (4–6)	4(4–4.5)	4 (4–7)	
Cholesterol, mmol/l	5.6 ± 1.1	5.5 ± 0.9	5.6 ± 1.1	0.90
BNP, ng/l	58 (37–124)	78 (33–123)	58 (41–125)	0.72

BMI body mass index, *ACE* angiotensin converting enzyme, *ARB* angiotensin II receptor blockers, *CAD* coronary artery disease, *Statin use* hypercholesterolemia with statin use, *Diabetes* blood glucose lowering diabetic medication, *ASA* low dose daily acetylsalicylic acid, *Hypothyreosis* continuous thyroid hormone supplementation, *AI* aromatase inhibitor, *BNP* b-type natriuretic peptid

* Independent samples *t*-test was used for normally distributed variables and Mann–Whitney *U* test for non-normal variables; Fisher's exact test was used for categorical variables

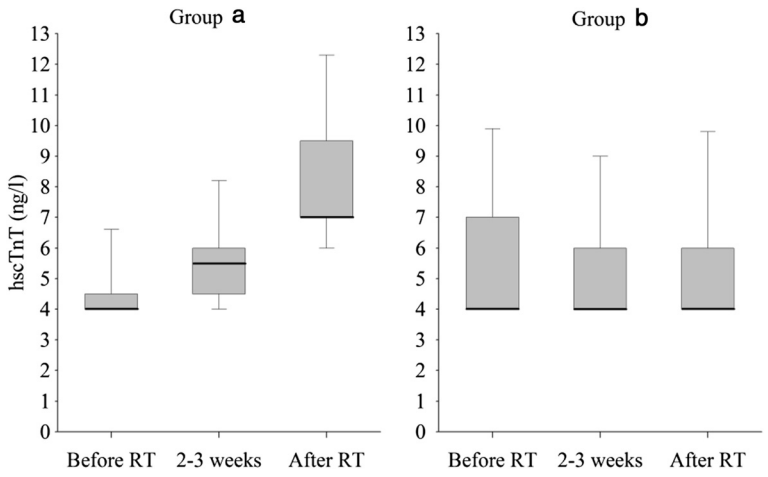

Fig. 1 Box plot figures to describe high sensitivity cardiac troponin T (hscTnT) before RT, at weeks 2–3 and after RT in patients with increased hscTnT release (Group **a**, $N = 12$) and in patients without hscTnT release (Group **b**, $N = 46$). Borders of the box represent the upper and lower quartiles, bold lines the medians and error bars above and below the box represent the 90th and 10th percentiles

Table 2). In the heart and left ventricle, the difference between groups was significant in all those Gy points from 5 to 20 Gy, but in the LAD the difference between groups was significant only at dose levels of 15 and 20 Gy.

Echocardiographic findings

In group A, the increase in hscTnT was accompanied by minor changes in the echocardiographic measurements as presented in Table 3. Measured before and after RT, the interventricular septum thickened ($p = 0.01$) accompanied with a change in LV diastolic function as the deceleration time increased ($p = 0.008$) among group A more than in group B. In other LV diastolic parameters, the mitral E-peak decreased in both groups. The decrease in the LV end diastolic and end systolic diameters in the group A were not statistically significant compared with group B ($p = 0.06$ and $p = 0.11$, respectively).

ECG

Normal sinus rhythms were present in all ECG recordings with no signs of atrial or ventricular abnormalities. RT caused moderate T-wave alterations in 3/12 (25 %) recordings among group A and 13/46 (28 %) among group B ($p = 0.82$).

Discussion

We demonstrated that high sensitivity cardiac troponin T levels increased during the whole breast adjuvant RT in every fifth patient. In addition, the increase in the hscTnT release associated positively with cardiac radiation doses and with minor changes in LV's measurements suggesting that RT caused subclinical myocardial damage.

