

Long-term cardiac function in breast cancer survivors after multimodal treatment

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Abbreviations

ACE: angiotensin converting enzyme
AI: aromatase inhibitors
AMP: adenosine monophosphate
ANP: atrial natriuretic peptide
ALND: axillary lymph node dissection
AS: Agatston score
BC: breast cancer
BCSs: breast cancer survivors
BCT: breast conserving treatment
BMI: body mass index
BNP: brain natriuretic peptide
CAC-score: coronary artery calcium- score
CCV: cardiac and cerebrovascular events
CI: confidence intervals
CMF: cyclophosphamide, methotrexate, 5-fluoruracil
CRN: Cancer Registry of Norway
CO: cardiac output
⁶⁰Co: cobalt 60
CRP: c-reactive protein
CT: computerized tomography
CX: circumflex
CVD: cardiovascular disease
D5%: dose delivered to 5% of the heart
DD: diastolic dysfunction
DIBH: deep inspiration breath-hold technique
DM: diabetes mellitus
DNA: deoxyribonucleic acid
DVH: dose volume histogram
DVT: deep venous thrombosis
EBCTCG: Early Breast Cancer Trialists' Collaborative Group
ECG: electrocardiogram
EF: ejection fraction
ER: estrogen receptor
FEC: 5-fluoruracil, epirubicin, cyclophosphamide
Fig: figure
FQ: fatigue questionnaire
FS: fractional shortening
FU: fluorouracil
GLS: global longitudinal strain
HADS: hospital anxiety and depression scale
HbA1C: haemoglobin A 1c
HER-2: human epidermal growth factor receptor 2
HF: heart failure
HL: Hodgkin's lymphoma
HR: hazard ratio
HUNT: Health Study of Nord- Trøndelag County
ICD: international classification of diseases
IHD: ischemic heart disease

IMLNs: internal mammary lymph nodes
 IMLNM: internal mammary lymph node metastasis
 IMRT: intensity modulated radiation therapy
 LAD: left anterior descending artery
 LMA: left main coronary artery
 LNM: lymph node metastasis
 LV: left ventricle
 LVD: left ventricular dysfunction
 LVEF: left ventricular ejection fraction
 MCE: major coronary events
 MI: myocardial infarction
 MRI: magnetic resonance imaging
 MRM: modified radical mastectomy
 MV: mega volt
 NBCG: Norwegian Breast Cancer Group
 NRH: The Norwegian Radium Hospital
 NSD: nominal standard dose
 NYHA: New York Heart Association
 OR: odds ratio
 PF: parasternal field
 POF: premature ovarian failure
 RCA: right coronary artery
 RM: radical mastectomy
 RNA: ribonucleic acid
 ROS: reactive oxygen species
 RR: relative risk
 RRHD: radiation related heart disease
 RT: radiotherapy
 RV: right ventricle
 SD: systolic dysfunction
 SNB: sentinel node biopsy
 STV: speckle tracking echocardiography
 SV: stroke volume
 TDI: tissue doppler imaging
 TF: tangential field
 TIA: transient ischemic attack
 TNM: tumor, node, metastasis
 Top: topoisomerase
 UICC: union internationalis contra cancerum
 VD: valvular disease

List of papers

Paper 1:

Long-term cardiac mortality after hypofractionated radiation therapy in breast cancer

Kristin Holm Tjessem, Safora Johansen, Eirik Malinen, Kristin V. Reinertsen, Turi Danielsen, Sophie D. Fossa, Alexander Fossa:

International Journal of Radiation Oncology Biology Physics 2013;87:337-343

Paper 2:

Dose distribution in the heart and cardiac chambers following 4-field radiation therapy of breast cancer: a retrospective study.

Safora Johansen, Kristin Holm Tjessem, Kristian Fosså, Gerhard Bosse, Turi Danielsen, Eirik Malinen, Sophie D. Fosså

Breast Cancer: Basic and Clinical Research 2013;7:41–49

Paper 3:

Coronary calcium score in 12-year breast cancer survivors after adjuvant radiotherapy with low to moderate heart exposure – Relationship to cardiac radiation dose and cardiovascular risk factors.

Kristin Holm Tjessem , Gerhard Bosse, Kristian Fosså, Kristin V. Reinertsen , Sophie D. Fosså, Safora Johansen, Alexander Fosså:

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Paper 4:

Cardiac function in long term breast cancer survivors after multimodal treatment. Comparison to a matched normal population

Gabor Kunszt, Kristin Holm Tjessem, Håvard Dalen MD, Sophie Dorothea Fosså, Kristin Valborg Reinertsen, Alexander Fosså, Svend Aakhus

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1. Background

1.1. Epidemiology, diagnosis, staging, and tumor characteristics

1.1.1 Breast cancer (BC) epidemiology

BC is the most common malignant disease among women in Norway and worldwide. In 2013, 3220 women were diagnosed with BC in Norway (1). The national mammography screening program was initiated in 1995 and has led to increased and earlier identification of BC. Nevertheless, there has been a true increase in BC incidence (1). In addition, the population has increased and the inhabitants are older, resulting in an increasing prevalence of BC in Norway (1).

The mean age at BC diagnosis in Norway is 59 years (1). In recent years, BC mortality has decreased, most likely due to earlier detection and improved treatment (2;3). The 5-year relative survival rates for all stages of BC have increased from 87.7% in 2003-2007, to 89.0% in 2009-2013 (1). The prognosis is closely related to the stage of the disease at diagnosis, 5 year survival for women with stage I disease is 98.9 %, whereas for those with disseminated disease (stage IV) it is 25.1% (1).

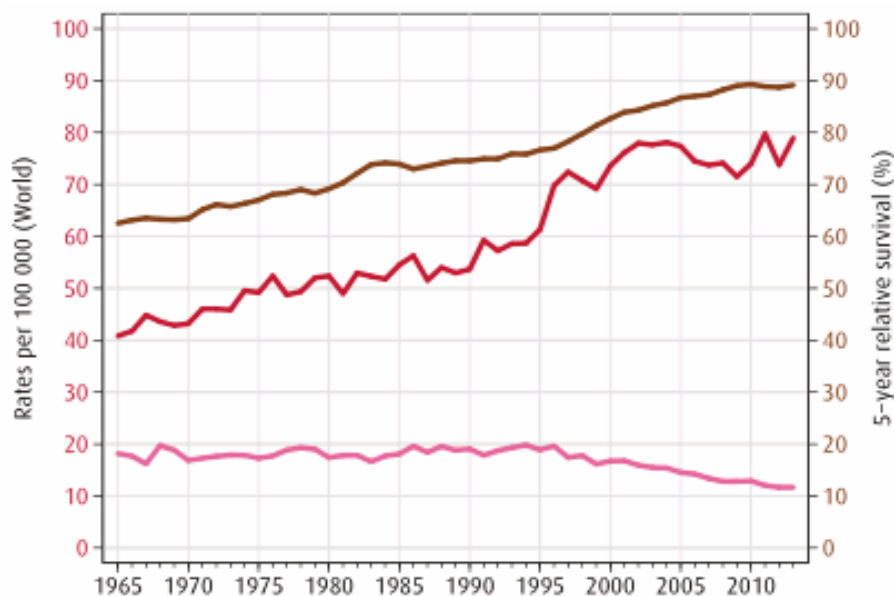


Figure (fig) 1: The incidence, mortality rate and 5-year relative survival for BC patients 1965-2013 (1)

1.1.2 Risk Factors

The risk factors for development of BC are diverse and include female sex and increasing age as well as high socio-economic status (4). It is estimated that around 25% of BCs are due to hereditary factors. 5 % of all BCs are due to genetic germline mutations in the genes BRCA1, BRCA2, TP53, PTEN or ATM. The remaining 20% of hereditary BC risk may be determined by the combined effect of many low-risk polymorphisms, some of them not yet identified (5). In addition, early menarche, late menopause, nulliparity and late (>30 years) first pregnancy are well-known risk factors (4;6). The use of the oral contraceptive pill has been found to increase the risk of BC, but the risk drops 10 years after cessation (7). A high alcohol intake (≥ 3 drinks/day) increase the risk of BC (8), and obesity poses a risk in postmenopausal women (5). Pre-invasive breast conditions, such as atypical epithelial hyperplasia, and prior radiotherapy (RT) towards the chest wall also predispose to BC (4;6).

1.1.3 Diagnosis

In the 1980's, most women with BC presented with a palpable breast lump that was further investigated by radiological and/or pathological means (9). This was later known as the "triple test". A triple test today comprises a clinical examination, radiological assessment (mammography, ultrasound and/or MRI) and a sample for pathological examination (fine needle aspiration or core needle biopsy). Since its foundation in 1988, the Norwegian Breast Cancer Group (NBCG) has issued updated guidelines for diagnosis and treatment of BC. Prior to this, guidelines were issued by the Norwegian Surgical Association by "Blåboka" from 1981. In 1995/96, the Norwegian BC screening program was initiated in selected counties in Norway, offering all women aged 50-69 years a screening mammography every second year. From 2005, the offer has been extended to be nationwide (10).

1.1.4 Stage, TNM classification and tumor characteristics

BC is classified into prognostic groups based on primary tumor size, number of metastatic lymph nodes and the presence or absence of systemic metastasis (11). Prior to 1997, the Norwegian classification differed from the internationally accepted staging system in that T2 node negative disease was classified as stage I in Norway, but stage II internationally. From 1997 the Unio Internationalis Contra Cancrum (UICC) classification (12) has been used, also in Norway. The TNM (tumor, node, and metastasis) classification denotes cancer distribution at the time of diagnosis and summarizes findings from clinical examination as cTNM, and from pathological assessment as pTNM. There have been some changes in the TNM

classification from 1980 up until now. Table 1 outlines the TNM and Stage classification for 1975, 1998 and today as they appear relevant to the patient cohorts studied in the present thesis.

In addition to the extent of the primary tumor, tumor grade is important in the prognosis of BC. The grade is based on histopathological examination of nuclear polymorphism, mitotic count and tubule formation. The results are graded from I (highly differentiated) to III (poorly differentiated) (13). More recently, Human Epidermal Growth factor receptor 2 (Her-2) and Ki-67 status have been recognized as important prognostic markers and indicators for the choice of adjuvant treatment

The first report on HER-2 gene amplification in BC tissue was published in 1987 by Slamon *et al.* (14). HER-2 is a member of the epidermal growth factor receptor (EGFR/ERBB) family. The HER-2 gene is amplified in 15-25% of breast tumors and its amplification is a marker for more aggressive disease (15). In recent years, Ki-67 has been incorporated in algorithms for adjuvant treatment (16). The Ki 67 protein serves as a cellular marker of proliferation, and the fraction of Ki67- positive cells correlates with clinical tumor aggressiveness (17).

1.1.5 Prognosis of Breast Cancer, stage II and III

The prognosis of BC has improved over the past few decades. The patients included in this thesis were treated in 1975-1991 and 1998-2002, respectively. With some exceptions in the 1975-91 cohorts, they all had stage II or III disease. The 5-year survival for stage II patients was 57.8% in 1975, 83.5% in 2000 and 89.1% in 2010. Correspondingly, stage III patients have improved their 5 year survival rate from 45% in 1975 to 62.9% in 2000 and 75.5% in 2010 (1). These increased survival rates may be explained both by improved treatment and by the implementation of the mammography screening program.

Tumor	1975	1998	2014
T1	Tumor ≤ 2 cm A: not fixated B: fixated to fascia/muscle	Tumor ≤ 2 cm with no fixation to underlying muscle	Tumor ≤ 2 cm with no fixation to underlying muscle
T2	Tumor $>2-\leq 5$ cm A: not fixated B: fixated to fascia/muscle	Tumor $>2-\leq 5$ cm with no fixation to underlying muscle	Tumor $>2-\leq 5$ cm with no fixation to underlying muscle
T3	Tumor >5 cm	Tumor >5 cm with no fixation to underlying muscle	Tumor >5 cm with no fixation to underlying muscle
T4	Tumor of any size with direct extension to skin/chest wall	Tumor of any size with direct extension to skin/chest wall or ulceration of the skin	Tumor of any size with direct extension to skin/chest wall or ulceration of the skin
N1	Mobile axillary LN A: not thought to contain tumor B: thought to contain tumor	Mobile axillary LN A: not thought to contain tumor B: thought to contain tumor	Palpable axillary LN A: not thought to contain tumor B: thought to contain tumor
N2	Fixated axillary lymph nodes	Fixated axillary lymph nodes	Nodes >2 cm or fixed or ipsilateral IMLNM without axillary LNM
N3	Supra/infraclavicular LNM or arm oedema	Ipsilateral internal mammary LNM	Ipsilateral supra- or infraclavicular LNM or ipsilaterale IMNLM in the presence of axillary LNM
M0	No clinically appearant metastasis	No clinically appearant metastasis	No clinically appearant metastasis
M1	Distant metastasis present	Distant metastasis present (supraclavicular LNM included in M1)	Distant metastasis present
Stage			
I	T1-2a,N0,M0	T1,N0.M0	T1,N0,M0
II	T1-3, any N, M0	T1,N1,M0, T2,N0-1 ,M1	T1,N1,M0 T2,N0-1,M0
III	T4,any N,M0	T1-2, N2-3, M0 T3-4,anyN, M0	Any T, N2-3, M0 T3-4, any N, M0
IV	Any T, any N, M1	Any T, any N, M1	Any T, any N, M1

Table 1: TNM classification and differences between the 1975, 1998 and 2014 classifications based on information from (9,12,16)

LN: lymph nodes, LNM: lymph node metastasis, IMLNM: internal mammary lymph node metastasis

1.2 Treatment of localized breast cancer

The present thesis reports on patients with BC treated with locoregional radiotherapy (RT) at the Norwegian Radium Hospital (NRH) in a time period spanning from 1975 to 2002.

Localized BC is BC confined to the breast and the axillary, supra/infraclavicular and internal mammary lymph nodes. The following will cover basic principles of surgical treatment of localized BC and the adjuvant treatment offered to patients in this period. Focus will be on the development of national treatment practice and issued guidelines as they relate to the patient cohorts studied in the thesis. As the main focus of the thesis is RT and long term effects, special emphasis will be placed on the development of RT as part of adjuvant treatment. It does not aim to be a full coverage of all aspects of treatment of localized BC.

1.2.1 Surgery

The surgical procedure of choice for BC prior to 1980 was radical mastectomy (RM) where the breast, underlying muscle (pectoralis major and pectoralis minor) and axillary lymph nodes were removed. RM was first performed by William Stewart Halsted in 1882 (18), and represents in today's view a disfiguring operation that could leave the patient with painful side effects such as neuralgia, contractures and arm edema. For this reason, RM was replaced by modified radical mastectomy (MRM) in the late seventies, where the breast is removed together with the majority of the axillary lymph nodes. The pectoralis major muscle remains intact, whereas the pectoralis minor muscle is removed totally or partially depending on the technique. This method is less mutilating, but as effective as RM in terms of local control and survival (19). Towards the end of the eighties, breast-conserving treatment (BCT) became available. BCT at that time involved removal of the tumor with at least 1-2 cm margins in conjunction with axillary lymph node dissection (ALND) (20). Survival for women with BC stage I and II BC after BCT followed by post operative RT is equal to those treated with MRM (21) and RM (22)

Still, MRM may be the preferred surgical procedure for some BC patients. BCT is generally not recommended when the tumor is >5 cm or when there is multifocality (23). If the patient is unable to complete, or there is a contraindication to post-operative RT (pregnancy, previous RT, heart disease, thoracic malformation), MRM is the treatment of choice (23). Relative BCT contraindications include a large tumor in a small breast, a very large breast or a connective tissue disorder. BCT today should include the whole tumor without evidence of tumor in the resection margins. Postoperative RT is always recommended after BCT (16).

To further reduce the extent of surgery and post-operative complaints, sentinel node biopsy (SNB) was pioneered by Armando Giuliano in 1994 (24). SNB provides precise information regarding the extent of axillary lymph node metastasis (25). The procedure was introduced in Norway and at the NRH around year 2000. For SNB, a radioactive substance (^{99m}Tc) bound to filtered sulfur colloid is injected into the tumor area the day before surgery. Just prior to initiation of surgery, methyl blue ink is inserted into the same area. The radioactive substance and the ink allow peroperative identification of the tumor draining lymph node(s), the so-called sentinels. This/these node/s, usually 1-2, are removed prior to the planned BCT or MRM. If no tumor cells are found by frozen section analysis in the sentinel node(s), axillary dissection is omitted. If the sentinel nodes are positive for metastasis, ALND is done (26).

Depending on the location of the primary tumor, the draining lymph nodes may include nodes in other locations, especially internal mammary lymph nodes (IMLNs). These are, however, not surgically removed.

1.2.2 Adjuvant therapy

In Latin, "*adjuvare*" means to aid or to contribute. As such, adjuvant therapy aims to eradicate micrometastatic residues not removed by surgery. Adjuvant therapy is given after surgery to target micrometastatic foci present locally in the breast/thoracic wall or axilla, in locoregional nodes other than the axilla (supraclavicular nodes or IMLNs) or tumor cells spread hematogeneously to distant sites. The central dogma is that such micrometastatic foci are present in a proportion of patients and that adjuvant treatment may reduce the risk of relapse and death of BC.

Adjuvant therapy for BC may consist of RT, chemotherapy, hormonal therapy and more recently, targeted therapy and bisphosphonates. Even though adjuvant BC therapy clearly has improved BC-free survival for most risk groups of operated patients (27;28), some of the positive effects of adjuvant treatment are offset by toxic side-effects (29;30). During the past decade, numerous studies have focused on this issue, balancing the treatment effect with the risk of later sequelae and modifying the adjuvant treatment to avoid these complications (31-34).

In some patients with BC, mainly in those with tumors >5 cm and/or stage III BC, chemotherapy and/or hormonal therapy may be given upfront to reduce tumor size locoregionally. This may allow for a less radical and safer surgical approach. At the same

time, distant micrometastatic disease is targeted. The term neoadjuvant treatment is used for this scenario.

As the patient cohorts included in this thesis are recruited over several decades from 1975 onwards, they have received various adjuvant treatments. The following sections describe the historical development in adjuvant treatment of BC with the main focus on the time periods when the patients included in this thesis were treated. Guidelines from the NBCG (Norwegian surgical association until 1988) issued from 1981 onwards, have been followed. Systematic treatment recommendations prior to 1981 have been difficult to identify retrospectively and the level of detail for the earliest time periods are therefore less accurate. The presentation is done chronologically.

Radiotherapy

The German physicist Wilhelm Conrad Röntgen, discovered on November 8, 1895 electromagnetic radiation in a wavelength range known as X-rays or Röntgen rays. Henri Becquerel, a French physicist discovered the natural radiation from radioactive substances a year later (35). RT for BC was first attempted just after the discovery of Röntgen, in 1896. It was not implemented in the treatment of BC before 1920 (36).

RT takes advantage of:

1. The possibility to direct ionizing radiation towards the tumor or areas with suspected micrometastatic disease with at least some level of anatomic precision, and at the same time minimizing the dose to healthy organs. As such, different physical steps may be followed such as imaging techniques to map the tumor in the patient, fixation to minimize uncertainties from positioning, treatment planning based on two-dimensional (2D) simulator films or three-dimensional (3D) CT images, standardized or individualized field arrangements, and energy and beam quality considerations.
2. The difference in how tumor cells and normal tissue reacts to radiation. In general, tumor cells do not have the same abilities when it comes to repairing and surviving radiation induced damage as do most normal cells/tissues.

Multiple studies have demonstrated reduced risks of loco-regional recurrence, increased disease-free survival and overall survival after treatment with loco-regional RT in BC patients (27;37). Post-operative RT was first found beneficial for women treated with MRM. The

Early Breast Cancer Trialists Cooperative Group (EBCTCG) metaanalysis from 2005 demonstrated a reduction in local recurrence rate from 23% to 6% as well as an absolute risk reduction in 15 year BC mortality of 4.4 % in women with node-positive BC treated with locoregional RT (37). This study included all node-positive BC patients, and there were still uncertainties regarding the benefit of RT in women with only one to three positive lymph nodes. The 2014 EBCTCG meta-analysis confirmed that post-mastectomy RT reduced both local recurrence and BC mortality, also in this subgroup (38). Similarly, the rationale for RT after BCT is to reduce the risk of a local and/or regional recurrence, and to increase the chance of survival (37). In women with pN0 disease RT reduced the absolute 15 year risk of BC death by 3.3% and in women with pN+ disease by 8.5%. By every four recurrences prevented, one BC death is avoided (39).

Fields, Energies and Indications

The field arrangements applied for stage II and III BC have become more personalized due to newer RT equipment, and treatment facilities have become more personalized. However, the main focus has always been to target the areas where there might be residual disease, and where there is risk of local recurrence.