High sensitivity cardiac TnT release

Cardiac troponin T is a well-established biomarker of cardiac damage in myocardial infarction due to ischemic heart disease. Moreover, it can detect cardiac damage in other clinical conditions such as heart failure [16, 17] and LV hypertrophy [18, 19]. The hscTnT values in healthy individuals have varied in different studies. The 99th percentile differed from 12 ng/l [20] to 20 ng/l [21] and is largely shown to be influenced by patient's age, gender and comorbidities [22]. By excluding patients with underlying cardiac diseases, it is estimated that the median values (IQR) of hscTnT for women <55 years as well as <75 years are <3 (<3 - < 3) ng/l and the upper normal 99th percentile limit is 3.4 ng/l for women <55 years and 11.4 ng/l for women <75 years [22]. The weekly within person variation of hscTnT in healthy individuals is estimated to be 8 % [23] -32 % [24]. Diurnal changes in hscTnT are also observed with a decline in values by 0.8 % per hour from 8:30 to 14:30 [23]. Based on these results, a relevant increase in hscTnT levels during RT was predefined to exceed 30 % from individual baseline in this study. In our patients, the serum samples at the baseline and at the end of the RT were taken in the morning within a 2 h maximum time difference in 55 patients. In the remaining three patients (all in the group B), the time difference was 2.1, 4 and 5 h. The hscTnT levels in the group A increased 40 %–325 % from baseline and, therefore, the change cannot be explained by normal weekly or diurnal variation.

In cancer patients, elevated troponin T and I levels have been reported after various chemotherapy regimens [25–27]. In addition, the elevation of TnT during or shortly after chemotherapy correlated with long term negative changes in echocardiography [25, 27]. The

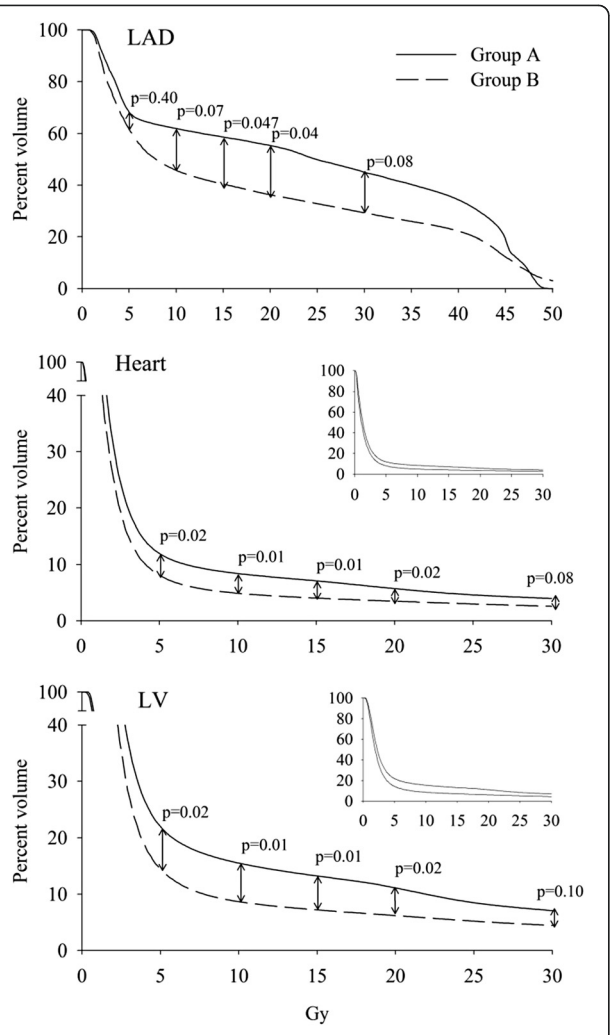

Fig. 2 Dose volume histogram (DVH) curves for left anterior descending artery (LAD), heart and left ventricle (LV) in patients with increased high sensitivity cardiac troponin T (hscTnT) release (Group A, *N* = 12) and in patients without hscTnT release (Group B, *N* = 46)

Table 2 Radiation doses to cardiac structures in patients with hscTnT increase >30 % from baseline during RT (Group A) vs. patients without hscTnT increase (Group B)