The field arrangements used at the NRH from 1975-1991 included tangential (TF), parasternal (PF), axillary and/or supraclavicular fields, and was based on national treatment guidelines at the time as well as the clinical situation of the individual patient including diagnostic information from mammography, surgery or other clinical information. The fields were then applied to the individual patient according to anatomical landmarks and x-ray simulator films. Phantoms were applied for calculation of the dose distribution. TFs did not cover the IMLNs and were used postoperatively in patients after BCT, in those with tumor-positive margins after mastectomy, preoperatively to downstage larger tumors or applied as definite therapy in inoperable stage III patients. The PF included the ipsilateral IMLNs, and applied in most women with stage II and III disease up until 1985, when the PF was omitted in most patients with stage II disease. A supraclavicular field including the axillary apex was applied together with a PF in patients with positive axillary lymph nodes with a centrally or medially located tumor. An axillary field was added when ALND was deemed non-radical. In patients with loco-regional recurrence combinations of the fields above were used according to the individual situation. RT was given mainly by ⁶⁰Cobalt (⁶⁰Co) units or linear accelerators with

5 MV photons. Electron beams (10-16 MeV) were used with increasing frequency over time in PFs.

Towards the end of the eighties, patients with four or more positive lymph nodes were given RT towards the chest wall, irrespective of resection margins, as well as to the ipsilateral axillary and IMLNs. Fields directed towards the supra- and infraclavicular nodes were omitted (40). Stage III patients were considered individually. Some of the patients received neoadjuvant treatment, whereas some were treated with primary surgery followed by postoperative RT. In some cases, the RT was split in half and given both pre- and postoperatively (40). RT alone was given if the tumor was >5 cm, mastitis carcinomatosa, when the patient refused, or for some reason was unsuitable for surgical treatment (40).

During the nineties, the field arrangements again included the supra/infraclavicular lymph nodes as these were found to be the most common site of nodal recurrence when not irradiated (41). Consequently, the fields applied in the late nineties were an anterior supraclavicular/axillary field, a posterior field covering the axilla, two TFs and an electron field covering the IMLNs.

In the 1998-guidelines, age and the number of lymph node metastases were taken into consideration (42;43). All premenopausal (age <55) women with stage II disease were given RT towards the chest wall and regional lymph node regions (41). In 1999, the use of ^{60}Co was discontinued and the photon energy of choice was mainly 5 or 6 MV.

Treatment planning at the NRH was based on CT-scans from year 2000. By this, 3D delineation of the volumes targeted by RT and the surrounding normal tissues to be spared, allows for a personalized refinement of the radiation fields and also of beam energies and qualities. The fields applied after year 2000 consisted of two TFs that covered the caudal part of the target volume (chest wall after MRM or breast after BCT with the ipsilateral IMLNs), and one anteroposterior field as well as an oblique field that covered the cranial part of the target volume (axilla, infra- and supraclavicular nodes and the cranial part of the ipsilateral IMLNs). All fields were irradiated with photons. From 2005 the inclusion of ipsilateral IMLNs in the radiation fields was no longer recommended (44).

Fractionation

The most common way to deliver radiation is through fractionation. Historically, this has been done by delivering the prescribed dose of RT in daily fractions of about 2 Gy with 5 fractions per week. For higher total doses, longer treatment time would be necessary than for lower doses. An increase in dose per fraction relative to 2 Gy is termed hypofractionation, and a decrease hyperfractionation. Due to different radiation sensitivity of tumor cells and normal healthy tissue cells, fractionation can be utilized to further potentiate the effect of radiation on malignant tissue, and to reduce unwanted unwanted radiation effects in healthy tissue (35;45).

Different radiobiological models have been proposed to describe and partly explain the differences in radiation sensitivity in relation to fractionation. One early example of such a model is the Nominal Standard Dose (NSD) model by Ellis (46). In more recent years, the Linear Quadratic Model (LQ model) advocated by Douglas and Fowler in 1976 (47;48) has been widely used. The LQ-model is based on the assumption that a lethal lesion is an unrepaired DNA double-strand break produced by either a single hit (α - linear), or by two less severe hits by to separate radiation tracks (β^2 - quadratic).

The LQ model can be used to describe the relationship between the total isoeffective dose and the dose per fraction in fractionated RT. It is essentially a mathematical formula fitted to a cell survival curve or any other experimental or clinical dose-response curve. The continuously downward bending form of such curves can be fitted as a second order polynomial, with a zero constant term to ensure that the survival fraction or tissue function is 1 or 100 % at zero dose. LQ survival curves are continuously bending, and the bendiness is determined by the α/β ratio. This is the dose at which the linear contribution of damage equals the quadratic contribution of damage.

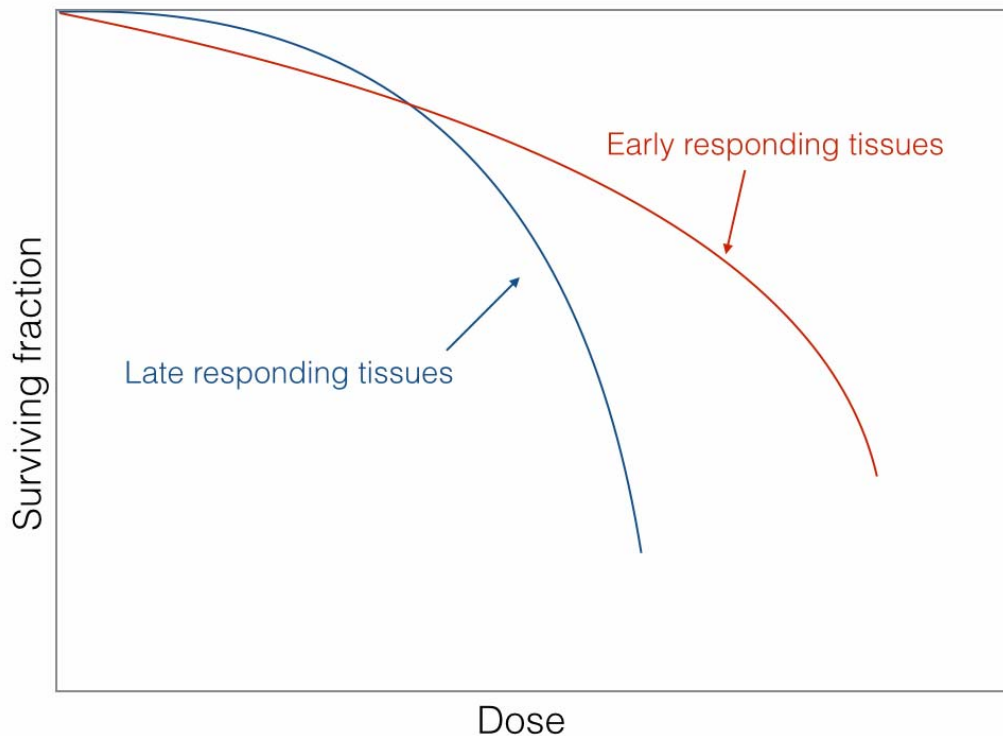


Fig 2: Difference between early and late responding tissues in response to fractionation.

In experimental and clinical RT, some normal tissues (such as skin, mucosa and bone marrow) react to treatment with early sign of damage within days and weeks, and are termed early responding tissues. Other tissues display detrimental reactions to RT much later, months or even years after treatment, hence called late responding tissues (such as connective tissue, kidney, lung). Early and late responding tissues have different responses to fractionation and this difference is reflected by the means of α/β ratios. Early responding tissues are typically characterized by α/β ratios of 7-20 Gy whereas for late responding tissues α/β ratios of 0.5-6 Gy may be found to fit the dose response curves. Hence, late responding tissues are sensitive to changes in fraction size whereas early responding tissues are relative insensitive to fraction sizes. It is important to note that there are uncertainties in the available estimates for α/β ratios across human tissues and the various side effects caused by RT in the clinical setting.

The heart and the different cellular components of this organ together are normally considered a late responding tissue with α/β ratio of 2-3 Gy and thus, toxic effects will often not become evident before years after treatment (49;50). It was previously thought that BC, like most malignant tumors, reacts as an early responding tissue to RT and hence would be insensitive

to fraction size. Recent reports have refuted this, and current consensus is that BC, with α/β ratio of 3-5 Gy, would be quite sensitive to changes in fraction size (51-53).

Large daily fractions have the potential to cause detrimental effects in healthy tissue. For tumors with high α/β ratio, RT is commonly given as hyperfractionated regimens to spare the surrounding tissues for late effects. This is however, not ideal for tumor eradication when the tumor, like BC, has a low α/β ratio and hence would be sensitive to change in fractionation. In these cases, a hypofractionated regimen might be more appropriate for tumor eradication, but reduction in total dose must be implemented to avoid toxic effects to the surrounding tissue.

In the middle of the sixties, hypofractionated RT for BC was introduced. Lack of RT capacity promoted the initiation of hypofractionation, allowing for less demanding treatment-schedules. As mentioned above, radiobiological models in the sixties and early seventies, such as the NSD, were in place to adjust for the difference in fractionation. Later, numerous weaknesses were identified in this formula (48). Most importantly, it did not account for the difference in radiosensitivity of different tissues.

Two fractionation patterns were used at the NRH from the mid seventies: 4.3 Gy x 10 given as two weekly fractions or 2.5 Gy x 20 applied as 4 weekly fractions. The regimens were in use contemporarily between 1975 and 1991, but the 4.3 Gy x 10 schedule was gradually superseded by the 2.5 Gy x 20 regimen. In Norwegian guidelines issued in 1981, no recommendation was made regarding fractionation (9).

During the early 80'ies it became evident that hypofractionated RT resulted in an unacceptably high level of late side-effects (54), and this was supported by the then proposed LQ-model. On an international level, hypofractionated RT for BC was therefore largely abandoned. In 1988 the NCCG recommended 2 Gy x 25 to a total of 50 Gy over 5 weeks (55;56), and this fractionation regimen remained the mainstay for many years.

Chemotherapy

The importance of adjuvant chemotherapy for BC is well-known, and advances in this field throughout the last decades are in part responsible for the improvement of BC prognosis (57;58). During the seventies, multiple clinical trials evaluated chemotherapy in the adjuvant setting (59;60). Amongst the trials were the Scandinavian Adjuvant Chemotherapy Trial, testing the effect of a short course of chemotherapy (5-Fluorouracil (5-FU)) after mastectomy (61). From 1981, adjuvant chemotherapy was given to all BC patients younger than 70 years (9). The combination given was Vincristine, Cyclophosphamide and 5-FU on the day of the surgery, and Vincristine, Cyclophosphamide and Methotrexate on the 7th postoperative day. Based on an EBCTCG metaanalysis from 1992 demonstrating increased recurrence-free survival after prolonged chemotherapy as compared to perioperative chemotherapy, perioperative chemotherapy was abandoned in the early nineties in Norway (62). In addition, the use of chemotherapy was restricted to stage II patients <55 years with lymph node metastases (62). Nine courses of Cyclophosphamide, Methotrexate and 5-FU (CMF) became the treatment of choice (63). Throughout the nineties the use of chemotherapy became more immersive and was also given to women <55 years with stage I node negative disease with more aggressive tumor characteristics (grade II-III) (62). Women aged 55-65 years with hormone receptor (HR) negative disease were also included in the recommendations for adjuvant chemotherapy (41). In addition, adjuvant chemotherapy was given to all stage II women <55 years (62). Stage III patients were frequently given neoadjuvant chemotherapy (41).

Towards the end of the century, evidence emerged that anthracycline containing chemotherapy was more beneficial for BC patients than the CMF-regimen (57). In addition, combinations of two or more chemotherapeutic agents were proven useful and more effective than one alone (28). Hence, from 1999, 5-FU, Epirubicin and Cyclophosphamide (FEC) were given to women <55 years. From 2001, all women deemed candidates for adjuvant chemotherapy were given 6 courses of FEC (57;64).

Patients with BCs harboring HER-2 amplifications benefit from the addition of trastuzumab, a humanized monoclonal antibody targeting surface HER-2 on the tumor cells. The benefit of adding trastuzumab to regular chemotherapy has been demonstrated clearly also in the adjuvant setting. Patients with HER-2 positive tumors and hormone receptor (HR) positive

status have less effect of antiestrogenic agents alone and should be offered chemotherapy (65). Due to its aggressive nature, a higher dose of anthracyclines (compared to standard FEC) is given to HER-2 positive patients (66).

In postmenopausal women, adjuvant treatment with zoledronic acid has shown beneficial effects, and is therefore recommended for all postmenopausal women given adjuvant treatment today (67)

Medical indications for adjuvant chemotherapy today are based on the characteristics of the tumor; the tumor grade, the stage of the BC, HR and HER-2 status and the percentage of Ki67 positive tumor cells. Recent analyses have shown that the addition of taxanes to anthracycline-containing regimens may be beneficial for certain groups (such as HER-2 positive patients) in need of adjuvant chemotherapy (58;68). The BC patients examined in this thesis did not receive Trastuzumab or bisphosphonates, and very few received Taxanes. The use of these drugs is therefore not discussed further.

Hormonal therapy

The concept of endocrine treatment of BC is old. In 1889, Albert Schinzinger proposed a connection between a high level of estrogen and poor BC prognosis (69). In 1896, George Thomas Beatson described the first ovariectomy as a part of treatment for BC (69). During the forties, it became evident that systemic use of antiestrogens could benefit patients with BC, and from around 1950, diethylstilbestrol was commonly used in the treatment of postmenopausal patients with advanced disease (70). Surgical and radiological castration were also considered treatment options, primarily for pre-menopausal women (71). Hormonal treatment takes advantage of the hormonal dependence of the tumour either by lowering the body estrogen level or preventing it from acting on the BC cell (72). Two out of three malignant breast tumors express estrogen and/or progesterone receptors (HR-positive tumors), and will thus grow in response to estrogen and progesterone binding (73). HR positivity is associated with a favourable prognosis (72).

In Norway, adjuvant hormonal treatment was applied from the mid seventies (74), initially given to all patients under the age of 70 with T3 or T4 tumors, or with fixed axillary lymph

node metastasis or supraclavicular lymph node metastasis (9). Nafoxidine was the first anti-estrogen applied in this setting (74). In the late seventies, testing for HR status was initiated and treatment was restricted to patients with HR positive tumors (9;73). Premenopausal patients were treated with surgical or radiological castration with 10-12 Gy towards the ovaries (9).

The recommended treatment from the late seventies however, was Tamoxifen for 3 years postoperatively. In principle, Tamoxifen was initially given in addition to surgical and radiological castration in premenopausal women, and as solitary hormonal treatment for postmenopausal women (9).

Tamoxifen is a selective estrogen receptor modulator that acts as an antagonist in breast tissue, and as an agonist in other tissues. Adjuvant use of Tamoxifen for 5 years has shown considerable effects on survival with a 31% reduction in annual BC specific death rate and treatment for 5 years is significantly more effective than 1-2 years of treatment (28). Tamoxifen has, in addition, estrogenic effects in postmenopausal women, which is positive due to its lipid lowering effect, and for protection of the skeletal system (75;76). Moreover, it reduces the risk for contralateral BC in pre and postmenopausal women (77).

In the late eighties, Tamoxifen for 2 years was given to all HR positive women diagnosed with lymph node positive BC or with large primary tumors (>5cm) (40). Some premenopausal women were still treated with surgical or radiological castration, but this practice became more sporadic and was discontinued during the nineties. In 1994, patients with T2 N0 tumors (2-5 cm), were included in the recommendations for Tamoxifen. The duration of Tamoxifen treatment was extended to 5 years in the 1998 guidelines on the background of published evidence by the EBCTCG (77) that demonstrated decreased recurrence and mortality rates on prolonged Tamoxifen treatment. In 2001, women <40 years with T1c grade 2 and 3, N0 were included in the recommendations.

Today, the NBCG recommend prolonged endocrine treatment for all premenopausal and some postmenopausal women. All premenopausal women are offered Tamoxifen for 5 years. If a patient, after treatment for 5 years, still is premenopausal, or her menopausal status is uncertain, she is recommended to continue Tamoxifen for another 5 years. If she is postmenopausal after 5 years on Tamoxifen, treatment with an aromatase inhibitor (AI) for 5

years is recommended (16). All postmenopausal women with HR positive disease are recommended treatment with an AI, either for 5 years, or for 2-3 years followed by 2-3 years of Tamoxifen. If Tamoxifen is chosen as primary hormone treatment, it should be given for 10 years, alternatively replaced with an AI after 2-5 years and subsequently treated with an AI for 5 years (16). AI's inhibits the action of the enzyme aromatase on the conversion of androgens to estradiol in peripheral adipose tissue, the main source of estrogen in postmenopausal women. Large randomized trials convincingly demonstrate a survival benefit of prolonging anti-estrogenic treatment, and in recent years, the use of AI has been proven more beneficial than tamoxifen in postmenopausal BC patients (78;79).

1.3. Cancer survivorship and late effects

1.3.1. Cancer Survivor

The incidence of cancer is increasing, and the prognosis of many different cancer types is improving (1). For these reasons, there are an increasing number of people surviving and living with possible side effects of their treatment. In 1996, the National Coalition for Cancer Survivorship advocated the definition of a cancer survivor as being any person diagnosed with cancer, from the time of initial diagnosis until his or her death (80).

1.3.2. Long term and late effects after breast cancer treatment

In principle, sequelae after cancer therapy may arise during or after therapy. It has been proposed to classify long term effects as adverse effects or complications of cancer therapy that appear during treatment and that continue beyond treatment cessation (81). A late effect is a side effect that occurs months or years after treatment (81). For the purpose of this study, these two terms are used interchangeably and both include complications arising during treatment and years to decades after treatment.

There are a number of long term effects after BC treatment. The present thesis focuses on cardiovascular disease (CVD), one of the most serious late effects. Other potential late effects are mentioned below but the detailed discussion of these is considered beyond the scope of this thesis.

Second malignancies after BC, that is any other malignant tumor diagnosed after BC, represent another serious late effect. They may occur sporadic, due to genetic factors, or be related to BC treatment (30). The risk of second primary contralateral BC is about 0.5%-1% per year, and risk factors include young age at primary diagnosis and/or radiation at young age, hereditary or familial BC, lobular cancer histology and multicentricity (82). The risk of developing a second malignancy in breast cancer survivors (BCSs) compared to the general population has been evaluated by Kirova *et al.* in 2008. They reported increased age-standardized incidence ratios for leukaemia (2.07) and gynaecological cancers (1.6) in BCSs after multimodal treatment (83). A Danish study reported a significantly increased hazard ratio (HR) of 1.34 for development of second cancers in RT-associated sites among irradiated women compared to non-irradiated breast cancer survivors BCSs (84).

About 20 % of BC patients are below 50 years and premenopausal at BC diagnosis, and consequently at risk of developing premature menopause during or after BC therapy (1). Amenorrhea is a common consequence of systemic BC therapy in young women (85). For most patients amenorrhea is temporary, but even if menses do resume, many of the young BCSs are at increased risk of premature ovarian failure (POF) and thus infertility (86).

Osteoporosis is defined as a skeletal disorder characterized by compromised bone strength predisposing to an increased risk of fracture (87). Women developing POF are at increased risk of developing osteoporosis due to accelerated loss of bone mineral density (88). Tamoxifen has a preventive effect for bone loss in postmenopausal women, and the opposite effect in premenopausal women (89),

Arm/shoulder problems may arise after surgery and RT of the chest wall and axilla. The cohort of stage II and III BCSs treated from 1998-2002 evaluated for CVD in the present thesis have previously been evaluated for arm/shoulder complaints previously. Nesvold *et al.* found that 20% of the BCSs treated with MRM followed by RT had lymphoedema, the corresponding number being 8% in the group treated with BCT. The prevalence increased with time after surgery with a higher number of metastatic lymph nodes as well as higher BMI (90).

BC-related pain was reported by 50% BCSs examined 1-3 years after BC surgery (91). Pain was most frequently localized in the breast area, followed by the axilla. Factors associated with pain included young age and adjuvant RT, but not chemotherapy (91). Shoulder pain has been reported more prevalent after MRM than after BCT (90).

A significant proportion of BC patients gain weight during adjuvant therapy and the proportion of fat mass is increased at the expense of lean mass (92). Obesity may not only increase the risk of CVD, but also poses a separate risk of cancer recurrence (93).

Thyroid disease, especially hypothyroidism, may be more common in BCSs than in the general population. A possible common etiological link between BC and thyroid disease has been debated as both conditions most commonly appear in postmenopausal women (94), but results are conflicting (95;96). In a case-control study based on the NRH cohort from 1998-2002 mentioned above, Reinertsen *et al.* found that the prevalence of hypothyroidism among

BCSs was 18%, compared to 6% among age-matched, cancer-free controls (97). The prevalence of hypothyroidism before BC diagnosis was similar to that of the control group, indicating an effect of treatment, most likely RT.

Cancer survivors, including BCSs, may report memory and concentration difficulties, suggesting cognitive impairment following diagnosis and treatment of cancer. When investigating cognitive functioning, an important distinction must be made between objective cognitive functioning, measured with standardized neuropsychological tests, and subjective cognitive functioning, measured with self-report questionnaires. Multiple studies have found lack of correlation between the two (98).

While most patients experience “normal” distress during and after BC diagnosis and treatment, there is a subset that experience clinically significant depression and may be in need of psychiatric intervention (99). Some studies find an increased rate of depression among BCS (100), but these findings are disputed by others (101).

Fatigue is a nonspecific, multidimensional construct generally thought to involve subjective feelings of tiredness, weakness, and/or lack of energy (102). It is also associated with the sense of cognitive limitations (103). While this description also may apply to many healthy individuals, patients with fatigue cannot alleviate the symptoms by rest (101). Chronic fatigue is defined as fatigue above a certain level for six months or longer (104). Persistent fatigue is fatigue at two assessment-points (105). Reinertsen et al evaluated the course of chronic fatigue in the 1998-2002 treated cohort of BCS and found that 33% of women were affected 4 years after treatment and 39% of them 7 years after treatment. Taking the two timepoints together, 23% of the BCSs had persistent fatigue (105).

1.4 Cardiovascular disease (CVD)

1.4.1 Cardiovascular disease- epidemiology, sex differences and risk factors

CVD is a group of diseases that involves the heart, the blood vessels or both (106). It is estimated that 17.3 million people died from CVDs worldwide in 2008, and 80% of these cases occurred in low- and middle-income countries. CVD is thus the leading cause of death globally (106). Since the seventies, CVD mortality rates have declined in many high-income countries but have increased at a fast rate in low- and middle-income countries (107).

CVD is classified under I00-I99 in the International statistical Classification of Diseases and related health problems, 10th edition (ICD-10). The current thesis focuses on the main adverse effects of BC treatment on the heart e.g ischemic heart disease (IHD) (ICD-10: I20-I25) and heart failure (HF) (I50). To a lesser extent the thesis has evaluated conduction abnormalities (I44-I49) and valvular disease (VD) (I34-I37).

The main clinical manifestations of IHD are stable angina pectoris, unstable angina pectoris, myocardial infarction (MI), and chronic IHD. HF can be subdivided into systolic HF (Ejection Fraction (EF) <50%) (108) and diastolic HF (where the EF is preserved) (109). Diastolic dysfunction (DD) often arise prior to systolic dysfunction (SD) and overt HF (110).

As CVD in its many forms is common in the general population, the risk factors are diverse. BC patients will have an underlying individual risk of CVD independent of their cancer treatment. These risk factors may be important for the later incidence of CVD and interact with treatment induced risk. A short review of accepted risk factors for the most common forms of CVD is therefore presented here. The potential impact of treatment on the development of CVD follows thereafter. The risk of CVD may be determined by non-modifiable traits such as family history, increasing age and male sex. In addition, a number of modifiable, life-style related risk factors have been identified, some of which are discussed below.

Elevated cholesterol and triglycerides are some of the most common risk factors for CVD. A study published in 1994 identified that the reduction of IHD by lowering serum cholesterol was age dependent (111). A 10% reduction of serum cholesterol in a 40 year old man would decrease the risk of IHD by 50%, while the same reduction in a 50 year old would reduce the risk by 40%. Similar results were found for women (111). Current international guidelines

aim primarily to lower low density lipoprotein (LDL) cholesterol, the efficacy of which in terms of reducing the risk of CVD has been proven in various clinical trials (112;113).

Obesity is a risk factor for hypertension and CVD (114). Unfortunately, weight gain and obesity are not uncommon among BCSs (92). An association between high childhood BMI and later IHD has been found, thus supporting the idea that atherosclerotic disease is a continuous process beginning in childhood (115).

Physical activity was recognized as one of the main preventors of CVD many decades ago. Morris *et al.* published a study in 1953, suggesting a link between physical activity and reduction in the risk of CVD (116), a correlation later confirmed by others (117).

Systolic and diastolic blood pressures both show a continuous and independent relationship with the risk of developing CVD (118). In recent years it was even suggested that values at the high end of normal (systolic pressure of 130 to 139 mm Hg, diastolic pressure of 85 to 89 mm Hg, or both) are associated with a risk of CVD (119). In a study of adults with their first myocardial infarction (MI), hypertension was the most common risk factor among the traditional cardiovascular risk factors (120).

Diabetes mellitus (DM) is associated with a 2- to 3-fold increased risk of developing CVD (121), the association being stronger for women than men (122). Further, DM is interlinked with metabolic syndrome, a disorder of energy utilization and storage, diagnosed by a co-occurrence of three out of five of the following medical conditions: abdominal (central) obesity (waist circumference > 88 cm), elevated blood pressure (systolic >130 / diastolic >85), elevated fasting plasma glucose (>5.6mmol/L), high serum triglycerides (>1.7mmol/L), and low high-density cholesterol (HDL) levels (<1.3 mmol/L) (123).

Smoking is a major risk factor for CVD, well known since the Albany and Framingham studies published in 1962, describing the relationship between cigarette smoking, MI and sudden death (124). Later, smoking cessation was found to be the single most effective measure one could undertake to avoid mortality from CVD (125).

The mechanism whereby alcohol interacts with the cardiovascular system and the risk of CVD is not entirely known. Alcohol has been found to decrease the risk of ischemic stroke

and IHD at low levels of consumption (5-10 g/day) (126), however, this protective effect is not observed at higher levels of consumption (127;128). Binge drinking, even by light to moderate drinkers, leads to an increased risk of ischemic events by increasing the probability of clotting and abnormal contractions of the heart chambers (128).

In addition, and pertinent to survivors of cancer therapy, exposure to RT and chemotherapeutic drugs are important risk factors for CVD, and discussed in the section below.

1.4.2 Cardiovascular disease - a late effect.

As stated above, adjuvant treatment reduces the rate of locoregional relapse, prevents distant metastases and improves survival of BC patients. However components of adjuvant treatment have also been found to increase the risk of CVD (29;31;32;110;129).

This thesis analyzes the cardiac effect of different adjuvant BC treatment over four decades. The main focus is on RT, but the effect of chemotherapy and hormonal treatment is also evaluated. It is important to balance the gain of adjuvant treatment with possible hazards done by it. This remains a continuous and important task as treatment modalities progress and change. Moreover, some of the late effects do not occur until decades after treatment and knowledge about late effects caused by outdated treatment may give valuable information when developing new treatment modalities (29).

1.4.3 The pathophysiology of cardiovascular toxicity from radiotherapy.

The heart was, for a long time, regarded a radioresistant organ. The background for such an assumption may in part have been the postmitotic state of cardiac myocytes and the low proliferative activity of endothelial cells and connective tissue (50). It was initially thought that doses total heart doses >40 Gy were required to cause irreversible damage in irradiated cardiac tissue (129;130). However, since the sixties, it has been recognized that incidental radiation to the heart increases the risk of cardiac mortality (33;131).

Recent studies show that considerably lower doses are sufficient to cause irreversible damage to the heart, and today the heart is viewed as of the critical dose-limiting organs in radiation for example in BC (27;33;132;133). The heart represents in many aspects a typical late responding tissue. After whole heart irradiation of >30 Gy, cardiac disease typically manifests within two years, whereas at lower cardiac doses latency periods are much longer

(33). There is no known lower dose threshold in which there is seen no cardiac damage (33), and subclinical abnormalities have been noted in up to 50% of patients receiving irradiation towards the heart (133).

All structural and functional components of the heart, including the pericardium, myocardium, valves, conduction system and the coronary arteries are susceptible to radiation damage. Radiation related heart disease (RRHD) comprises pericarditis, pericardial fibrosis, HF, and IHD (33). The true frequency of RRHD is not known as it is difficult to clinically differentiate from CVD due to other causes. A review of the different pathological processes is provided below, with the emphasis on HF and IHD as the most common clinical complications reported for BCSs.

Heart Failure

HF as a long-term effect after RT is thought to involve myocardial fibrosis. Myocardial fibrosis generally occurs in patches, often in the anterior wall of the myocardium (33), typically in specific patterns that correspond to the areas receiving the highest radiation doses (134). The pathological processes leading to HF are complex and involve more than one pathway. From autopsy findings it is known that the total amount of collagen in the heart increases after RT and that the amount of collagen type I (the most abundant form of collagen, found in skin, tendons, organs and bone) increases relative to type III (found in granulation tissue with much greater distensability than type I) (130). This alters the compliance of the cardiac wall and hence contributes to DD (135). The parietal lining is typically more affected than the visceral (33)

Myocytes are terminally differentiated cells and relatively resistant to radiation damage (136). One of the main theories of radiation related HF is therefore that myocyte damage results from indirect toxicity caused by microvascular changes (137). Damage to the microvasculature via inflammatory and thrombotic pathways lead to increased capillary permeability and subsequent loss of vasculature, focal ischemia and interstitial fibrosis (136;138). Further, this injury may subsequently lead to chronic ischemia and DD due to diffuse myocardial fibrosis (139). Another effect of radiation is increasing reactive oxygen species (ROS) production; further creating an inflammatory environment in which other types of injury can be exacerbated (140).

Ischemic Heart Disease

There are two hypotheses of the biological mechanisms whereby radiation leads to increased morbidity and mortality from IHD. The first hypothesis is that radiation increases the frequency of MI by interacting with the pathological pathways of age-related coronary atherosclerosis, resulting in accelerated atherosclerosis seen at a younger age than it would normally occur (33;141). The second hypothesis is that radiation increases the lethality of MI by reducing the heart's tolerance to acute infarctions as a result of microvascular damage. According to the latter hypothesis, the MI may occur due to pathologies unrelated to radiation (33;129).

Endothelial dysfunction and diffuse atherosclerosis have been identified as causes of ischemia without evidence of blockage of the coronary arteries in women not treated for BC (142). It appears that atherosclerotic plaque in women may be less fibrotic and contain more of the so-called foam-cells, thus giving the potential for reversibility of plaque, but nevertheless a greater potential for plaque rupture (143).

The pathogenesis of IHD after RT is multifactorial and there is evidence to support both theories mentioned above. The pathological changes in the heart seen after RT clearly resembles that found among individuals with IHD in the general population (144). The left anterior descending artery (LAD) and the right coronary artery (RCA) are most commonly affected, as in the general population (145). Disease of the left main artery (LMA) appears more often in patients treated with radiation than in general IHD, possibly reflecting its exposed position relative to the irradiated volumes in the chest (145). Autopsy findings identified that the median layer of smooth muscle in the coronary arteries tended to be much thinner in patients treated with RT than in the control population, and the media and adventitia were more densely thickened with fibrous tissue (146). Narrowing of the lumen generally appeared more proximal in patients treated with RT compared to IHD from other causes and more often included the ostia of the coronaries (146).

As mentioned above, radiation activates the inflammatory pathway (136) and this has been increasingly recognized as important for development of radiation related atherosclerotic disease (147). The first morphological changes seen in irradiated hearts is lymphocyte adhesion and extravasation from irradiated capillaries. Intimal proliferation of myofibroblasts

with lipid-containing macrophages forming plaques may fissure and lead to thrombosis (148). This reduces the arterial lumen to various degrees ultimately resulting in IHD.

Pericardial fibrosis and pericarditis

The risk of pericardial disease was more pronounced in the sixties and seventies when radiation doses towards the heart were higher (50;146). Extensive fibrous thickening of the pericardium occurred with older techniques (146). Dense collagen and fibrin replaced normal adipose tissue (33). Changes after RT also include pericardial adhesions, excessive pericardial fluid and local inflammation resulting in pericarditis. Pericardial effusion is characterized by a variable amount of exudate in the pericardial sac. If the effusion increases rapidly, cardiac tamponade may result, but this complication is seen only rarely today (33).

Valvular disease

VD has been reported in up to 71% of individuals exposed to doses >30 Gy after RT to the mediastinum and may be due to fibrotic damage (33;146). The heart valves are avascular, thus, the pathogenesis cannot be explained primarily by microvascular changes. The mechanism underlying development of VD after RT is not completely understood, but it has been suggested that valvular damage results after injury to the surrounding myocardial endothelium (130). Another theory is that higher pressure from the systemic circulation is important. This is supported by the fact that the valves on the left side of the heart generally are more affected than the ones on the right side (130).

Conduction abnormalities:

Fibrosis secondary to RT may alter the conduction system and cause arrhythmias (149). Multiple abnormalities have been reported after RT including atrioventricular nodal bradycardia, all levels of heart block, and sick sinus syndrome (150). Strender *et al.* conducted a study of BCSs and found increased ECG abnormalities 6 months after adjuvant RT, but these were reversible (151). However, a recent review done by the Danish Breast Cancer Collaborative Group reported that RT was not associated with an increased risk of receiving a pacemaker due to arrhythmias (152).

1.4.4 The pathophysiology of cardiovascular toxicity from chemotherapy

Different chemotherapy regimens are used in the treatment of BC, and as mentioned above, these have changed over the past few decades. Included below is a brief summary of the most important cardiotoxic agents used in BC therapy, their mode of action, the mode by which they cause cardiotoxicity, and the clinical implications.

Anthracyclines

Anthracyclines are compounds originally derived from *Streptomyces* species and their anti-tumor activities were established in the 1960s. Anthracyclines are red aromatic polyketides and occur in a variety of forms due to structural differences in the aglycone and the different sugar residues attached (153). They are among the most effective anticancer drugs developed (154), acting on multiple levels inhibiting DNA and RNA synthesis. They exert their effect by intercalating between base pairs of the DNA/RNA strands and by inhibiting the topoisomerase II enzyme, thus blocking DNA replication and transcription. In addition, anthracyclines produce ROS that may cause DNA damage or lipid peroxidation (31;155). It has also been suggested that anthracyclines mediate their effect through p53 and subsequently induction of apoptosis (31).

The mode by which anthracyclines cause cardiotoxicity is complex and multifactorial. Studies have identified oxidative stress as one of the main contributors of myocardial injury (31;130;155). Increases in ROS in the heart occur through the formation of anthracycline-iron complexing or the redox recycling of the quinone and semiquinone moieties of the anthracycline (31) (fig 3). ROS then rapidly interacts with various cellular components causing lipid peroxidation and membrane damage. Additionally, ROS reduces the contractility by altering the permeability in mitochondrial membrane and hence affecting the intracellular influx of calcium (156). ROS generation also induces nitric oxide synthase and peroxynitrate that subsequently trigger cell death and hence, may lead to myocardial dysfunction (157). Myocytes have a limited antioxidant reserve to protect the cells from effects mediated by ROS, and anthracyclines further increase the myocytes susceptibility to ROS by suppressing other potentially protective antioxidant enzymes (130). In addition, recent studies have identified damage of cardiac progenitor cells within the myocardium as a source of damage (158).

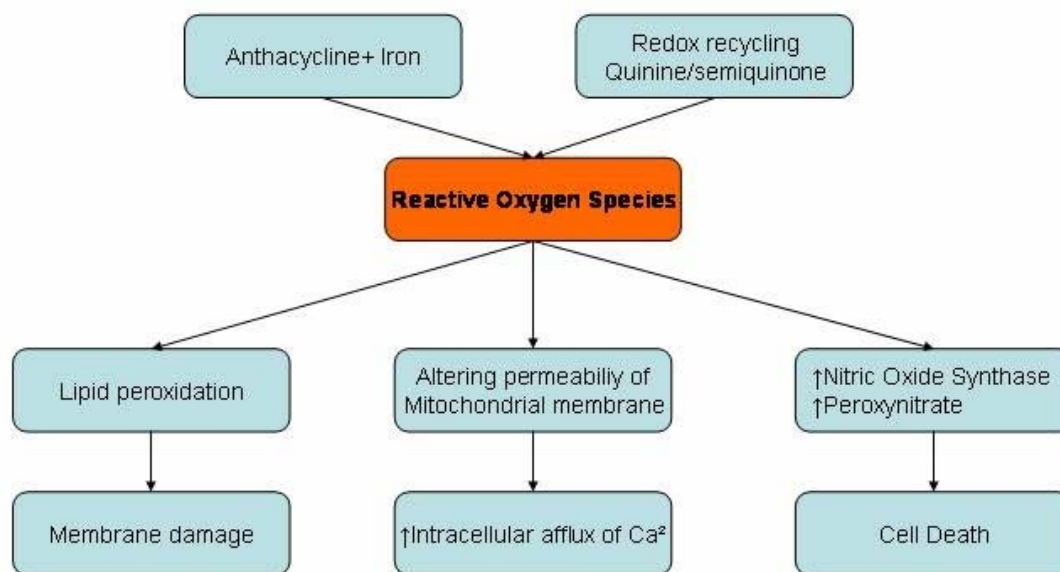


Fig 3: The mechanism of ROS-mediated cardiotoxicity of anthracyclines

Recently, an alternate explanation for anthracycline-mediated cardiotoxicity has been proposed (159). This mechanism involves the topoisomerase II enzymes. The isoenzyme Top 2B is expressed in normal cells, whereas top 2A is expressed in cancer cells only. As mentioned above, the binding of anthracyclines to topoisomerase II is one of the mechanisms of cellular damage and tumor eradication by anthracyclines. Research in mice has demonstrated that cardiomyocyte specific deletion of the top 2B enzyme conferred protection against doxorubicin-induced damage to the cardiac myocytes (160). Mice lacking the top2b gene in cardiac muscle did not develop HF whereas those who did have this gene developed a decrease in EF. If these findings are further confirmed, a top 2A specific anthracycline may be a potential way to avoid cardiotoxicity (160).

Several risk factors for increased cardiotoxicity by anthracyclines have been identified and include older age, high cumulative anthracycline dose, long time since treatment, the addition of other cytotoxic drugs such as cyclophosphamide, trastuzumab and taxanes, and mediastinal irradiation (32).

Clinically, the cardiotoxic effects mediated by anthracyclines are diverse and range from acute to late effects. Acute effects are typically arrhythmias, but acute HF and MI have been

described (110). Late effects of anthracyclines are mainly progressive cardiac dysfunction resulting in HF. DD has been seen with cumulative doses $>200 \text{ mg/m}^2$ of Doxorubicin whereas SD has been reported at cumulative doses of $400\text{-}600 \text{ mg/m}^2$ (32). Cardiotoxicity after treatment with lower doses has been less studied, but one study found congestive HF after administration of a Doxorubicin dose of 250 mg^2 in a cohort of survivors after hematopoietic cell transplantation after a median of 3 years (161). The patients given Epirubicin as a part of the FEC-regimen in this thesis received a cumulative dose of 360 mg/m^2 of Epirubicin, corresponding to an equivalent isotoxic Doxorubicin dose of 241 mg/m^2 .

Doxorubicin and Epirubicin are the two anthracyclines most commonly used in the treatment of BC. The two show similar response rates, but differ in terms of late effects. Doxorubicin is associated with an increased risk of cardiotoxicity compared to equimolar doses of Epirubicin (162).

Cyclophosphamide

Cyclophosphamide is an alkylating agent mediating its anticancer effect through attachment of an alkyl group to DNA (163). During the seventies and eighties, acute adverse cardiac effects were reported in up to 43% of patients after administration of alkylating drugs (164). Histopathological changes after high doses of cyclophosphamide ($>7\text{g/m}^2$) indicate that the cause of myocyte damage is endothelial destruction followed by extravasation of toxic metabolites (164). Cyclophosphamide-associated cardiotoxicity occurs during or soon after (within 3 weeks) administration as acute or subacute onset of HF (165).

The risk of cardiotoxic effects from cyclophosphamide appears to be more related to the amount of a single dose ($> 1.5\text{g/m}^2$ daily) rather than the cumulative dose (166). The dose of cyclophosphamide as a part of the CMF ($600\text{mg/m}^2/\text{dose}$) and FEC ($600\text{mg/m}^2/\text{dose}$) regimens are below this limit and in later years, cardiotoxicity from cyclophosphamide appears to be a minor problem in adjuvant BC treatment (167).

5-Fluoruracil

5-FU is an antimetabolite inhibiting cell metabolism involving purine and pyrimidine nucleosides (168). The mechanism underlying 5-FU cardiotoxicity is unknown, but clinically it manifests as acute coronary events. Vasospasm and coronary artery thrombosis have been suggested as pathophysiological correlates (169). The risk of developing cardiotoxicity from

5-FU appears to be greater in patients with pre-existing IHD and in patients treated with high doses of 5-FU ($>800 \text{ mg/m}^2/\text{day}$) (169). The corresponding doses of 5-FU as part of the CMF and FEC-regimens are $600 \text{ mg/m}^2/\text{dose}$. The cardiotoxicity seen after 5-FU administration is largely reversible, at least in patients without pre-existing CVD (170).

Taxanes and Trastuzumab

Taxanes and Trastuzumab are both cardiotoxic agents currently used in adjuvant BC treatment. However, as only a minority of the patients included in this thesis were treated with taxanes and none were treated with Trastuzumab, these agents are not further described.

1.4.5 Hormonal treatment and cardiovascular disease.

Normal levels of estrogen have protective effects on the cardiovascular system. Estrogens have been found to improve vascular function and to slow the rate of atherosclerosis, and the development of cardiac hypertrophy (171). In addition, estrogens have beneficial effects on the lipid profile (171). Higher levels than normal, however, may increase the risk of thromboembolic events (172).