Cardiac structure	Group A (N = 12)	Group B (N = 46)	*p* value*
Heart			
Dmean (Gy)	4.0 ± 1.8	2.8 ± 1.3	0.02
Dmax (Gy)	48.9 ± 5.3	43.9 ± 11.0	0.13
V5 (%)	12.0 ± 6.2	8.1 ± 4.6	0.02
V10 (%)	8.4 ± 5.1	4.9 ± 3.4	0.01
V15 (%)	7.1 ± 4.4	4.0 ± 3.1	0.01
V20 (%)	5.7 ± 3.3	3.5 ± 2.8	0.02
V30 (%)	4.0 ± 2.6	2.6 ± 2.3	0.08
AUC (%.Gy)	403 ± 172	291 ± 134	0.02
LV			
Dmean (Gy)	6.7 ± 3.3	4.5 ± 2.6	0.02
Dmax (Gy)	47.8 ± 5.8	41.0 ± 12.2	0.07
V5 (%)	22.0 ± 12.3	14.5 ± 9.2	0.02
V10 (%)	15.5 ± 10.2	8.6 ± 7.0	0.01
V15 (%)	13.2 ± 9.2	7.2 ± 6.4	0.01
V20 (%)	11.1 ± 7.7	6.2 ± 5.9	0.02
V30 (%)	7.1 ± 5.4	4.4 ± 4.7	0.10
AUC (%.Gy)	668 ± 333	468 ± 260	0.03
LAD			
Dmean (Gy)	23.8 ± 10.1	17.5 ± 10.8	0.07
Dmax (Gy)	43.4 ± 11.8	37.9 ± 15.8	0.27
V5 (%)	68.3 ± 24.2	61.7 ± 24.1	0.40
V10 (%)	61.9 ± 26.1	45.7 ± 27.7	0.07
V15 (%)	58.6 ± 26.3	40.0 ± 28.0	0.047
V20 (%)	55.4 ± 26.3	36.2 ± 28.3	0.04
V30 (%)	45.0 ± 25.3	29.3 ± 27.8	0.08
AUC (%.Gy)	2422 ± 1053	1793 ± 1097	0.08

hscTnT high sensitivity cardiac troponin T, *RT* radiotherapy, *Dmean* mean radiation dose to the structure, *Dmax* maximal point radiation dose in the structure, *V30/20/10/5* the volume of structure receiving 30Gy/20Gy/10Gy/5Gy dose, *AUC* area under the curve, *LV* left ventricle, *LAD* left anterior descending coronary artery
*Independent samples *t*-test

effects of adjuvant breast cancer RT on TnT levels were not detected in earlier studies with less sensitive troponin methods [28, 29]. In a study by Nellessen et al. [11], thoracic RT for breast or lung cancer in 23 patients increased troponin levels measured during and at the end of RT. It is, however, noteworthy that the majority (16/23) of these patients had received prior chemotherapy.

Our patients were, as described earlier, previously untreated by chemotherapy. Therefore, these observed changes represent the radiation induced damage to myocardium. The RT-induced harmful process in the heart is thought to evolve from the clotting of myocardial microvessels and the subsequent hypoxia leading to myocyte damage [12]. The absolute levels of hscTnT at the end of RT in our patients were below the clinically used threshold for myocardial infarction (50 ng/l). Nevertheless, measurable (>4 ng/l) troponin levels indicate worse cardiac long term prognosis in other clinical conditions [17, 18].

Cardiac radiation doses

In the previous studies, the mean cardiac radiation dose has varied from 4.9 Gy (retrospective analysis) [4] to 9 Gy (prospective analysis) [5]. In our study, the mean heart dose in the group A was significantly higher (4.0 ± 1.8 Gy) than in the group B (2.8 ± 1.4 Gy). As presented in Table 2 and Fig. 2, also the measured dose-volume points for the whole heart were significantly different between the groups. In group A, larger volumes of the

Table 3 Echocardiographic measurements at baseline(before RT) and the change from baseline measured at the end of RT in patients with increased hscTnT release (group A) and in patients without hscTnT release (group B)