Traditional endocrine therapies, such as Tamoxifen and oophorectomy, have not been associated with cardiotoxicity (155). Indeed, tamoxifen has even been found protective against MI and IHD. As a selective estrogen receptor agonist/antagonist it has a generally beneficial impact on serum lipids, a potential mechanism for its cardioprotective effect (173). The positive effects on the heart are unfortunately offset by a higher incidence of other vascular events. A review article reported a relative risk of stroke of 1.49 for patients on tamoxifen compared with controls. The corresponding risk of deep venous thrombosis was also increased by a relative risk of 1.87 (172).

AIs have been associated with a slightly higher risk of CVD, but results are conflicting (174;175). No significant differences between AI and tamoxifen have been found on the risk of MI (176). In addition, the use of AIs lower the risk of thromboembolism compared to Tamoxifen (175).

1.4.6 Investigation and diagnosis of cardiotoxicity

It follows from the above that cardiac damage from treatment most often manifests itself as IHD, HF and/or valvular disease. The paragraphs below highlight diagnostic test in use to

detect cardiac abnormalities in asymptomatic and symptomatic cancer survivors. The tools used are not specific to cancer patients/survivors, but their relevance to cancer survivors will be presented.

For IHD, stress ECG and myocardial scintigraphy are non-invasive procedures used in the clinical setting to evaluate myocardial perfusion. However, these modalities are largely insufficient to detect subtle changes. For example, the use of stress-ECG for the diagnosis of IHD has substantial limitations in women with regards to predicting significant angiographic plaque (177). The gold standard for investigation of IHD is angiography, but this is not normally performed in asymptomatic individuals, being an invasive procedure with its inherent risks like myocardial infarction (MI), arrhythmias and stroke (178).

There is currently strong evidence that Coronary Artery Calcium (CAC) computerized tomography (CT) scan can be used as a predictor of later IHD in asymptomatic individuals (179;180). A CAC-scan offers information about the presence and extent of calcified plaques in the coronary arteries. Becker *et al.* evaluated prospectively the predictive value of CAC in 1726 asymptomatic individuals with a mean observation time of 40.3 months. Individuals at risk of future MI and cardiac death could be identified within an asymptomatic population based on higher CAC-scores independent of concomitant risk factors. At the same time, future cardiovascular events could be excluded in patients without coronary calcifications (180). The use of CAC-score in cancer survivors at risk of developing IHD has not been extensively evaluated yet. Andersen *et al.* found that Hodgkin's lymphoma (HL) survivors with a prior diagnosis of IHD had increased CAC compared to HL survivors without symptomatic CAD (181). To the best of our knowledge, no prior longitudinal studies confirming the predictive value of CAC-scores on later development of IHD have been done in patients/survivors after cancer therapy.

For myocardial disease, the use of conventional 2D echocardiography has been the mainstay of monitoring myocardial function in cancer survivors (32;110). The European Association of Cardiovascular Imaging and the American Society of Echocardiography recommends screening echocardiography every five years in asymptomatic cancer survivors (182). However, resting left ventricular ejection fraction (LVEF) assessment by standard 2D echocardiography does not detect early myocyte damage due to cardiotoxic therapy (183), and is a poor predictor of later HF (184). Accordingly, tissue Doppler imaging (TDI) have been

evaluated as a novel technique for the use of monitoring cardiac function in cancer survivors (32). TDI makes use of Doppler measurements in the myocardium to assess velocities, strain and strain rate (155). Strain and strain rate are an ultrasonic method of quantifying regional deformation based on principles derived from mechanical engineering (185). These parameters appear more sensitive for detecting reduced myocardial activity than LVEF-measurements (185;186)

Cardiac Magnetic Resonance Imaging (MRI) has emerged as a highly valuable tool for assessment of cardiac function (155), and the method is currently evaluated for use after oncological treatment (187). Unfortunately, the high costs and low availability make cardiac MRI less useful in a routine clinical setting (32).

The use of biomarkers to monitor cardiac function is an area of much interest. A biomarker is a marker that is easily accessible, for instance a substance in circulating blood that reliably identifies patients with evidence of cardiac damage or individuals at risk of developing such complications (188). IHD and HF are both late effects, frequently with a long asymptomatic latency period, and hence, easy and early identification of individuals at risk is an attractive idea.

The fact that elevated serum troponin levels correlate well with LVEF decline in CVD from causes other than cancer treatment indicates a potential usefulness of troponins as a monitoring agent (155). Troponins are small proteins found in cardiac and skeletal muscle. Three types of troponins exist; troponin I that inhibits the interaction of myosin with actin, troponin T binds the troponin components to tropomyosin, and troponin C contains the binding sites for Ca^{2+} and hence promotes contraction (189). Troponins are released in response to myocardial injury regardless of cause. Troponin T and troponin I have been proven equally useful in the diagnosis of MI and HF in general cardiological patients (190;191). The usefulness of monitoring BCSs is suggested by elevated levels of troponin I found immediately after RT in left-sided BC as opposed to right-sided BC (192). Studies have also found a link between elevated troponin I and risk of cardiac events following chemotherapy (193).

The peptide hormones, atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) are secreted in response to myocyte stress along with their n-terminal parts; NT-pro ANP and NT-pro BNP. The latter is the peptide currently measured in blood and correlates with the amount of stress occurred in the cardiac muscle (194). The natriuretic peptides are widely used biomarkers in diagnosis and prognosis of HF, but recent research has identified that mid-regional pro atrial natriuretic peptide (immediate precursor protein of ANP) might be superior in that regard (195). However, it has still not become widespread in clinical use, and proBNP remains the main biomarker in use for that purpose today.

There is conflicting evidence as to the usefulness of pro-BNP for monitoring the development of CVD in cancer survivors. Pro-BNP has been found less useful than troponins (110;155). However D'Errico *et al* reported higher pro-BNP-levels in patients where a large heart volume was included in the radiation field than in patients with lower cardiac exposure (196). These levels were elevated 5-22 months after RT for left-sided BC (196). Sandri *et al.* found that persistently elevated levels of pro-BNP after administration of different regimens of high dose chemotherapy to be associated with later development of systolic or diastolic HF (197). It follows from the above that the use of pro-BNP as a biomarker for monitoring cardiovascular health in cancer survivors remains controversial.

1.4.7 Prevention of cardiotoxicity from cancer treatment

Prevention of cardiotoxicity from cancer treatment is an area of growing interest. Prevention from treatment-related cardiotoxicity can broadly be categorized into three categories; primordial prevention, primary prevention and secondary prevention (155).

Primordial prevention is any prophylactic intervention or therapy before or during cancer treatment to prevent anticipated injury (155). Primary prevention is therapy provided to selected asymptomatic patients with elevated risk or early signs of cardiac damage but with preserved normal findings on diagnostic tests, in order to treat early injury and prevent progression of disease. Secondary prevention is treatment after detection of disease to prevent impairment (155). Treatment of any form of CVD specifically in cancer survivors has been insufficiently studied and current recommendations are largely based on treatment guidelines derived for patients without former cancer treatment (155).

For prevention of cardiac damage from RT, the largest effect is potentially generated from primordial prevention. Risk reduction of RRHD may be ensured both by minimizing the radiation dose and the cardiac volume exposed to RT (130). In addition, fractionation may influence the risk of late cardiac effects.

In the last two decades, advances have been made in treatment planning techniques, thus reducing the dose to the heart in BC patients receiving adjuvant RT. Prior to year 2000, when CT dose-planning was initiated at the NRH, manually planned standardized field arrangements were used. For TFs, x-ray simulator images were used to direct the angles at which the opposed TFs were applied to avoid unnecessary cardiac radiation. The PF field was the main field contributing to cardiac radiation dose. From the mid eighties, this field was given as electron beams with energies of 10-16 MeV with the advantage of a finite dose depth range after which the dose falls rapidly. Hence, radiation to structures of the heart with a larger distance to the anterior thoracic wall was reduced compared to the previously used photon beams of ^{60}Co or 5MV. However, with the standardized fields and the 2D treatment planning based on anatomical landmarks and x-ray simulator films, no information on individual cardiac doses were available and refinements for cardiac protection in the individual patient were not possible.

3D-conformal RT adopts CT imaging as treatment planning equipment. A 3D image of the target and surrounding tissue is formed, including information regarding tissue density and depth. This allows for individualized refinements of field arrangements to optimize dose distribution to target volumes and at the same time record and adjust for doses to normal organs such as the heart (130).

There are still gaps in knowledge, as to whether there is a threshold dose to the heart below which there is no risk applied, and it is debated which part of the heart is most radiosensitive (33;129). Some studies have suggested that the dose to the LAD is, in fact, more important than the dose to the heart (198), but this has not been confirmed by others (132). For routine clinical purposes, current Norwegian recommendations state that the mean dose to the heart should not exceed 2 Gy, with no recommendations as to the LAD dose (16). Previously, it was advised to keep the volume of the heart that receives 25 Gy or more to a minimum of 5%, a guideline inferred by the NBCG in 2005.

As discussed previously, different fractionation regimens have been used in the delivery of RT for BC over the last decades. Discussions as to which regimen is the optimal with regards to the balance between killing cancer cells and protecting the heart and other surrounding normal tissues are ongoing. Contemporary studies done on CVD after hypofractionated RT do not seem to find an elevated risk of cardiac disease; however, their observation time does not yet exceed 10 years (199-201).

The introduction of deep inspiration breath hold (DIBH) technique (202), intensity modulated RT (IMRT) (203), and proton therapy have further reduced cardiac dose during RT for BC (133). DIBH technique has been in use at some institutions for about a decade. DIBH takes advantage of the mobility of the heart and lung and applies radiation when the patients hold their breath in deep inspiration, thus removing the vital organs out of the radiation field. This results in lower doses to the heart and lungs (202). IMRT utilizes inverse algorithms, whereby the radiation is given to maximize the target dose, and at the same time minimizing the dose to surrounding tissues (130). Proton therapy has the advantage that dose depth curves peak and stop abruptly in the tissue at predicted points (Bragg peak principle), and hence may further minimize the dose to underlying organs. This is in contrast to photons and electrons currently used in routine RT (130). Chang *et al.* studied the use of proton therapy for BC with promising results as to the dose received by normal tissues (204). However, for any of these novel techniques the impact on long term cardiac effects is not yet clear.

Radiation causes inflammatory and thrombotic lesions, and anti-inflammatory and/or anti-thrombotic medication may therefore play a role in prevention of RRHD (205). Studies on age-related atherosclerosis in hypercholesterolemic mice reported promising results in inhibiting and stabilizing the plaque with acetylsalicylic acid, but the effect was not seen in radiation induced IHD (206). Similar results have been reported for Clopidogrel, a drug that inhibits platelet aggregation, in this setting (207). Due to their anti-inflammatory, anti-thrombotic and anti-fibrotic effects, statins have been suggested as candidates for reducing radiation-related IHD. One experimental study found a statin-induced reduction in pulmonary and cardiac fibrosis after thoracic radiation in mice (208).

BCSs have elevated risk of CVD in the presence of traditional risk factors for cardiac disease compared to BCSs without such risk factors (33;34;49;132;137). For these individuals, the focus on primary prevention may be most relevant. Controlling other cardiac risk factors such

as smoking, hypertension, obesity and diabetes may be of critical importance before and after radiation. BCS that opt for a healthier diet and increase their physical activity are likely to have better psychosocial and physical health, including cardiovascular health (209). Similarly, ensuring correct treatment of preexisting CVD prior to initiation of potentially cardiotoxic treatment for BC may be important. Admittedly, little research is done on treatment of patients with early myocardial damage prior to RT, but Daher *et al.* have called for more cardiological investigations prior to cancer treatment, in order to initiate primary or secondary prevention (210) .

As with RT, the focus on prevention from cardiotoxicity after chemotherapy is mainly on primordial prevention.

The use of continuous infusion contra bolus infusion has been proven to reduce cardiotoxicity from anthracyclines, however, this is not commonly applied in clinical practice (211;212). Liposomal or polyetyleneglycolcoated (pegylated) delivery of chemotherapy is an area of much interest, especially in the delivery of anthracyclines. Liposome encausulated anthracycline delivery or pegylated Doxorubicin have been developed to specifically target tumor cells rather than cardiac cells (31). The encapsulation is thought to enable the drug to escape the capillaries of the tumors more easily than through the endothelial tight junctions of the heart, thus accumulating in the tumor compared to normal organs (212). The ongoing study “Liposomal doxorubicin-investigational chemotherapy-tissue Doppler imaging Evaluation (LITE)” compares left ventricular function in 80 BC patients with BC treated with either standard Epirubicin or liposomal Doxorubicin. Results from the pilot study published in 2012 showed that the use of liposomal Doxorubicin reduced the risk of cardiotoxicity (213).

The use of drugs with potential cardioprotective effects have been investigated in combination with chemotherapy, some with promising results. Carvedilol, a non-selective beta-adrenergic blocker, was added to Doxorubicin or Epirubicin in a case control study including 50 patients, and the LVEF six months after treatment was significantly higher in the group given Carvedilol (69.9 vs 52.3%) compared to placebo (214). The angiotensin II converting enzyme blocker Valsartan attenuated pathological LV remodeling and DD in 20 patients examined on day 3, 5 and 7 after CHOP (Cyclophosphamide, Doxorubicin, Vincristine, Prednisolone) infusion for non-Hodgkin lymphoma (215). Statins, due to their pleiotropic effects, have also been proposed as possible agents for preventing cardiotoxicity from anthracyclines.

Atorvastatin was found to reduce the rate of anthracycline-induced SD six months after administration of chemotherapy in a study including 40 patients and controls (216). Finally, animal studies done on the use of calcium-channel blockers and prostacycline analogues have shown protective effects when given simultaneously with anthracyclines (31;110;156). The abovementioned studies regarding the use of Carvedilol, Valsartan and statins are all limited by their small sample sizes. Larger studies are needed for confirmation of these findings before they can be implemented in routine practice. Of relevance, the ongoing "The prevention of cardiac dysfunction during adjuvant breast cancer therapy (PRADA) study", is a Norwegian randomized controlled trial investigating the effect on LV function of adding a beta-blocker (Metoprolol) and/or an angiotensin receptor blocker (Candesartan) to a Epirubicin-containing regimen +/- Trastuzumab in BC patients. The authors aim to include 120 patients, and the cohort will thus be larger than in previous studies (217).

Prevention of anthracycline-mediated cardiotoxicity using anti-oxidant drugs was supported by the theory of involvement of ROS in the development of cardiotoxicity. Dexrazoxane is a chelating agent that limits formation of anthracycline-iron complexes that are believed to generate ROS (130;218). An alternate theory involves dexrazoxane-mediated prevention of anthracycline binding to Top2 (159). Studies done on BC patients given anthracyclines with or without additional dexrazoxane, have revealed that LVEF reductions were less likely to occur in women receiving dexrazoxane (218;219). The use of dexrazoxane is however restricted due to concerns that it might lower the antineoplastic effect of anthracyclines and hence increase the risk of relapse and secondary malignancy (220;221). Other studies does not find this association (130;219;222).

Amifostine has been propagated as a cardioprotective agent without compromising antineoplastic effects (130). Results from animal studies suggest that Amifostine scavenges ROS in normal tissue, and thus prevents ROS from acting on cardiac structures (223). The acidic pH of the tumor tissue hampers the conversion of Amifostine into its active metabolite, possibly explaining its protective effect on normal tissue. However, Amifostine may be less cardioprotective than dexrazoxane, perhaps because dexrazoxane prevents the formation of radicals, whereas amifostine scavenges them (130).

ROS generated by the cardiotoxic agents may be neutralized by antioxidants, endogenously and exogenously. First generation antioxidant drugs such as N-acetylcystein, vitamin D and E

all showed promising results in animal studies, however, these have not been proven effective in the clinical setting (110). In addition, the use of antioxidants in combination with cancer treatment may counteract the antineoplastic effect of both chemotherapy and RT, and is still discouraged by many oncologists (224).

The use of biomarkers may help identifying patients in need of primary prevention. Cardinale *et al.* selected 114 patients on the basis on their troponin levels and randomized them to additional treatment with the acetylcholinesterase (ACE) -inhibitor Enalapril or placebo(225). After 1 year follow-up, LVEF decline and HF was found only in the placebo group.

Guidelines for patients with asymptomatic SD advocate the use of an β -adrenergic blocker or ACE-inhibitor, however, these guidelines are developed for early stage heart failure of any cause, and not specifically for patients in the oncological setting (155;226). Published guidelines aiming at cancer survivors recommend HF to be managed similarly to HF from other causes, although it is not clear whether patients with cancer or cancer survivors respond similarly. In addition, specific advice regarding timing and duration of treatment are not given (226).

2. The current thesis

2.1 Introduction

The patient cohorts included in this thesis are BCSs treated from 1975 to 1991 (study group 1) and from 1998 to 2002 (study group 2). The groups provide a long observation time, an important feature in cancer survivorship studies, both to evaluate long term tumor control and unwanted late effects of cancer treatment.

Evaluation of treatment is an ongoing and continuous process. As mentioned in the previous section, some late effects will develop years to decades after treatment. The treatment given in the study groups examined in this thesis are now partly out-dated. Nevertheless, current knowledge on long term effects can only be based on data collected from previous treatment techniques. In addition, the information regarding positive and negative effects of treatment in the past enables us to develop and improve established treatment in terms of survival while at the same time, avoiding late effects.

From 1975, the patients included in study group 1 received hypofractionated RT for BC. The treatment regimen involving 4.3 Gy x 10 was later found to result in unacceptably high level of late local effects and was stopped in 1986. Two women received compensation claims due to mutilating late effects developed after treatment with this schedule. As a consequence, a task force was appointed to evaluate the scientific basis for the introduction of hypofractionated RT in 1975, whether the regimen was evaluated properly during the years it was in use and whether the practice of 4.3 Gy x 10 should have been abandoned earlier than actually done. In 1996, women who were still alive after treatment with the 4.3 Gy regimen were invited to attend a clinical consultation focusing on late effects. For comparison, women treated with the more conventional regimen (2.5 Gy x 20) during the approximate same time period were invited. The task force found significantly more local late effects in women given the 4.3 Gy schedule. They concluded that the introduction of the regimen itself in 1975 could not be criticized due to the level of knowledge regarding hypofractionated RT at the time. However, they found it reprehensible to continue the treatment from 1983 when sufficient evidence was available showing that hypofractionated regimens of this kind were harmful to the surrounding tissues (227).

This example illustrates the importance of evaluating former treatment modalities and the need for continuous evaluation of the treatment applied today.

Many of the late effects after cancer treatment are relatively common also in the general population. It is therefore important to compare the BCSs with a control population to ascertain to which degree the observed effects are attributable to the treatment itself.

2.2 Study Aims

Based on the background and introduction outlined above, the current thesis pursues the following study aims:

- 1) To explore long-term IHD mortality in BCS compared to the normal population in relation to the degree of hypofractionation of RT
- 2) To examine coronary calcium distribution as an early marker of IHD in BCS and the relationship to treatment factors such as cardiac radiations doses.
- 3) To examine the degree of SD and DD and valvular function measured by echocardiography and tissue Doppler imaging in BCSs both in relation to treatment factors and compared to a control population.
- 4) To explore the additional influence of traditional risk factors of CVD together with treatment parameters on long term cardiovascular health in BCSs.

2.3 Patients and Methods

2.3.1 Study Populations

The current thesis studies 2 separate patient cohorts presented in 4 papers. Study group 1 is included in paper 1. Study group 2 is the cohort included in papers 2, 3 and 4, but each of these papers has a separate selection from this cohort.

Study group 1

Study group 1 consists of 1566 women treated for BC (stage I-IV) with locoregional RT by two different hypofractionated RT regimens from 1975-1991. 10.7% of the patients had stage IV disease, but as the focus was on cardiac mortality from locoregional RT and patients dying of BC were censored at the time of death, the inclusion of these patients were deemed appropriate. Patient selection was based on NRH's RT registry. The eligibility criteria were: 1) Females with no malignancy prior to first-time diagnosis of BC (non-melanoma skin cancer allowed) 2) Loco-regional RT with treatment start 1975-1991. Patients with re-irradiation of the same regions were excluded. As a control group for study group 1, Statistics Norway supplied ten randomly selected cancer-free controls per patient matched on age, education and county of residence.

Cancer registry of Norway/Statistics Norway provided information on cause of death. ICD- 9 and ICD-10 were used to classify causes of death as BC (ICD-9 174, ICD-10 C.50.9), IHD (ICD-9 410-414, ICD-10 I20-I25), CVD (ICD9 390-459, ICD 10 I00-I99) and other causes. Since it was the most prevalent cause of cardiac death, only death from IHD was considered in the analysis in paper 1.

Study group 2

Study group 2 consists of women treated for stage II and III BC with postoperative loco-regional RT at the NRH from 1998-2002. The study was planned in 2003 and initiated in 2004. The eligibility criteria were 1) age <75 in 2004/2005, 2) no recurrence of BC, 3) no other cancer except basal cell carcinoma, in situ carcinoma of the uterine cervix, or prior or simultaneous surgery for contralateral BC with no adjuvant treatment.

Altogether 719 eligible women were treated from 1998-2002. 200 patients died, developed metastasis or other cancers and were therefore not invited to participate. In 2004/2005, 415 of these women were invited to participate in the study, of them, 318 (76%) completed a mailed

questionnaire and underwent an outpatient clinical examination (T1). In 2007, 310 of the women participating in 2004/2005 were asked to complete a follow-up mailed questionnaire and to provide new blood samples (T2). In addition, 104 BCSs, all treated with manually planned RT and not previously contacted, were invited to take part in T2. These patients were not identified in 2004 due to an administrative error. 334 patients altogether returned the questionnaire and had blood samples drawn at their general practitioner's office. 405 BCSs participated at T1, T2 or both (fig 4). For paper 2, only patients treated with CT-planned RT enrolled at T1 were included; a total of 216 patients.

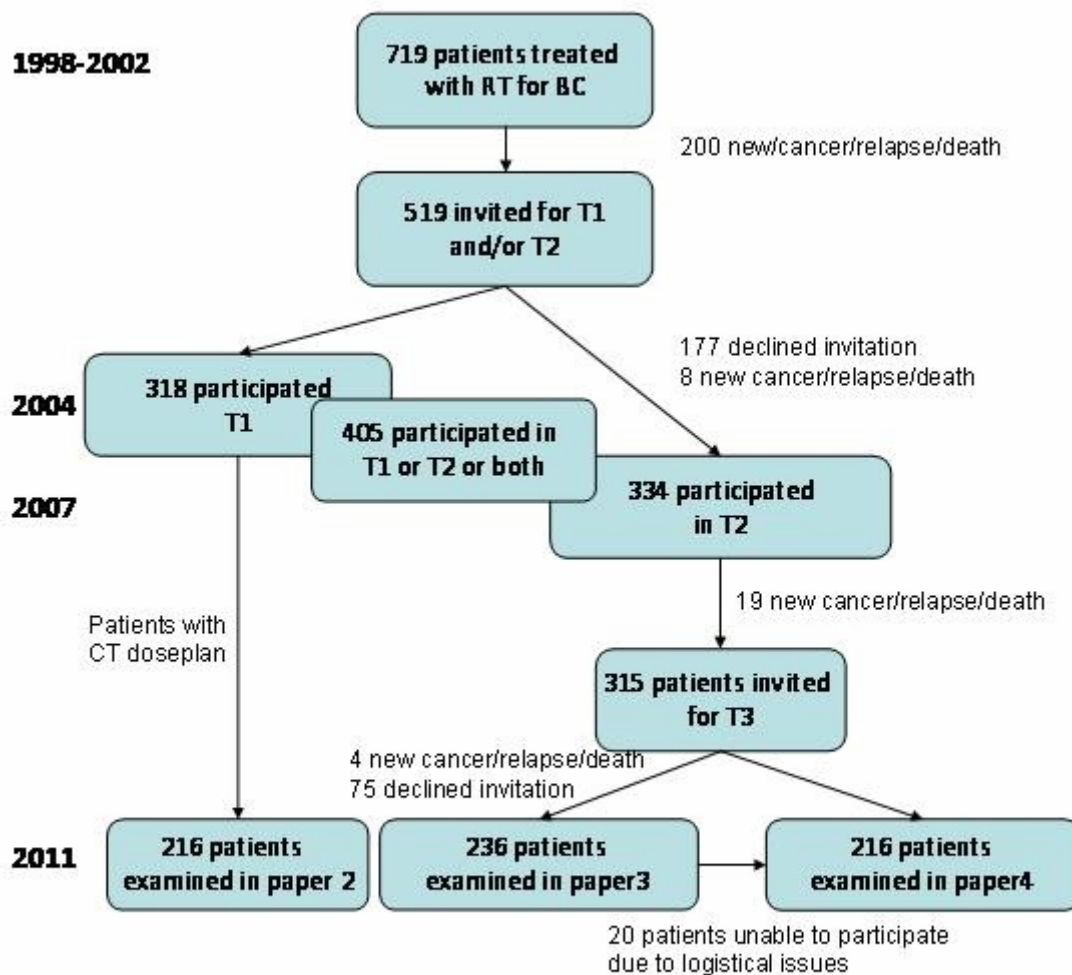


Fig 4: Overview of study group 1 at assessment points T1 (2004), T2 (2007) and T3 (2011).

In 2011 315 of the BCSs from the T2 cohort were still alive and without recurrence/metastasis. Along with a new mailed questionnaire, they were invited for a clinical examination (T3), blood sampling, echocardiography and CT CAC-score. 236 women (76%) accepted the invitation. Of the 236 women, 178 (75.4%) had attended at all three time points, whereas 58(24.6%) attended only at T2 and T3. All 236 women were included in paper 3. Due to logistical constraints, 20 patients did not undergo an echocardiography; therefore only 206 patients are included in paper 4.

The control population for echocardiographic parameters for study group 2 was extracted from the Health Study of Nord-Trøndelag County, Norway (HUNT) (228). This is a cross-sectional study of 50839 (54%) inhabitants of Nord-Trøndelag County in the mid region of Norway where a complete echocardiographic examination was performed in 1296 subjects without known CVD, DM or hypertension. Patients and controls were matched 1:1 on age, sex, body weight and systolic blood pressure.

2.3.2 Treatment

Study group 1

In the seventies and early eighties the majority of Norwegian BC patients underwent RM or MRM with or without ALND. Towards the end of the eighties, BCT became available. Adjuvant chemotherapy and/or hormone treatment was frequently used in patients with stage II and III disease, but its impact on late effects is not considered in paper 1 as none of the registries available provided reliable data.

Locoregional RT for BC from 1975-1991 was given by two different fractionation patterns, either 4.3 Gy x 10 (1975-1986), or 2.5 Gy x 20 (1975-1991). The choice of fractionation was originally based on the stage of disease, but practical considerations were also taken into account. The 4.3 schedule was considered well suited for patients living far from the hospital whereas the 2.5 schedule predominantly was offered to patients living nearby the hospital and to in-house patients.

Standardized field arrangements were used based on the extent of disease of the individual patient. The fields used were PF, TF, axillary or supraclavicular fields, dependent on the clinical situation. These are described in more detail in the background section. Altogether, 8 different combinations of these fields were identified. During the seventies and eighties,

treatment was mostly given as photon therapy from ^{60}Co units or linear accelerators with energies of 5MV. Over time, the use of electron beams for parasternal irradiation was favoured due to concerns of cardiac doses from photon therapy to the central mediastinal structures.

Study group 2

According to national guidelines for BC stage II/III at the time, treatment consisted of surgery (BCT or MRM) and postoperative locoregional RT with the possible addition of adjuvant chemotherapy and/or hormonal therapy. RT field arrangements are described in detail in the background section, and depicted visually in figure 5. Prior to year 2000, RT was still manually planned based on standardized field arrangements. RT for the PF was given with either photon or electron energy. Photons were from ^{60}Co units (corresponding to $\sim 4\text{MV}$), 5MV or 6 MV, whereas the energies applied for the PFs with electron energy depended on the depth of the IMLNs from simulator films. The fields were simulated with x-rays and angled to avoid the heart contour as much as possible. Patients treated from year 2000 and onwards received 3D conformal RT planned with CT-scans. Photon energy of mainly 6 MV was used for all treatment fields. To avoid unwanted radiation towards the heart and lungs, these organs are delineated in the planning system as organs at risks. Measures were taken to avoid these organs in the treatment field and calculations made to ensure these organs received a dose of radiation as low as possible.

The fractionation was the same throughout the entire period; the breast/chest wall received a total dose of 50 Gy given in 25 fractions over five weeks. After BCT, women under 50 years received an electron boost of 10-16 Gy to the tumor bed (6–12 MeV). After mastectomy, all stage III patients and women with stage II with positive resection margins were given a bolus covering the chest wall; the remaining patients received a bolus covering the mastectomy scar with a 3 cm margin.



Fig 5: Illustrations of locoregional RT before (left) and after year 2000 and the implementation of CT-based 3D-based RT (right).

2.3.3 Methods

Cardiac Dosimetry paper 1

In study group 1, the standardized fields mentioned above were applied in 8 different field combinations. We simulated the most frequently applied field arrangements on CT planning images of a contemporary BC patient of normal build using the RT planning system Oncentra v.4.1 (Nucletron BV, Veenendaal, The Netherlands). Both left- and right sided disease were evaluated. 4 MV photons were used for the tangential, supraclavicular and axillary fields to mimic the corresponding depth dose curves of ^{60}Co and 5 MV Linac beams. For the PF, either 4 MV photons or 12 MeV electrons were used. The heart was delineated on CT images. Resulting doses to the heart were extracted from the planning system, and converted to equivalent dose in fractions of 2 Gy using the linear quadratic cell survival formalism and $\alpha/\beta=3$ Gy (45). Dose volume histograms (DVH) for the heart were derived and the equivalent dose delivered to 5 % of the volume ($D_{5\%}$) used as the representative dose to the heart.

Cardiac dosimetry paper 2, 3 and 4

Patients with a CT based dose planning procedure from study group 2 were evaluated. A clinical oncologist delineated the heart on the CT images at the time of treatment as part of their regular treatment planning. Treatment planning and dose calculation were preformed using the Helax-TMS system. The slice thickness was 1 cm and the CT-scan was done without

contrast. For the purpose of present studies, a radiologist revised the delineation on archived CT-images. The cranial limit of the heart included the right atrium and excluded the pulmonary trunk. The caudal contour of the heart was the caudal myocardial border. The pericardium was included in the heart volume; the inferior caval vein was excluded. In addition, the right and left atria and ventricles were contoured separately. The atrial septum was often well discernible due to its lower attenuation than the surrounding blood and myocardium and was drawn in the middle of the septum. With the CT scan technique used, the coronary arteries were not consistently visible; thus, the coronary arteries were not contoured. The position of the pulmonary, mitral and tricuspid valves and the intraventricular septum were not reproducibly located because of cardiac motion and the lack of contrast. To have reproducible results, we defined borders from certain key structures that were reliably identified on the available scans:

1. The atrioventricular groove/coronary sulcus that passes around the heart and separates the atria from the ventricles
2. The interventricular groove that separates the ventricles and descends towards the *apex cordis*
3. An area of fibrous/fatty tissue where the atrial septum meets the atrioventricular plane, anatomically corresponding to the *trigonum fibrosum dextrum*
4. The pulmonary artery bifurcation.

The border between atria and the ventricles was defined as straight lines drawn between the coronary sulcus on both sides (1) and through the *trigonum fibrosum dextrum* in the middle (3). The border between the ventricles was a straight line between the *apex cordis* (2) and 3. The atria were readily discernable, and a line was drawn between them for division (fig 6).

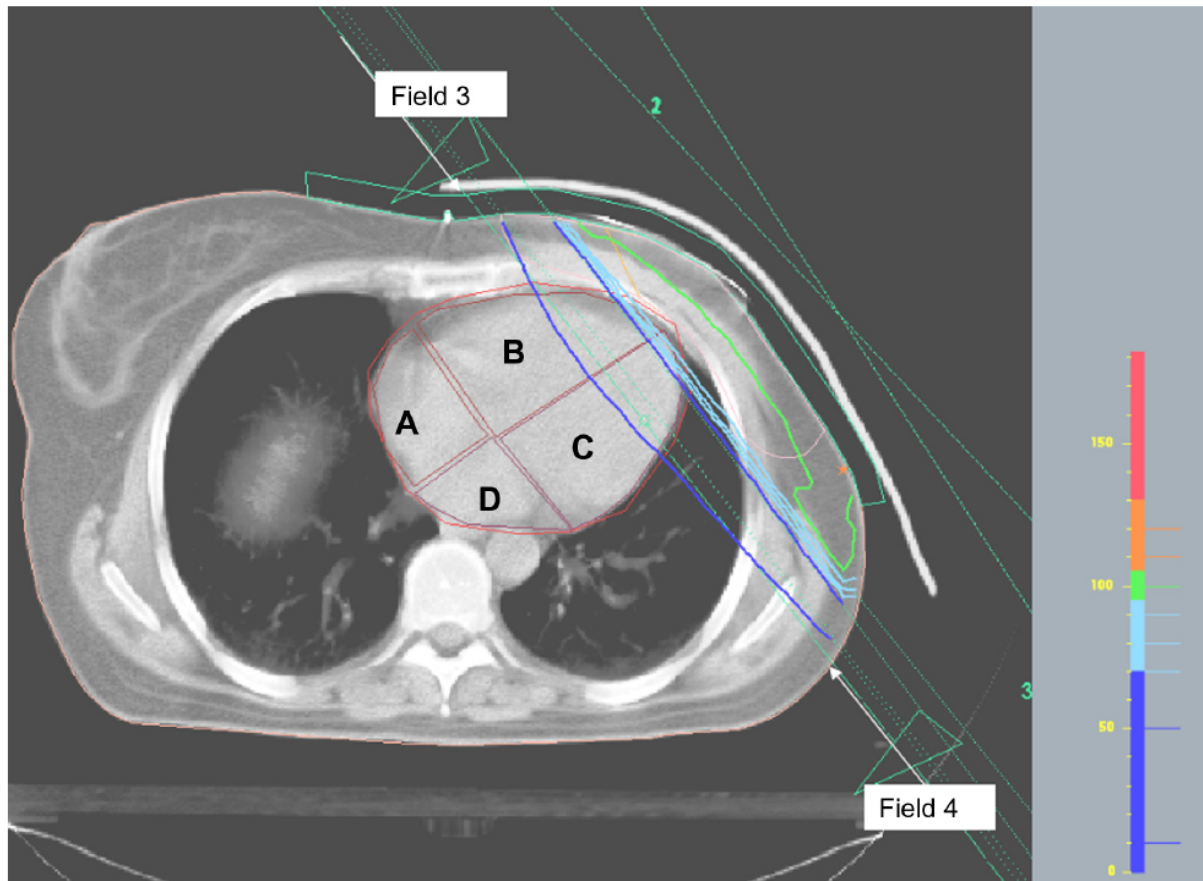


Fig 6: Radiation fields covering the caudal part of the breast/chest wall. The arrows indicate the radiation direction. In addition, delineation of the whole heart and four cardiac chambers in a patient with a left-sided BC is shown. A = right atrium, B = right ventricle, C = left ventricle, and D = left atrium.

For each patient, DVHs for the whole heart and each of the four delineated cardiac chambers were obtained. Mean, median and maximum doses were extracted. Further, the dose delivered to 5% of the whole cardiac volume or each chamber ($D_{5\%}$) was reported for every patient as well as the volume percentage of the respective structure receiving more than 25 Gy (V_{25Gy}). Cohort-based DVHs displaying means and standard deviations were generated.

The impact of boost on cardiac radiation doses was not investigated because no dose plan data were available on the Helax-TMS system. Accordingly, using the more modern treatment planning system (Oncentra), the contribution of the boost was estimated to be $D_{5\%}$ of 0.2 Gy for a boost dose of 16 Gy measured on a patient of normal built.

Potential excess IHD at 15 years was calculated as individual normal tissue complication probability (NTCP) estimates using the relative seriality model (229). This model employs the

DVH for the heart and accounts for the serial and parallel architecture of tissue subunits and for the binomial nature of cell kill. The dose at 50% response, D50, and the maximal relative slope, γ , were used to describe the dose-response curve. The parameters used were D50 = 52.3 Gy and $\gamma = 1.28$. Fractionation sensitivity (α/β -ratio) of 3 Gy was assumed.

CT-CAC-score

CT-CAC scans were done without contrast and were acquired within a single heartbeat with prospective electrocardiography (ECG)-triggering on a 320-row CT system (Aquilion ONE, Toshiba Medical Systems, Japan). Scan parameters were 120 kV, 150-350 mA, 0.35 sec gantry rotation time and 120-160 mm scan length. 3 mm axial images were reconstructed consecutively. CAC scoring was performed on a Vitrea workstation (Toshiba Medical Systems, Japan) and expressed as Agatston score (AS) with standard density threshold 130 Hounsfield units. Percentile ranks as compared to healthy age-matched women were calculated based on published data (230).

Echocardiography

A comprehensive echocardiography was performed by an experienced physician sonographer using high-end echocardiographic scanners (Vivid 7 or Vivid E9 GE Vingmed Ultrasound, Horten, Norway) according to recommendations. At least three cardiac cycles were obtained and stored digitally for subsequent analysis in dedicated software (GE EchoPac vs. 112). Left ventricular (LV) wall thickness, internal dimensions and fractional shortening (FS) were assessed by M-mode from parasternal long axis view, and LV mass was calculated (231). LV volumes and ejection fraction (EF) were obtained by modified Simpson's biplane method (232). Stroke volume (SV) and cardiac output (CO) were estimated from the area and the velocity integral of LV outflow tract. Global longitudinal strain was assessed using 2-dimensional (2D) speckle tracking echocardiography (STE) with semiautomated analysis in three apical views and optimal frame rate for underlying grayscale images. Mitral and pulmonary venous flow velocities and isovolumic relaxation time were recorded by PW Doppler. Left atrial volume was derived from the biplane method of discs. Peak systolic (s') and early diastolic (e') mitral annular velocities were recorded by PW tissue Doppler in septal and lateral position. SD was defined as either EF <55% or FS <27%. Subclinical SD was identified by peak s' in septal position <6 cm/s and in lateral position <6.7 cm/s and by global longitudinal strain <18% using 2D STE (Chahal NS). DD was defined by septal e' <8 cm/s or lateral e' <10 cm/s and graded as normal, mild, moderate or severe dysfunction with

simultaneous estimation of LV filling pressures (233). Right ventricular function was assessed by lateral tricuspidal annulus (LTA) peak systolic velocity (s') using PW TDI and by displacement of LTA on color TDI. Central venous pressure and peak systolic pulmonary pressure were estimated from inferior vena cava dimensions and tricuspid regurgitation pressure drop. Right atrial (RA) size was obtained. Valvular regurgitations were graded as minimal, mild, moderate or severe and valvular stenosis was graded as mild, moderate or severe (234). Valvular incompetence was defined as regurgitations of more than mild or stenosis of any severity (235).

Blood Tests

C-reactive protein (CRP), proBNP, Troponin T and cholesterol were measured in serum and Haemoglobin A1c (HbA1c) in full blood. Peripheral venous blood was drawn into empty glass tubes and tubes containing ethylenediamine tetraacetic acid (EDTA). Samples from T1 and T3 were analyzed immediately. Serum from 2007 was stored in aliquots at -80 ° C and later thawed once for analysis. The following assays were performed on the MODULAR Analytical platform, P800 _ E170 modules (Roche Diagnostics, Basel, Switzerland): ProBNP was analyzed by an electrochemiluminescence immunoassay (Roche proBNP II) with a detection limit of 35 pmol/L. CRP was measured with a high sensitivity, particle-enhanced immunoturbid assay with a detection limit of 0.5ug/L in 2004 and using a particle-enhanced immunoturbidimetric assay (Tina-Quant CRP Gen.3) with a detection limit of 4.0 mg/L in 2011. Troponin T was determined using a high sensitive immunoassay (Roche hs-TnT) with detection limit 10 ng/L). Total cholesterol was measured enzymatically on Kodak Ektachem/Vitros in 2004 and later on Roche 6000, c501/Modular analyzer. HbA1c was analyzed by an immunoturbidimetric method (Roche Modular, Roche Diagnostics GmbH, Mannheim, Germany).

Clinical data and examination of patients in study group 2

For study group 1, clinical data were extracted from the patients' medical records at the NRH. Causes of death were ascertained in a subset of patients by comparing external records (local hospital, family doctor). For study group 2, clinical data, including type of BC surgery, clinical and pathological BC classification and adjuvant treatment were collected from the patients' medical records. A clinical examination was performed at T1 and T3, and the current thesis focuses on findings from T3. The clinical examination consisted of a general medical history with focus on cardiovascular history and risk factors including family history,

hypertension, hypercholesterolemia, DM, medications, smoking history and diet. Cardiac function as assessed by the New York Heart Association (NYHA) classification was noted for all patients (236). Measurement of blood pressure, as well as general examinations of heart, lungs, breast and the associated lymph nodes were performed. Height and weight was recorded and Body Mass Index (BMI) calculated ($\text{weight [kg]}/\text{height [meters]}^2$). The LENT-SOMA classification was used for evaluation of late effects in the skin (237). Shoulder/arm mobility was measured.

Questionnaire

For study group 2, questionnaires were developed and sent to the patients in 2004 and 2007 (97;105). A similar, but modified questionnaire was constructed for T3 in 2011 focusing on CVD, its symptoms and risk factors using questions from HUNT 2 and 3 (238). Patients were asked if they ever had a MI, angina, palpitations, syncope, heart failure, hypertension, stroke or diabetes and if they were taking medications to treat any of these disorders. All of the questionnaires included questions regarding sociodemographic factors. The fatigue questionnaire (FQ) (239), hospital anxiety and depression scale (HADS) (240) and questions from EORTC QLQ BR 23 (241) were also enclosed in all questionnaires. 178 (75.4%) patients answered three questionnaires, while 58 (24.6%) answered questionnaires only in 2007 and 2011. Papers 3 and 4 of the current thesis focus on information from the 2011 questionnaire. For variables analyzed for study purposes, the information gathered from the out-patient clinical examination was used and matched with the information on the questionnaire for quality control.