	Group A (N = 12)		Group B (N = 46)		
	Before RT	Change	Before RT	Change	P*
LV dimensions					
LVEDD (mm)	44.0 (42.0 - 45.5)	-2.0 (-3.5 - 0.0)	46.0 (44.0 - 47.0)	-0.5 (-2.0 - 2.0)	0.06
LVESD (mm)	30.0 (27.5 - 31.0)	-1.5 (-4.5 - 1.5)	31.0 (29.0 - 33.0)	0.0 (-2.0 - 2.0)	0.11
IVS (mm)	11.0 (8.5 - 11.5)	1.0 (0.0 - 1.0)	10.0 (9.0 - 11.0)	0.0 (0.0 - 1.0)	0.01
PW (mm)	10.0 (9.5 - 11.0)	1.0 (0.0 - 1.0)	10.0 (9.0 - 11.0)	0.0 (-1.0 - 1.0)	0.50
RV functions					
Tapse (mm)	23.5 (21.0 - 25.0)	-1.0 (-3.5 - 0.5)	24.5 (21.0 - 28.0)	-2.0 (-4.0 - 0.0)	0.63
RV s' (cm/s)	11.3 (9.5 - 14.1)	-0.2 (-0.6 - 1.5)	12.0 (10.3 - 13.2)	-0.5 (-1.4 - 0.7)	0.34
RV Ee' ratio	4.2 (3.3 - 5.0)	0.7 (-1.6 - 2.5)	3.9 (3.3 - 4.9)	0.1 (-0.3 - 0.8)	0.88
LV functions					
EF (%)	62.5 (60.0 - 64.5)	1.5 (-4.5 - 3.5)	62.0 (58.0 - 65.0)	0.0 (-4.0 - 4.0)	0.87
Mitral E (cm/s)	68.8 (58.5 - 79.0)	-4.3 (-6.0 - 0.3)	74.0 (64.7 - 84.8)	-3.8 (-10.8 - 3.9)	0.94
Dt (ms)	204 (192 - 236)	32 (9–64)	230 (208 - 261)	0 (-29 - 21)	0.008
EA ratio	0.8 (0.7 - 0.9)	0.0 (-0.1 - 0.1)	1.0 (0.8 - 1.1)	0.0 (-0.1 - 0.1)	0.99
IVRT (ms)	113 (89 - 122)	3 (-6 - 21)	103 (90 - 123)	4 (-10 - 21)	0.92
Ee' ratio	8.9 (6.9 - 10.9)	0.6 (-2.0 - 1.5)	9.3 (7.4 - 10.6)	-0.3 (-1.4 - 0.6)	0.30

LV left ventricle, *LVEDD* left ventricular end diastolic diameter, *LVESD* left ventricular end systolic diameter, *IVS* interventricular septum thickness, *PW* thickness of left ventricle's posterior wall, *RV* right ventricle, *tapse* tricuspid annular plane systolic excursion, *RV's* systolic tissue doppler measurement of right ventricle's free wall, *RV Ee'ratio* the ratio of early tricuspid inflow to annular diastolic velocity, *EF* ejection fraction, *Mitral E* first peak of diastole, active filling, *Dt* deceleration time during diastole, *EA ratio* ratio of diastolic peaks E and A, *IVRT* isovolumic relaxation time, *Ee'ratio* ratio of early transmitral flow velocity (E) to early diastolic velocity of the mitral valve annulus (e)
*p value between groups

cardiac structures received more radiation. These findings show, that increasing cardiac doses associate with myocyte damage measured by hscTnT release. In animal models, even doses as low as 2 Gy for the whole heart induced cardiac remodelling and fibrinogenesis [30]. In a retrospective study on breast cancer patients, the risk of major cardiac event increased linearly with every increasing Gy for the heart [4]. Absolutely safe cardiac doses cannot be extrapolated either from our results or from previous data. Furthermore, it is still unclear, whether a low dose for a larger cardiac volume is more detrimental than a higher dose for a smaller volume. However, our DVH data suggest that if the mean dose for the whole heart is under 2 Gy at least the risk of acute myocyte damage is low. This can easily be reached in majority of node-negative patients, if vDIBH (own institutional experience) or Active Breathing Control (ABC) [31] is utilized.

Increasing doses to LV correlate with myocardial perfusion defects [6, 7, 32]. In our patients, the mean dose to LV was higher in the group A and the difference in V5 Gy, V10 Gy and V20 Gy was also significant.

The LAD runs in the sulcus between the left and right ventricle. Its anatomical location in relation to chest wall varies from one patient to another. The LAD is, however, generally at the most anterior part of the LV and therefore, the radiation doses to LAD in left sided breast cancer RT can be substantial. In coronary vessels, the acute RT induced damage may later manifest as thickening and fibrosis of the vessel walls and increased local atherosclerosis. The coronary arteries should be considered as a serial-type organ, where the damage to one small part affects the function of whole the unit. In our patients, the DVHs of the LAD differ clearly after 5 Gy and converge only at high doses. The AUC analysis of the LAD did not reach statistical significance due to variation of doses and the limited number of patients.