2.3.4 Statistical Analysis

Continuous variables were described using mean and standard deviation (SD) or medians and ranges as appropriate. Categorical variables were described as proportions and 95% confidence intervals. For continuous variables, groups of patients were compared using the student's t-test for normally distributed data and Mann-Whitney U-test for skewed data. For categorical variables, the Chi-square test was applied. Spearman's correlation coefficient was used to evaluate correlations between two continuous variables.

In paper 1, time to death of IHD and CVD was right truncated at 20 years and modeled with Cox proportional hazards model adjusted by age and stratified by matched set for comparison with controls.

For analysis of hypertension and hypercholesterolaemia as risk factors in paper 3, the patients were dichotomized as having reported a diagnosis of and/or treatment for hypertension and or hypercholesterolaemia. For multiple logistic regression analyses, patients were grouped according to AS <100 or \geq 100.

In paper 4, logistic regression analysis was applied to estimate the risk for SD and DD between patients and controls. Univariate and multivariate logistic regression model was used to identify independent risk factors for diastolic dysfunction within the patient group. Variables with statistical significance <0.15 in univariate analysis, or of clinical importance, were included in the multivariate analysis.

P-values of <0.05 were considered significant and all p-values reported were two-sided. In paper 3 Bonferroni correction was applied for multiple comparisons in univariate tests as requested by the reviewer. For paper 1, 2 and 3, SPSS 18 was used. For paper 4, SPSS 21 was used.

2.4 Main findings

2.4.1 Main findings paper 1

The aim of paper 1 was to evaluate long term risk of death from IHD after two different regimens of hypofractionated RT for BC. 1107 patients received 4.3 Gy x 10 (4.3 Gy group) whereas 459 patients received 2.5 Gy x 20 (2.5 Gy group).

Simplified dosimetry revealed that the PF resulted in higher doses to the heart compared to other fields. RT for left sided disease and photons for PF were associated with higher heart doses than RT for right sided disease or electron beams. 90.9% of patients in the 4.3 Gy group received RT including a PF, compared to 35.3% in the 2.5 Gy group (fig 7). In the 4.3 Gy group, 75.5% of patients with a PF received photon irradiation, PF, whereas only 29.6% of the patients with a PF in the 2.5 Gy group had their PF given with photon beams.

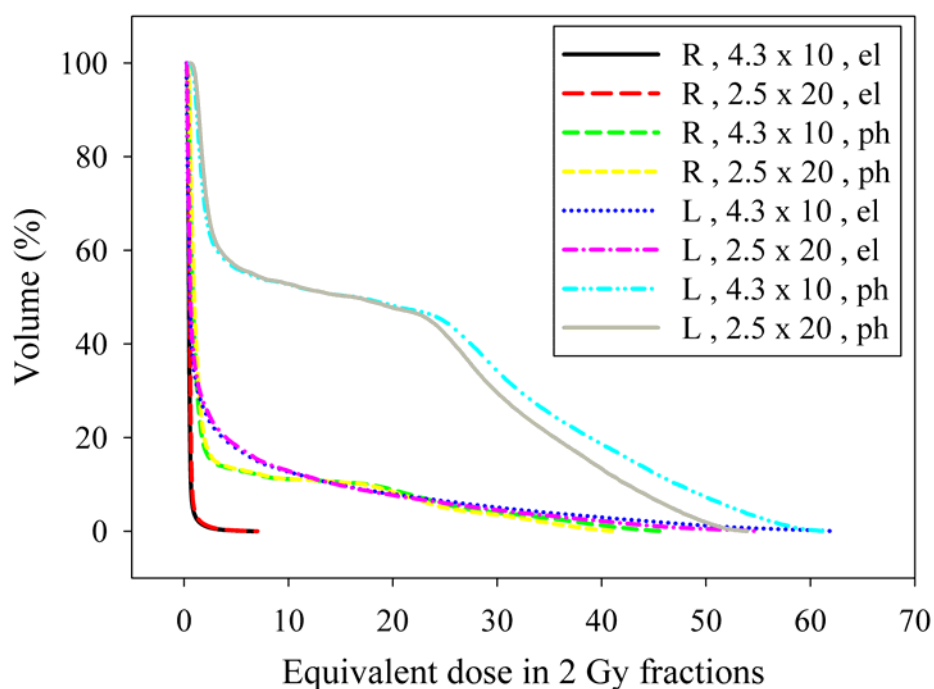


Fig 7: Dose to the cardiac volume from the PF converted to equivalent dose in fractions of 2 Gy. ph=photons, el: electrons, L: left, R: right

In the 4.3 Gy group, 40 patients (4%) died from IHD compared to 7 patients (2 %) in the 2.5 Gy group. Patients in the 4.3 Gy group had an increased risk of death due to IHD compared to those treated with 2.5 Gy (HR=2.37, 95% Confidence Interval (CI): 1.06-5.32, p=0.036), with the difference emerging after 10-15 years (fig 8)

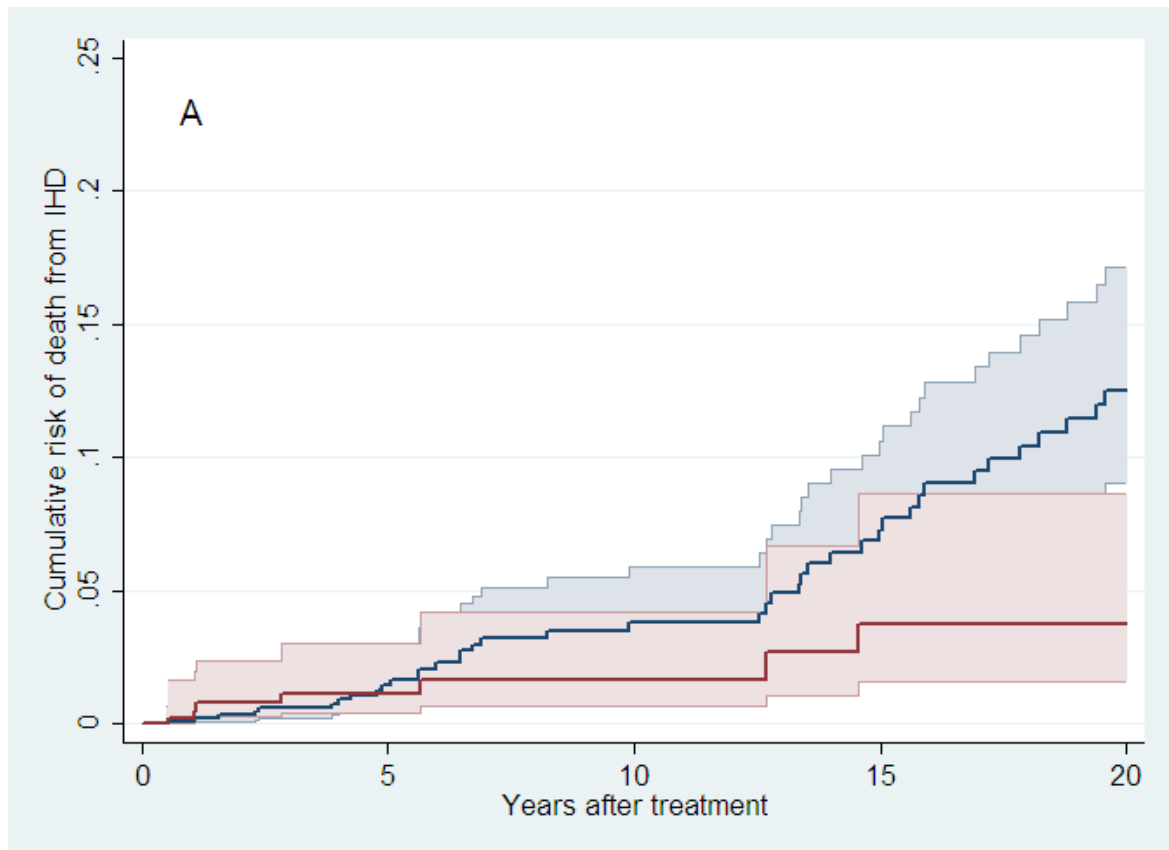


Fig 8: Cumulative risk of death due to IHD, comparing 4.3 Gy group (blue line) with the 2.5 Gy group (red line).

Patients treated for left-sided BC did not have increased risk of dying from IHD compared to those treated for right-sided BC. PF irradiation was not associated with increased mortality from IHD comparing patients from both fractionation schedules. However, within the group of patients who received a PF, photon beams were associated with an increased risk of dying from IHD compared to electron beams (HR=2.56, 95% CI:1.12-5.84, p=0.025). Treatment before 1984 was associated with an increased risk of dying from IHD (HR=2.87, CI: 1.34-6.17 p=0.006) compared to treatment from 1984 onwards.

In addition, all analyses were done with CVD as an end-point, with similar results as with IHD.

Patients in the 4.3 Gy group had increased risk of dying from IHD compared to their controls (HR=1.59, 95% CI:1.13-2.23, $p=0.008$). No elevated risk of death from IHD was found in the 2.5 Gy group compared to controls. Compared to controls, patients treated with parasternal photon fields had an increased risk of dying from IHD (HR=1.79, 95% CI:1.21-2.67, $p=0.004$). Treatment with parasternal electron beams was not associated with an increased risk of dying from IHD when comparing patients and controls. An increased risk of dying from IHD (HR=1.67, 95% CI:1.18-2.36, $p=0.004$) was found in patients receiving RT before 1984 when compared to controls, but not in patients treated from 1984-1991.

Treatment with 4.3 Gy x 10 retained its association with an increased risk of IHD when entered into multivariate analysis, but with borderline significance only (HR=2.90; 95 % CI: 0.97-8.76; $p=0.057$). The use of PFs with either photons or electrons was not associated with increased risk.

2.4.2 Main findings paper 2

Paper 2 evaluates doses to the whole heart and the individual cardiac chambers after locoregional RT for BC. 216 patients, 111 with left-sided BC and 105 with right-sided BC treated between 2000 and 2002 with CT-dose-planned RT were included.

Cohort-based medians of the whole heart mean dose for left- and right-sided tumors were 3.2 (0.7-7.9) Gy and 1.3 (0.6-4.1) Gy, respectively. Compared to women with right-sided tumors, those with left-sided tumors had significantly higher radiation doses to the whole heart and the ventricles, whereas the doses to the atria did not differ. In 13 (11.7%) patients with left-sided cancer, 5% of the heart volume was exposed to 25 Gy or more.

The individual NTCP estimates for IHD were low for the whole population included in this study, with a maximum probability of 2.8%. Moderately higher risks for patients with left-sided disease were identified compared to patients with right-sided disease.

The dosimetric findings for patients participating at T3 are further used to analyse the influence of cardiac radiation doses on CAC (paper 3) and on echocardiographic findings (paper 4) for patients that participated in T3.

2.4.3 Main findings paper 3

Paper 3 investigates CAC score in relation to treatment parameters and other risk factors of CVD in BCS. In a longer perspective, it aims to identify early, by CAC score analysis, BCSs at risk of IHD after RT. 236 BCSs having a cardiac CT scanning at T3 were examined. Five of these 236 individuals had experienced a stroke or a transient ischemic attack, 9 had atrial fibrillation, 3 had angina and 2 had a pulmonary embolism.

The median AS for the entire study group was 2 (0-1831). 132 of the 236 women (56.7%) had detectable calcium deposition in one or more of their coronary arteries. 104 (44.0%) had AS of 0, hence no CAC. No differences were found with regards to the distributions of calcium depositions in the different coronary arteries according to cancer laterality ($p=0.389$).

The CAC score observed in the BCS studied did not differ from the expected percentile distribution of CAC scores in the normal population ($p=0.468$) (fig 8). 103 women (43.6%) had AS under the 25th percentile according to their sex and age. 20 (8.5%) women were between 25-50th percentile and 58 (24.6%) were between 50th -75th percentile. 36 (15.3%) women were between the 75th -90th percentile, whereas 19 women (8.1%) were above the 90th percentile.

The distribution of calcium depositions in the coronary arteries were similar for patients with left- or right sided BC (LAD [$p=0.664$], circumflex artery (CX) [$p=0.209$], LMA [$p=0.363$] and RCA [$p=0.521$], respectively)

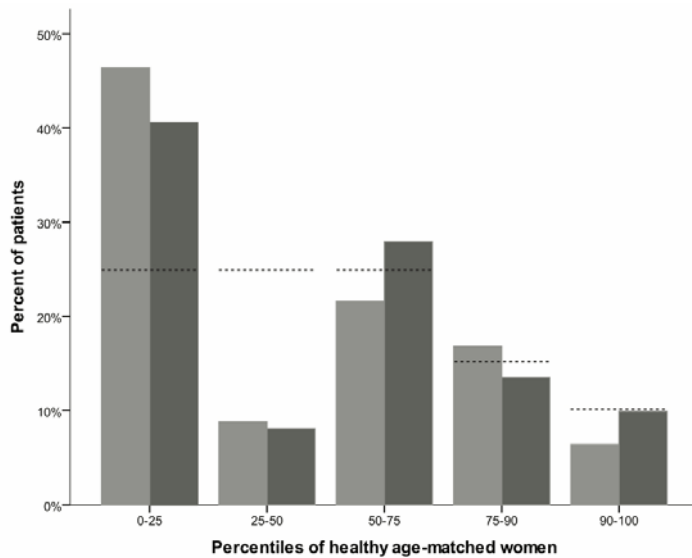


Fig 9. CAC observed in BCSs expressed as percentiles of AS of healthy matched women. Light grey columns represent left-sided and dark grey columns right-sided BC. Dashed lines depict percentages of patients expected within each percentile range if equal to healthy women.

Because CT dose planning was introduced in 2000, dosimetric data were available in 106 patients only. The AS did not differ between the group with dosimetric data and the group without these data ($p=0.650$). The cohort-based median of the mean dose for the whole heart was 2.5 (0.5-7.0) Gy, and for patients with left sided and right sided BC it was 3.0 (1.5-7.0) Gy and 1.5 (0.5-5.5) Gy respectively ($p<0.001$). The median D5% across the population was 4.0 (1.0-37.0) Gy. One patient only had a D5% greater than 35 Gy. This patient, with a D5% of 37 Gy, had an AS of 0. There was no correlation between the D5% to the whole heart and the total AS. No correlations were identified between the D5% to the left ventricle (LV) and AS of LAD, CX nor LMA. Likewise, the D5% to the right ventricle (RV) was not correlated with AS of RCA. Additionally, all analyses were done with mean dose as a variable instead of D5% with similar results.

As mentioned above, 19 patients altogether had previous CVD. These individuals did not have significantly higher AS compared to the remaining 217 patients ($p=0.242$).

Higher age ($p<0.001$) was significantly associated with increasing AS, as was a history of hypertension ($p=0.022$). Diabetes and a self-reported history of hypercholesterolemia were not associated with a high AS.

Significant correlations with increasing AS were found for increasing levels of proBNP at T1/T2 ($p<0.001$) and T3 ($p<0.001$), and for increasing levels of cholesterol at T1/T2 ($p=0.022$). HbA1C, only measured at T3, was significantly correlated with increasing AS ($p=0.022$). Cholesterol values at T3, levels of TroponinT at T1/T2 and T3 were not associated with AS, neither was CRP measured at T1/T2 nor at T3.

Age retained its significant association with AS (Odds Ratio (OR)=1.11; 95 % CI:1.05-1.17; $p<0.001$) when entered into multivariate analysis, as did cholesterol at T1/T2 (OR=1.77, 95%CI:1.24-2.51, $p=0.001$).

2.4.4 Main findings paper 4

In paper 4, the findings from echocardiography in 216 patients participating at T3 were analysed for the influence of treatment factors and other traditional risks factors on early findings of cardiac disease in BCS.

No difference in the prevalence of SD was identified between patients and controls using TDI peak systolic velocities or 2D STE. On the contrary, DD occurred in 142 patients (66%) compared to 78 (36%) controls (OR=3.5, 95% CI:2.9-5.0), $p<0.001$) Of patients with DD, 8.5% had indirect evidence of elevated filling pressure compared to 3.8% of the control group ($p=0.309$). The difference in diastolic function between patients and controls were evident in all age groups. Patients with DD had reduced systolic function parameters, lower LV dimensions and increased wall thickness compared to those without DD. Univariate analyses indicated an association between conventional risk factors such as age, hypertension, DM, hypercholesterolemia, smoking and DD.

Treatment with anthracyclines displayed an appearant protective effect towards DD, however this effect was reversed when adjusting for age. A borderline significant association was identified between manual dose planning and DD.

In multivariate logistic regression analysis, age and manual dose planning were significantly associated with DD (OR 1.2 (1.1-1.3), $p<0.001$ and 2.5 (1.1-5.6), $p=0.03$ respectively) whereas anthracycline therapy was borderline significantly associated with DD (OR 2.4 (1.1-5.8), $p=0.06$).

When analyzing right ventricular function, no significant difference was identified in peak s' of lateral tricuspidal annulus between patients and controls, neither in the frequencies of reduced peak s' using a cut-off level of 10 cm/s. However there was observed a higher displacement of LTA in patients compared to controls, but without a difference in frequencies of reduced displacement using a cut off level of 1.7 cm. Moreover, different treatment planning of RT and the use of anthracyclines did not influence right ventricular function.

Eighteen patients (8.3%) had left sided VD. There were 8 (3.7%) patients with aortic valve dysfunction compared to 0 (0.0%) among controls ($p=0.007$), and 10 (4.6%) with mitral valve dysfunction compared to 1 (0.5%) control ($p=0.015$). Only one patient had aortic stenosis, the others were valvular regurgitations. No severe VD was observed. Different treatment planning of RT and the use of anthracyclines did not influence valvular function in logistic regression analysis.

2.5 Discussion

2.5.1 Methodological considerations

In epidemiology, and to some extent in clinical research, the design of studies may vary according to the time at which the study cohort is defined (prospective or retrospective design) and how it is followed over time (longitudinal or cross-sectional design). Concerning the time point at which the study is designed and the cohort to be followed is defined, there are two main types of studies: prospective studies define the study cohort in present and aim to gather defined data in the future, and retrospective studies look back on a population and events that have already taken place.

Study 1 is an example of retrospective design aiming in retrospect to identify patients with the different hypofractionated regimens and to evaluate if hypofractionated RT increased the risk of IHD. Paper 2-4 are part of a study where the study cohort was defined retrospectively, that is patients treated between 1998 and 2002 were identified in 2004, where the design from there on was to prospectively evaluate these patients for certain long term side effects and to follow the cohort for more than a decade.

One of the main weaknesses of a retrospective study is the potentially low quality of the information gathered from the past. In both cohorts of the present thesis, the use of registries to identify the study subjects may be associated with a certain level of inaccuracy. This is evident in the identification of cohort 2 (papers 2-4) where an administrative error was revealed in 2007 and a number of patients were invited to participate at a later stage than the main group. Furthermore, retrospective collection of data on the individuals, concerning for instance disease status and treatment may present with a possible loss of details. This lack of detailed and fully reliable information is evident from the level of BC related details for study cohort 1, where routine charts and registries from the seventies and eighties are used as main sources of information. Lastly, a retrospective design does not allow studying traits or factors that are defined as interesting at the time of study design, but that were not collected during the primary treatment of the patients. This is relevant to papers 3 and 4, where information on traditional risk factors and the presence of clinical diagnoses of CVD in each individual prior to BC treatment would have been of interest. Also, definition and inclusion of patients in the study cohort at the start of treatment could have increased the participation rate and reduced the bias introduced by loss of participants throughout the study. In study cohort 2 (papers 3 and 4) many patients from the original target population have been excluded over the years

due to death, other cancers or recurrent BC, or simply because they declined participation. The population of 236 (paper 3) and 216 (paper 4) women represent only 33% and 31 % of the originally treated patients, respectively. This high drop-out rate, immanent to all long term studies in cancer survivors, may lead to selection bias, as will be discussed later.

Studies may also vary in the design as to how often information is assessed in the study cohort. Cross-sectional studies collect information at one time point. Therefore, cross-sectional studies are suitable to assess prevalence rates and to detect associations between variables. The possibility of drawing inferences regarding causality is limited (242). To some extent, the cross sectional design is a characteristic of paper 2, 3 and 4. In paper 2, we assessed the cardiac radiation dose distribution to the heart and cardiac chambers on archived CT images from the treatment planning module performed at diagnosis. In paper 3 and 4, the prevalence of CAC and cardiac dysfunction, respectively, among 12 year BCSs were examined. Even though data on treatment factors were available from start of treatment about 12 years earlier and traditional risk factors were available from a time point 7 and 4 years prior to examination, no prior information on CAC or cardiac function was available for comparison.

In a longitudinal study, information is obtained from the same individual at more than one time. This design is suitable to estimate, for instance, changes in a trait over time, such as a certain disease or pathological finding and to establish a causal relationship between exposure and disease (242). Features of a longitudinal study are present in papers 1, 3 and 4. In paper 1, long term follow-up concerning causes of death is provided by registries in the Norwegian Health care system, i.e. death certificates gathered by Statistics Norway that allow tracing individuals for the date and cause of death over decades. Coupling of data from different registries and comparison of patients with matched controls are facilitated by personal identification numbers for each patient. Since the basic structure of how to collect and record data on causes of death have been operable for decades, continuous long term follow up of these endpoints are possible. Patients included in paper 3 and 4 were examined at T1, T2 and T3 and hence, information regarding risk factors and biomarkers were available from previous examination points. Data from paper 2 regarding cardiac dose were also available. This information was used to analyze for correlations of treatment factors and assumed risk factors detectable early after treatment and later development of CAC (paper 3) or DD (paper 4). Apparently, the prevalence of overt CVD such as reported diagnoses of IHD or HF was low in this cohort of BCS 10-12 years after treatment. Longer follow-up may be important for the

cohort to look at the correlation of CAC and later IHD as well as for DD and later overt HF. In addition, it must be admitted that a longitudinal study like this ideally should be initiated before treatment in order to record pre-treatment characteristics and risk factors.

Response rate

For patients included in papers 3 and 4, the response rate for the questionnaire and clinical examination was 76% (N=236), with 69% participation rate in paper 4 due to logistical constraints. The response rate at T1 and T2 were 77% and 81%, respectively. This compares favourably to the average response rate to mailed surveys reported by Asch *et al.*, being 60% (243). However, as mentioned above; only 33% of the original patients treated from 1998-2002 were examined in 2011. 35% of the originally treated patients declined participation. The second main reason for loss of informative cases is the underlying competing incidence of death, BC recurrence and other cancers as specified in the protocol. It is not possible to evaluate the cardiac risk profile and prevalence of CVD in the patients lost from the study cohort due to death, BC recurrence or other cancers prior to study start in 2004 or the patients not volunteering for inclusion. This may represent a major source of bias (see below)

Bias

Bias is a systematic error in the design or conduct of a study, and can be classified into selection bias, information bias and confounding factors (242).

Selection bias

Selection bias occurs when the selected patients differ systematically from their representative population, i.e; the study population is not representative for the population approached (242). Selection bias results from procedures used to select individuals for the study population and from other factors that influence the study population.

All patients included in this thesis were given RT at the NRH. Referral practices in Norway over time have nearly exclusively been dependent on the place of residence. In the seventies and eighties, approximately 80% of BC patients in Norway were referred to the NRH when considered candidates for RT, only patients in Hordaland County were referred to the Haukeland University Hospital in Bergen and patients in Oslo were treated at Ullevål University Hospital. Due to establishment of regional RT departments for the mid and northern health regions in Norway during the eighties, the corresponding referral rate had

decreased to about 40% in the period from 1998 to 2002 (244). Accordingly, the referred patients might differ from patients treated at other hospitals in Norway, especially for the latter treatment period. Differences of relevance to CVD may include for example sociodemographic factors and general health factors. Since many risk factors for CVD are associated with socioeconomic status, the referral practice may bias the selected cohort for the underlying risk of CVD. However, there is little reason to believe that the socioeconomic status and general health differs much in the 4 health regions in Norway served by the 4 different University Hospitals in charge of locoregional RT in BC patients. Lastly, the treatment recommendations issued by the NBCG are followed in all Norwegian treatment centers. Therefore, we believe that referral practices represent a minor source of bias and that the findings are indeed representative of Norwegian BC patients at the time.

As mentioned above, the number of patients studied at T3 with CAC score and echocardiography represent a minority of the originally treated patients from the period 1998-2002. 231 (32%) patients were not studied due to relapse of BC, new cancer or death, and 252 (35%) patients declined. To look for possible selection bias at T3 of patients with better cardiovascular health, patient charts were reviewed to determine the cause of death. Of the 436 patients volunteering to participate at T1/T2 (including patients only answering the questionnaire), 75 (17.2%) patients died before T3. 76% of the diseased patients died from BC, whereas only 4.0 % died from CVD, none of them from IHD. Due to privacy restrictions, an attrition analysis could not be done for women who were non-eligible at study start in 2004 (due to death, relapse or second cancer) or who declined to participate.

Furthermore; selection bias may result from the relative complexity of our study- including a clinical examination, and diagnostic interventions conducted during a full day at two different hospitals in Oslo. This may have lead to a selection of the healthiest BCSs, as patients with poor health may have declined because of the inconvenience of the study itself.

Information bias

Information bias occurs when information collected regarding the study participants is lacking or is incorrect.

Bias may arise from uncertainties in source documents and registries. Patients in study group 1 were studied retrospectively and source documents such as patients' charts, RT records and data from the CRN had to be reviewed. There were uncertainties and ambiguities that had to be controlled and double checked to ensure the highest level possible for all recorded data concerning disease and treatment parameters. In addition, in study group 1, a large proportion of women are reported to have died from BC. A concern may be that the cause of death often is reported by a doctor unfamiliar with the patient, and hence may be more prone to explain the cause of death as BC given their previous diagnosis of BC. To evaluate the relevance of such misclassification in our population, 100 patient charts were retrieved to review information regarding cause of death. We were able to determine the most likely cause of death in 97 individuals, and this coincided with the cause of death reported by the CRN/Statistics Norway. The high BC mortality is most likely due to the relatively large proportion of stage III (21.6%) and IV (10.5%) patients in our sample and comparable to findings by others reporting BC outcome from similar time periods (245). The level of accuracy concerning data on BC and treatment is higher and more easily accessible in modern patients' charts and registries, thus improving the quality of data extracted for study cohort 2.

One common form of information bias is recall bias. Recall bias may be relevant to studies that contain questionnaires or that in other ways rely on the patient's recollection of their past medical history. Information on past symptoms and any diagnosis of CVD in both paper 3 and 4 was based solely on the patients own report, either in the response to the mailed questionnaires or in the out-patient interview at T3. No external information from notes at their general practitioner's office, from local hospitals or available registries were consulted. In this setting, questions regarding cardiovascular health and risk factors may pose an issue of recall bias. If patients with a past medical history of CVD do not give correct information, underestimation of disease burden may arise. Alternatively; patients with a past history of CVD may be more prone to answering details concerning their cardiovascular health as the study in all appears more relevant to them.

Confounding factors

A confounder is a variable associated (positively or negatively) with other independent variables and the dependent variable. Confounding can interfere with our ability to correctly identify the causal impact of an exposure on a given outcome. To account for confounders, stratification and multivariate analysis may be used.

In paper 1, the two groups treated with either the 4.3 or the 2.5 Gy fractionation regimens were not fully balanced for all disease and treatment parameters. In retrospect, the basis for allocation to one of the two fractionation regimens was difficult to recapitulate in all individuals, but apparently the practical considerations regarding number of visits to the hospital (favouring patients living far from the hospital to be treated with the 4.3 Gy regimen) was only one of many factors. In addition, patients with the 4.3 Gy x 10 regimens more often received parasternal irradiation. Simplified dosimetry revealed that PFs contributed to the majority of the cardiac dose, and that photon beams resulted in higher cardiac doses than electron beams. For these reasons, possible confounding effects of field arrangements and beam quality were investigated further. We found no difference in cardiac mortality when comparing patients who had received parasternal irradiation and those who had not. However, there was an increase in death from IHD in patients receiving a PF with photons compared to electrons. In multivariate analysis, however, these treatment parameters did not reveal any statistically significant result, leaving the degree of hypofractionation as the only treatment factor independently associated with risk of death from IHD. The study, however, presumably lacks statistical power to detect the impact of parasternal irradiation on cardiac outcome.

In paper 3, using risk factors for CVD as independent variables and CAC as the dependent variable, higher age was significantly associated with increasing AS, as were a history of hypertension and high cholesterol values at T1. All were included in multivariate analysis, and of the three, age and hypercholesterolemia retained its significance, thus indicating that age is a confounding factor for hypertension.

In paper 4, treatment with anthracyclines was univariately associated with lower rate of DD. In light of the well-known negative effect of anthracyclines on cardiac function, this obviously raised the question as to a confounding effect. When age was stratified for, the apparent protective effect disappeared indicating a confounding effect of age in this analysis, as chemotherapy only was given to patients <65 years. Age is a well-known risk factor of DD.

Ultimately, the use of anthracyclines was positively associated with a higher risk of DD in multivariate analysis.

Internal validity

Internal validity refers to the extent to which one can conclude with confidence that an independent variable has in fact produced an observed effect and to which extent the results can be generalized to the whole group of BCSs approached in the study. To evaluate internal validity, representativeness, bias and possible confounders need to be considered (246).

Only 106 of the 216 patients examined for heart dose in paper 2 were included in paper 3 and 4. To check for internal validity of their parameters, i.e. to demonstrate that the 106 were a representative subgroup of the patients with CT-planned RT, the cohort based median of the mean dose for the two groups were compared and found to be very similar (for all 216, left-sided BC 3.2 Gy and right-sided 1.3 Gy; for the 106 participating in T3, left-sided 3.0 Gy and right-sided 1.5 Gy).

Again, the fact that only 33% of the originally treated BC patients were examined at T3 may limit the internal validity of the findings. It is not known whether these BCSs are representative for the entire cohort and thus, this may represent an issue of the internal validity of papers 3-4. Due to privacy restrictions, an attrition analysis could not be performed on the individuals not consenting to participate at any time point (39.4% of the patients). However, as mentioned above, an analysis of causes of death in BCSs consenting at T1 and/or T2, revealed a low rate of death from CVD, thus indicating that the difference in CVD burden did not differ extensively between the group examined at T3 and those who were not further assessed after T2.

External validity

External validity of a study addresses the ability of the results to be generalized to other populations than the approached study population, i.e. whether the results from the selected study group can be relevant to a general population of similar individuals.

For paper 1, external validity would address the question whether the results can be generalized to other BC patients treated with RT for BC in Norway from 1975-1991. In 1975, the NRH treated approximately 80% of all BC patients in Norway, with the exception of

patients treated at Haukeland and Ullevål University Hospitals. Towards the end of the eighties, RT was also available in Tromsø (1986) and Trondheim (1987). The fractionation regimens applied at the other hospitals were the conventional 2Gy x 25. The regional difference in fractionation limits the relevance of the results from paper 1 to the whole population of BC patients given RT at that time. However, a proportion of the cohort in paper 1 received a less hypofractionated regimen, 2.5Gy x 20, more similar to the conventional treatment given at the other hospitals.

Hypofractionated RT for BC has become increasingly used over the past decade, and the interesting question is how the regimens examined in paper 1 can be compared to the hypofractionated regimens applied today. The fractionation regimens applied today most commonly use daily doses of 2.5-3.2 Gy, and hence, the 4.3 Gy regimen examined in our paper may not be comparable to these contemporary schedules (52;53;199). Furthermore there are large differences as to the field arrangements, as well as the absence of 3D dose planning and respiratory gating in all patients in study group 1. The 2.5 Gy x 20 regimen examined in paper 1, however, would be comparable to some of the regimens applied today. Of interest, even in the lack of 3D dose planning and respiratory gating, no increased risk of death from IHD was found for these patients, supporting the safety of modern hypofractionated RT regimens for BC.

For papers 2- 4, external validity related to how the results would apply to other BCSs treated for BC stage II/III in Norway from 1998-2002. At that time, approximately 40% of all BC patients receiving RT in Norway were treated at the NRH. Moreover, the treatment guidelines at that time were issued by the NBCG and applied nationwide. Although the way guidelines are implemented at different hospitals and followed by individual physicians is known to vary, we are not aware of any systematic difference in RT application for Norway in that treatment period. However, the results of our study may not be applicable to the patients treated today mainly because changes in recommended target volumes (IMLNs not irradiated since 2005) and the use of respiratory gating may spare the heart tissue in a way not done 12-15 years ago.

At examination, study group 2 had a low level of heart disease. We do not know whether our results in these studies would apply to a population with a more pronounced risk profile or disease burden with regards to CVD.

Statistical power

A certain sample size is needed in clinical or epidemiological trials to ensure that differences between groups can be considered trustworthy (246). Even though one generally accepts results that would occur by random with a chance of 5 % or less (the generally accepted confidence level of p-value smaller than 0.05) as true, there is a chance that these results have occurred randomly (type I error). The possibility of type I errors was raised as a concern by the editor of Radiotherapy and Oncology when reviewing the submitted manuscript of paper 3. Testing for multiple variables in repeated tests will increase the likelihood of type I errors. To account for this, the traditional Bonferroni correction was applied whereby the p-values obtained from each test were multiplied by the number of tests performed (n=22). There is generally no clear consensus that Bonferroni correction is appropriate in hypothesis generating research, and the method is considered a conservative and strict approach to omit type I error. Thus, there is a possibility of loss of clinically relevant findings when Bonferroni correction is applied.

If the number of subjects and endpoints analyzed are too small, there is a chance that meaningful differences between groups go unnoticed due to a lack of power of the study (type II error). Type II errors are best avoided by doing upfront calculations on the number of patients needed to detect the differences of interest, either based on preliminary data in similar patients and interventions or based on assumptions on what would be considered clinically relevant differences worth knowing about. For the studies relating to CAC or echocardiographic findings in BCSs after more contemporary adjuvant treatment, no such calculations were possible due to lack of previous data.

In paper 3, the level of CAC in the BCSs was found to be similar to the general female population. Furthermore, the numbers of patients with a concomitant diagnosis of IHD or other forms of CVD were small. The possible correlation between CAC score and simultaneous diagnosis of IHD may therefore have gone unnoticed in this cohort, in contrast to what our group has found for HL survivors after mediastinal irradiation (247). Likewise, differences in CAC in relation to cardiac doses might have been detectable if the patient proportion had been larger. A common way to counteract difficulties from lack of power is to increase the population studied as the study progresses. Due to the longitudinal design of the study, relying on the data from two previous time points, we could not expand the cohort

investigated in a meaningful way. To expand study group 2, a national or international cooperation would have been preferable.

Relating to the risk of type II errors and the possible underestimation of the value of CAC scores, several observations from papers 2, 3 and 4 substantiate the impression that the general cardiovascular health in study cohort 2 is good, that the prevalence of IHD/CVD 10-12 years after treatment is low, and that future risk for IHD/CVD is low. First, attrition analyses reveal that very few patients (4 of 436) not included in T3, are dead of CVD. Second, only 9 patients attending T3 report any form of CVD. And finally, different NTCP-models applied in papers 2 and 3 suggest that the cardiac doses resulting from the RT (as measurable for the 216 patients with CT dose planned treatment) are associated with a low future risk of CVD. In paper 2, the relative seriality model was applied to calculate individual NTCPs (248). The NTCP estimates were very low for the whole population, with a maximum probability for IHD of 2.8% in one patient with left-sided radiation. If NTCP-values $\geq 5\%$ had been identified, the beneficial effect of RT could have been comprimized (37). In paper 3, an alternative NTCP formula presented by Darby *et al.* was applied to estimate the number of major coronary events (MCE, myocardial infarction or death due to IHD) relative to the radiation dose they had been exposed to. The estimated numbers of MCE are 2.8 (1.19%) for the current study population, 1.6 (1.22%) for the left sided BC group and 1.2 (1.11%) for the right sided group. In summary therefore, the chosen cohort of survivors after RT with low to moderate cardiac exposure may not fully reflect a possible value of CAC monitoring in patients with higher exposure.

The prevalence of DD in the cohort of 10-12 year survivors of BCS was, compared to relative normal findings in CAC, unexpectedly high. Depending on the rate of progression to overt HF in coming years, the cohort may be large enough to address the value of early detection of DD during further follow up.

Comparison to data from healthy subjects

The use of data from healthy control subjects allows interpretation of the clinical significance of our findings. For the analyses presented in paper 1, Statistics Norway supplied ten randomly selected cancer-free controls per patient matched on age, education and county of residence. Matching on relevant risk factors for CVD was not possible, mainly due to the scarce availability of this information both for the patients and in the general population. We

believe that matching on education and county of residence, factors reflecting the socioeconomic status of the individual would be the best available way to match for correlates of risk factors of CVD. Also, by selecting 10 controls per patient, we minimized the effect of random variation.

For the analysis of CAC in paper 3, published CAC scores in the general population published in 2001 were used as control material. This study presents a set of population based standards of CAC-score, and reports the distribution of CAC as a function of age and gender in a sample of 35246 subjects (230). Healthy asymptomatic individuals 30-90 years of age, and of socioeconomic status above the United States average, were studied from 1993-1999. Despite limitations concerning the comparability to Norwegian BCSs, we believe the data to be the best external comparator available. Limitations include recruitment in a different decade and different life-style factors in the two countries. Patients in study group 2 were examined with a multi-detector CT, whereas the normal individuals examined by Hoff et al were examined by electron-beam CT. However, it has been documented that the two methods have equivalent reproducibility for measuring CAC (250).

For the analyses in paper 4, a control group for echocardiographic parameters was extracted from the Health Study of Nord-Trøndelag County, Norway (HUNT) (228). The patients and their controls were matched 1:1 on age, sex, body weight and systolic blood pressure. The HUNT studies include more information on each individual, but the number of patients with an echocardiography is limited, and therefore matching on more items is difficult. The chosen parameters were considered the most relevant. In many respects, Nord-Trøndelag County is representative of Norway. However, the prevalence of higher education is lower than average for the Norwegian population (251). Low level of education and socioeconomic status are associated with a higher risk of CVD (252). However, as standards of living and access to health care is relatively homogenous throughout Norway we believe these differences to be of minor importance for the purpose of comparison.

2.5.2 Discussion of the main findings

Paper 1

Findings from paper 1 indicate that highly hypofractionated RT and parasternal photon beam irradiation may both be associated with increased IHD mortality in BC patients. Higher IHD mortality was found for women treated with 4.3 Gy fractions compared to 2.5 Gy fractions and for those treated with parasternal photon beams compared to electron beams. For both treatment parameters, the difference in IHD mortality also emerged compared to matched healthy women. In multivariate analysis, only fractionation retained a borderline significance.

The study was mainly inspired by recent developments that aim to reestablish hypofractionated RT regimens in treatment of BC patients. A number of recent studies have evaluated hypofractionated RT for BC, most notably the randomized controlled trials START A and B (52;53). The endpoints of these trials are loco-regional tumor relapse, late normal tissue effects and quality of life. START A compared three different patterns of fractionation: 3.2 x 13 (41.6 Gy), 3.0 Gy x 13 (39 Gy) and the conventional 2.0 Gy x 25 (50 Gy). The target volume included was either the whole breast or the whole breast with the addition of a supraclavicular field with or without the axillary chain. With a median observation time of 5.1 years, 3.2 Gy x 13 was found similar to the conventional regimen (2.0 Gy x 25) in terms of local tumor control and late normal tissue effects. The 39 Gy regimen had a lower rate of late local cosmetic effects, but was associated with an increase in loco-regional relapse. In the START B trial, 2215 women with early BC were randomly assigned to receive either 2.0 Gy x 25 (50 Gy) over 5 weeks, or 2.67 Gy x 15 (40 Gy) over 3 weeks (53). Median follow-up was 6 years, with lower rates of late effects after photographic and patient-reported assessments in the hypofractionated regimen compared to the conventional RT regimen.

A review of START A and START B was published in 2013 and long-term follow-up confirms that hypofractionated RT with doses of 2.67 Gy x 15 gave as good locoregional control as the conventional 2 Gy x 25, and with a lower rate of moderate to severe late effects, including IHD, after 10 years observation time (200). The START meta-analysis did not find subgroups with poorer response to hypofractionated treatment.

In the UK FAST trial, 915 women were randomly assigned to 50 Gy in 25 fractions or to a total dose of 28.5 or 30 Gy, given as once-weekly fractions of 5.7 or 6.0 Gy, respectively. After median follow up of 3 years, 28.5 Gy was comparable to the 50 Gy regimen with

regards to photographic breast appearance, but patients given the 30 Gy regimen had significantly higher risk of late adverse effects (51). The two hypofractionated regimen of the UK FAST trial are the two contemporary regimens that most resemble the 4.3 x 10 regimen evaluated in paper 1, and the early results of the UK FAST trial also seem to discourage the use of the most hypofractionated regimens.

Apart from the START review, there are, to our knowledge, no other prospective randomized studies that encompass the effect of hypofractionated RT on the cardiovascular system. Hence, other retrospective articles have been reviewed to evaluate the cardiac safety on hypofractionated RT for BC, some of which are discussed below.