Echocardiographic changes

LV's ejection fraction (LVEF), a widely used marker of the LV's systolic function in oncology, did not decrease in our patients after RT. This finding is similar than with Lo Q et al. [33], who detected subclinical cardiac dysfunction by 2-D strain imaging in LV after left-sided breast cancer RT, but LVEF remained unchanged. In addition, LVEF can remain within normal limits even in a presence of significant cardiac diastolic dysfunction. This entity of heart failure with preserved ejection fraction (HFpEF) is more common in elderly women and it is thought to have a multi-factorial etiology [34, 35]. It

might share some pathophysiology with radiation induced heart disease such as increased fibrosis markers [36] and proinflammatory changes [37].

Diastolic changes in LV's function were observed in both groups such as lower mitral E peak. As RT induces inflammation and later fibrinogenesis in tissues [12], it is logical that the early cardiac changes would be first present in the diastole, where the impaired relaxation and increased stiffness in ventricular walls cause impaired filling. The prolongation of diastolic deceleration time, a representative of increased ventricular wall stiffness, was apparent after RT in group A accompanied with increased septum thicknesses in contrast to group B. In addition, the LV diameters in both end systole and diastole decreased more in group A than group B- yet this failed to reach statistical significance due to small number of patients. Diastolic dysfunction can cause major cardiac morbidity such as dyspnea in mild exertion, fatigue and fluid retention [38]. These findings further support the value of troponin release as a simple marker of radiation-induced myocardial damage.

Confounding factors and limitations

Hypertension is a known additive risk factor for RT induced cardiotoxicity [4]. In our patients, 50 % of the group A patients had hypertension compared with 30 % in the group B. However, this difference was not significant. It is of interest, that none of the patients using thyroid hormone supplementation had increases in hscTnT levels. There is experimental data on the benefits of thyroid hormone use after myocardial infarction (MI) in rodents [39, 40], and the beneficial effects of high T3 levels on cardiac functions after MI have been described also in humans [41]. This finding is intriguing and should be validated in a larger population as no reliable and clinically relevant subgroup analysis could be performed in our study due to the relatively small number of patients.

Clinical implications

HscTnT is a sensitive marker of acute myocardial damage. In general, greater damage causes a greater troponin release and indicates worse prognosis. In this study, the increases in hscTnT values associated with increased cardiac radiation doses for the whole heart and LV. In daily RT treatment planning, at least the whole heart should be contoured as an organ at risk for left sided breast cancer RT in order to keep the cardiac dose as low as possible. Evolving radiation techniques will help to accomplish the cardiac dose sparing. Patient derived risk factors, such as hypertension, are still important and need special caution and guidance. Echocardiography can measure RT induced subclinical cardiac changes immediately after breast cancer RT and should be used in the later follow up of patients. Likewise, an expert consensus from 2013 recommends routine echocardiography after left sided breast cancer RT for all breast cancer survivors in 5–10 years to screen for radiation induced heart disease [42].

Conclusions

The elevation on hscTnT levels suggests that adjuvant RT causes subclinical myocardial damage in patients with left-sided breast cancer. As the increase in hscTnT associates with increased cardiac radiation doses all efforts should be made to keep the radiation to the heart as low as possible. Whether these acute subclinical changes in echocardiography increase the risk of excessive long-term cardiovascular morbidity or mortality in our patients will be addressed in the later follow-up.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

All authors participated in the study design, coordination and discussed the outcome during the study. TS did the heart contouring, collected the data and performed the RT data analysis with EB. ST did the echocardiographic measurements. PR and ST analyzed the ECGs. TS drafted the manuscript and ST, EB, VV, PR and P-L K-L read, corrected and approved the final manuscript.

Acknowledgements

We wish to thank Mrs. Tuija Poussa for the statistical analyses. The Authors also thank research nurses Virpi Räsänen, Hanna Näppilä and Emmi Vettenranta for their expert assistance during the study. Financial support was provided by the Pirkanmaa Hospital district Science center, Elna Kaarina Savolainen and Seppo Nieminen funds and Finnish cultural foundation, Pirkanmaa regional fund and Finnish Foundation for Cardiovascular Research.

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Received: 13 April 2015 Accepted: 5 June 2015
Published online: 10 July 2015

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