The effect of fractionation of locoregional RT for BC on the rate of fatal cardiac and cerebrovascular (CCV) events was evaluated by Stokes *et al* in a retrospective study including 4929 patients. They found that fraction sizes of 2.66 Gy did not increase the risk of CCV events after a median observation time of 11.7 years (201). Chan *et al.* evaluated 5334 women treated for BC with RT towards the breast/chest wall with TF. One group was treated with a hypofractionated RT regimen (up to 2.75 Gy/fraction) and another group with a conventional regimen (≤ 2 Gy/fraction). The authors reported no difference in cardiac or other cause mortality after an observation time of 15 years (253). The impact of fraction size on cardiac mortality was also evaluated in a retrospective population based study by Mahrin *et al.* including 7447 women irradiated with TF only (199). Median follow-up was 7.9 years. There was no difference in cardiac mortality in women treated with hypofractionated regimes (most frequently 2.5-2.75 Gy per fraction) compared to those treated with fractions of 1.8-2 Gy. However, there was a non-significant trend towards increased cardiac mortality for patients with left-sided BC treated with fractions >2 Gy. Patients included in the study were not treated with PF and thus, cardiac doses were probably lower than in our cohort. This is further reflected in a lower rate of cardiac deaths of 1.3% of the patients reported by Mahrin *et al.* to have died of cardiac events, compared to 3% dying from IHD alone in our series. We did not identify an increased mortality from IHD in BCSs treated with 2.5 Gy, the group that also less frequently received PFs, supporting the notion that moderately hypofractionated regimens without parasternal irradiation seem to be associated with little excess cardiac mortality. Based on these data, the NCCG decided in 2013 that treatment with 2.67 Gy x 15 is an option for patients with T1-2pN0 status as a part of BCT. To minimize the dose to the heart, treatment to left-sided BC patients is given via respiratory gated RT.

The study presented in paper 1 revealed no differences in cardiac mortality comparing left- with right-sided disease. This is in contrast to most studies demonstrating an increased risk of cardiac death after treatment for left sided BC (29). The most reasonable explanation for such as discrepancy is the lack of power inherent to our limited sample size. Frequent photon irradiation towards the right internal mammary chain in the present study may be another explanation. A right-sided PF is associated with a lower total cardiac dose, but important structures such as the right proximal coronary artery will receive similar doses as do structures on the left side in left-sided fields, probably putting the patient at risk of IHD and death (254).

The comparison of fractionation regimens in paper 1 is not based on randomization or an otherwise specified patient selection for the different regimens. Initially, it was thought that the 4.3 schedule would fit particularly well for patients living far from the hospital whereas the 2.5 schedule predominantly was offered to in-house patients and patients living nearby the hospital, simply due to logistical issues. This was, however, clearly not the only characteristic used for patient selection, evident when all available details of these patients were considered. Thus, the groups were not balanced for important patient and treatment parameters, all of which may have influenced the outcome. Some of these differences in patient selection we may have detected, while others may have passed unrecognized and thus represent unknown confounders in our analysis. We do however, not believe that differences in stage of BC between the groups is important, since the risk of death from IHD, once you have become a long term survivor, is probably not influenced by stage or other BC related parameters.

Further, PF's and photons versus electrons for parasternal irradiation were used differently in the two schedules. In particular, patients with the 4.3 Gy x 10 regimen more often received parasternal irradiation shown to contribute most to cardiac doses. This was evaluated as a possible confounding factor by comparing patients who had received parasternal irradiation and those who had not. There was no difference in death from IHD between the groups. However, there was an increase in death from IHD in patients receiving parasternal photon fields compared to electrons. The role and use of the PF in treatment of BC is debatable. Nilsson *et al.* found an increased risk of coronary artery stenosis after RT including parasternal photon beams both to the left and right internal mammary chain, and several studies have found increased risk of cardiac disease in patients irradiated with such fields (255;256). On the other hand, Højris *et al.* found no increase in morbidity or mortality from

IHD in a randomized trial comparing adjuvant radiotherapy including a parasternal electron field and no RT (257).

The main strength of this paper is the relatively large number of included patients from overlapping periods and the long observation time that enables us to look for late effects beyond the first decade. The higher cardiac mortality associated with the 4.3 Gy x 10 emerged after more than 10 years after treatment. This finding underscores the need for longer follow up of recent trials before final conclusions can be made on the safety of contemporary hypofractionated RT. Furthermore, our study compares BC patients to matched healthy controls, again showing a higher risk associated with the highly hypofractionated 4.3 Gy x 10 regimen, but not with the moderately hypofractionated 2.5 Gy x 20 regimen.

Paper 2

Paper 2 evaluates cardiac doses in a cohort of consecutive patients with stage II and III BC who received adjuvant RT planned with individual CT scans. The data presented in paper 2 serve as a background for the larger part of the project examining the cardiovascular health of these patients 10-12 years after treatment. The overall intent of paper 2 is to analyse cardiac doses for later correlation to clinical findings, most notably from CT-CAC and echocardiography.

The beneficial effect of adjuvant RT for BC has lead to increased research on long-term radiation-related adverse effects and the possible reduction of these. Cardiotoxicity is among the most severe long-term adverse effects and multiple studies have found that tumor laterality has great impact on radiation doses to the heart with additional impact of the RT technique applied (29;42;141). Due to recent advances in RT techniques, such as the introduction of CT-based treatment planning, IMRT and DIBH there has been a gradual decrease of cardiac doses during the last decades (133). The opportunity to achieve individual CT-based DVHs has lead to guidelines regarding cardiac radiation doses. From year 2005 onwards the NCCG recommended that not more than 5% of the heart's volume should receive >25Gy. 13 of the included patients in paper 2 (all left-sided) violated these criteria. The most recent guideline from the NCCG (2013) states that the mean dose received by the heart should not exceed 2 Gy, but also recommends DIBH technique for left sided BC. Hence, the mean dose for the right-sided patients included in paper 2 (1.3 Gy) is well below today's

recommended value, whereas the mean dose for the left-sided BC patients (3.2 Gy), without the use of gating, was above the threshold of 2 Gy (16).

For the last decade, it has been debated which part of the heart is most sensitive to RT, and thus should be chosen as the reference point for cardiac tolerance dose (136). The coronary arteries have been suggested, and in particular LAD being the most exposed coronary artery as reflected by its position on the anterior part of the heart (198). The coronary arteries were not delineated in our study due to difficulties in identifying them, mainly because of the lack of intravenous contrast. However, Darby *et al.* found that the mean dose to the heart was a better predictor of long term IHD than the mean dose to the LAD (132). Taylor *et al.* identified a mean dose to the heart of 13.5 Gy from manually planned left TF including the IMN. The corresponding dose to the LAD was 32 Gy; hence, the dose to the LAD was 2.4-fold higher than the mean cardiac dose (258). Due to differences in radiation techniques (slightly different field borders), there is an obvious limitation of applying this calculation on our patient cohort. However, with that in mind, our series resulted in a mean dose to LAD of ≤ 8 Gy for left-sided tumors. Hence, the introduction of CT-based RT may have led to a reduction of doses to the LAD for patients with left-sided tumors.

A limited number of studies have evaluated cardiac doses after contemporary RT for BC, thus comparison with other studies is difficult. As mentioned above, Taylor *et al.* found a mean cardiac dose of 13.2 Gy after left-sided treatment of the chest wall and IMLNs with TF with 6 MV photons, but the treatment was manually planned (258). In another study, Taylor *et al.* evaluated cardiac doses in 50 consecutive patients who received CT-planned RT for BC. They found a mean dose of 2.3 Gy after left-sided irradiation, lower than in our population, but the fields did not include the IMN (134). Another study compared the mean cardiac dose in patients with CT-planned TF's in patients who received manually planned RT. They found a significantly lower mean dose, 6 Gy versus 8 Gy after the introduction of CT-based treatment planning (259). Accordingly, the doses described in paper 2 appear moderate to low compared to other studies. However, a great variability in cardiac doses received were identified, in particular large doses were received by some patients with left-sided tumors. Inter-patient variations of the individual breast and thoracic anatomy (size, contour, lumpectomy vs. mastectomy) may explain some of the variability found in our study. Due to the heart's position mainly in the left thoracic half, these influences are predominantly recognized for left-sided tumors (260).

All patients studied in paper 2 received irradiation to the ipsilateral IMLNs. As presented above, the cranial part of the IMLNs was included in the anterior photon field covering the cranial part of the target volume. The caudal part of the ipsilateral IMLNs was included in the TFs. No separate PF was given. Including the IMLNs in the TFs results in a lower cardiac dose as opposed to a separate PF given as a direct anterior field. As mentioned above, Taylor *et al.* found that TFs including the IMLNs resulted in a mean dose of 13.2 Gy, whereas a separate photon PF resulted in a mean dose of 16.7 Gy (258). In contrast, IMLNs irradiated with a separate PF given with electrons resulted in a mean dose of 2.7 Gy. The EORTC trial 22922/10925 evaluated the survival and cardiac toxicity in approximately 4000 patients three years after elective irradiation of the IMLNs and medial supraclavicular nodes. The authors reported no increase in cardiac toxicity related to the irradiation of these structures. However, it was suggested that a follow-up of at least 10 years was needed to determine whether cardiac toxicity is increased after RT (261). As evident from the above, IMLN irradiation and the choice of beam energy to achieve this have been debated and remains controversial. The irradiation of the ipsilateral IMLNs was abandoned in Norway in 2005.

The cardiac dosimetry presented in paper 2 does not account for the contribution to the total heart dose from electron boost irradiation. This additional therapy was given to a subgroup of 36 patients under the age of 50 after BCT (see above). The dose planning system Helax-TMS (in use from 1994 to 2006), being based on a pencil beam algorithm, does not provide reliable dose estimates when heterogeneous tissue is irradiated by electron beams. Thus, the electron boosts were planned manually after CT based planning was completed. However, the estimated contribution by this electron boost was typically around a D5% of 0.2 Gy for a boost dose of 16 Gy. This value was determined for a patient of typical build on a contemporary treatment planning system using Monte-Carlo simulations (Oncentra planning system). Admittedly, dose contribution from boost irradiation may be somewhat higher in patients with medially located tumors. However, the contribution to the heart dose from electron boost fields is expected to be small for the 36 patients in question.

For each patient, DVHs for the whole heart and cardiac chambers were obtained. DVHs of the heart were employed to calculate individual NTCP in terms of IHD using the relative seriality model. These estimates indicate a low risk for IHD in this population as a whole. As mentioned previously, the low predicted (from the cardiac doses at the time of treatment) and

later observed (at the timepoint T3) rate of IHD in the study cohort reduced the likelihood of detecting correlations of dose and cardiovascular late effects

The main strength of paper 2 is the relatively large sample size of consecutive patients treated in a homogenous way in “real life”. The data is essential for correlation to long term cardiac outcome in the patients assessed at T3.

Paper 3

This paper found no excess CAC in BCSs treated with locoregional RT compared to the general population. Several lines of observation substantiate the conclusion that a low to moderate cardiac radiation dose does not greatly influence levels of CAC 12 years after treatment. No influence of laterality of the cancer, an important radiation related parameter, was detected when analyzing all 236 patients. A more detailed analysis was conducted in the 106 patients where dosimetric values were available and there was a lack of correlation between the regional calcium deposition and doses to the left and right ventricle.

Chang *et al.* evaluated the CAC-score of 20 BC patients treated with RT after a median observation time of 8 years (262). No increase in coronary calcium burden in these patients was identified, and there was no difference between patients with right and left-sided BC. Being a first pilot study of CAC-score after RT in BC, the authors called for larger surveys with longer observation time. Our study serves as an important contribution in that regard.

In recent years, evidence has emerged that CAC-score is a valid and reproducible predictor of IHD in asymptomatic individuals. Becker *et al.* studied prospectively the predictive value of CAC in 1726 asymptomatic individuals with a mean observation time of 40.3 months from the time of cardiac CT to clinical endpoint or study closure. They found that patients at risk for future MI could be identified within an asymptomatic population independent of concomitant risk factors. In addition, they found that future cardiovascular events, limited to the relatively short observation time of less than 4 years, could be excluded in patients without coronary calcifications (180). These findings have been supported by others (179). It is currently not known whether CAC-scores can have a similar predictive value in individuals after previous RT involving the heart. This remains an important question if CAC-score is to be used as a non-invasive test of present or future IHD in cancer survivors. Studies of HL survivors suggest that a relationship exists between CAC-scores and IHD also after RT (181).

However, this matter is still largely unexplored and no larger cross-sectional or prospective studies have been published.

Paper 3 is a part of a longitudinal study, but the patients examined at T3 are examined cross-sectionally. To address the question of CAC-scores as a predictor of CVD in general or IHD in particular, CAC-scores in patients with or without known CVD or IHD could be compared. The levels of CAC in the present cohort are within the range of the normal population and the prevalence of CVD in the 236 patients at T3 is low, with only 8% of patients reporting any form of CVD at examination. No significant difference in AS was found between these individuals and the individuals without a past medical history of CVD. Probably, the power of the study is therefore too low to prove a correlation. In survivors after mediastinal RT for HL, such an association between levels of CAC and coexisting IHD would seem to support this relationship, but in these patients, cardiac doses were considerably higher and follow up times longer (181).

It is well recognized that cardiac effects may develop decades after treatment, and it may be that our observation time of median 12 years is too short to fully evaluate the value of CAC measurements. Longer follow-up of these 236 patients is thus warranted to address this question. With the intended longitudinal design of this ongoing project and the continued high level of support from the patients, we believe this to be possible.

As described in the background section, there are two central theories regarding the development of IHD after RT. According to the first hypothesis, RT increases the frequency of MI by interacting with one or more steps in pathological pathway of age-related coronary artery atherosclerosis, resulting in an accelerated atherosclerosis seen at a younger age than it would normally occur (33;141). The second hypothesis is that radiation increases the lethality of MI by reducing the heart's tolerance to acute infarctions as a result of microvascular damage to the myocardium. The MI may occur due to pathologies unrelated to radiation (33;129). The normal distribution of CAC in BCS after RT may support the latter hypothesis, as the microvascular changes will probably not be visible on this type of investigation.

The current study explored the utility of CAC as a possible early marker of IHD and not death from IHD itself. The number of patients needed to correlate CAC scores to disease may be high. The need for high numbers of patients would be expected as results demonstrating

higher morbidity or mortality from IHD in irradiated BCSs are typically derived from meta-analyses of randomized trials or register data involving large number of patients and longer periods of follow-up (29;37;132). It follows from statistical power calculations that important changes in relative risk (two or threefold increase), with a low risk (1-2 %) in the reference group, large numbers of patients are necessary to demonstrate such changes with statistical significance. This may be even more pronounced if one chooses to study an early marker of subsequent disease.

For the 106 patients in our cohort with available dosimetric data, the median value for the mean cardiac dose was 3.0 Gy (left-sided BC), and 1.5 Gy (right-sided BC). With such low to moderate cardiac exposure, our study population has a low risk of developing CVD. Using a 1% risk of CVD within 10 year for otherwise healthy 50 year old Norwegian women as a background estimate (249), the proportional increase in the rate of major coronary events (MCE, MI or death due to IHD) per Gy of radiation may be estimated using the NTCP formula presented by Darby *et al.* (132). Using this formula, the estimated numbers are 2.8 MCEs for the current study population, 1.6 after treatment for left-sided BC group and 1.2 for those treated for right-sided disease. These low numbers correspond well with the low NTCP values for IHD identified in paper 2.

Brenner *et al.* used the same formula from Darby *et al.* to predict the increase in rate of MCE in a group of 48 patients treated for BC after 2005(263). They found an estimated excess lifetime risk of MCE ranging from 0.05% to 3.5%, with a typical value of 0.3% for contemporary radiation techniques. Applied to the numbers from paper 3, the current population has a comparable excess chance of 0.18% of developing a MCE (0.22 % for left-sided BC and 0.11 % for right-sided BC).

Paper 3 did not reveal any correlation of CAC and the use of adjuvant chemotherapy. Specifically, no impact of adjuvant anthracycline treatment was found. BC patients who received anthracyclines as a part of their adjuvant treatment did not have increased CAC, nor did they have more CVD at T3. The cardiotoxic effects mediated by anthracyclines are diverse and range from acute to late effects and from arrhythmias to HF, but do not typically include IHD (130). Therefore, a higher CAC-score after FEC would not necessarily be expected based on the biology of cardiac damage by anthracyclines. Limited power may be a plausible explanation as to why an increase in overall CVD was not observed.

Paper 3 describes the relationship between established risk factors of CVD and the presence of CAC in BCSs. Increasing cholesterol values measured 2-7 years after treatment, but 4-10 years before evaluation of CAC, were positively correlated with AS in multivariate analysis. In addition, a history of hypertension was univariately associated with increasing AS. These risk factors may be modifiable by changes in lifestyle, such as smoking cessation, diet alterations, physical activity and possibly medication; hence the impact of these risk factors on later IHD or CVD in BCS may be modified by lifestyle changes in similar fashion as in the general population. It has been shown previously that women with pre existing risk factors, such as hypertension and hypercholesterolemia, have a greater absolute increased risk of developing CVD from RT than other women (49;132). Together, these data imply that there may be value in reducing risk factors of CVD at the time of diagnosis and during follow-up.

Cardiac biomarkers such as Troponin T and proBNP have been used to predict cardiac damage from cardiotoxic chemotherapy with promising results (264). In the current study, women with high AS had significantly higher levels of proBNP at both data collection points, which is both 5-7 years prior to and at the time of CAC-CT. However, proBNP were not associated with AS in multivariate analysis. With the cautious note that the relationship between AS and later CVD in BCSs has not been shown, neither by us nor other groups, it remains unclear whether these biomarkers will prove better than traditional risk factors in early prediction of cardiovascular complications after cancer treatment.

In summary, the findings in paper 3 suggest an important link between traditional cardiac risk factors in BC patients at the time of diagnosis and during follow-up, and the development of CAC. We have not managed to establish an association between CAC-scores and simultaneous signs of CVD or IHD, and the prospective predictive value of the CAC-scores determined 10-12 years after treatment needs to be evaluated further. However, with external evidence from other sources, BCS and their health care providers may address issues of risk factors and possibly avoid later cardiovascular complications.

Paper 4

Paper 4 identifies a more than threefold risk of development of LV DD in long term BCSs compared to healthy controls. No significant impairment in LV systolic function was revealed even when more sensitive echocardiographic methods such as TDI and GLS were applied.

For myocardial disease, the use of conventional 2D echocardiography has been the mainstay of monitoring myocardial function in cancer survivors (32;110). Unfortunately, LVEF assessment as evaluated by 2D echocardiography does not detect early myocyte damage due to cardiotoxic therapy (183), and is a poor predictor of later HF (184). In that regard, cardiac MRI may have advantages in the assessment of cardiac function (155), and the method is currently evaluated for use after oncological treatment (187). Unfortunately, the availability is limited by its high costs, and the application of contrast. As a result, more sensitive echocardiographic techniques such as 2D STE and TDI are evaluated for detection of early cardiac alterations. STE and the evaluation of GLS may be useful tools in detecting early LV dysfunction after administration of RT and chemotherapy (265). In HL survivors, all treated with mediastinal RT two decades before examination with echocardiography, Tsai *et al.* reported reduced GLS in survivors also treated with anthracyclines compared to subjects treated with RT only. No comparison to the general population was performed (266). In paper 4, despite the difference in peak septal s' between patients and matched controls, there was no difference in the prevalence of SD. With relatively small doses of anthracyclines and low to moderate cardiac radiation exposure of the BCSs studied, it may well be that the effect of age on LV systolic function is of greater influence than the impact of cancer treatment.

On the contrary, the current study identified that LV diastolic parameters were markedly different between patients and controls matched for age, gender, weight and systolic blood pressure. A comprehensive assessment of LV diastolic function displayed a remarkably increased prevalence of DD in the BCSs. This finding is consistent with other studies reporting assessment of diastolic function as helpful for detecting cardiac alterations despite normal EF (267;268). Findings of DD with preserved EF is common in the general population, and it has been shown to predict the development of HF as well as other cardiac events (109). Likewise, changes in LV diastolic function after treatment of cancer patients may precede changes in detectable systolic abnormalities by many years (269). In addition, DD has been identified as an important risk factor for cardiac and all cause mortality, also in survivors after mediastinal RT for HL (109;139).

Treatment of DD to prevent progression and development of diastolic HF primarily involves treating the underlying risk factors and co-morbidities like hypertension, diabetes and hypercholesterolemia. There are still uncertainties regarding the efficacy of these treatments on clinical outcome (270). However, there are indications that asymptomatic DD leaves the heart vulnerable for added stress and thus may progress to systolic failure, thus, early identification and treatment with established HF treatment may be of value (268).

The most common cardiotoxic late effect of anthracyclines is progressive cardiac dysfunction that may ultimately lead to HF. Univariate analysis of anthracycline-containing treatment identified an apparent protective effect of anthracyclines on DD. The obvious explanation for this is that chemotherapy was given to the younger patients exclusively (<65 years old). When controlling for age, the protective effect was eliminated. Ultimately, in multivariate analysis, anthracyclines were associated with an increased risk of DD with borderline significance. This is more in line with previous published findings (267).

The current study identifies manual treatment planning, as opposed to 3D CT dose- planning as a risk factor for DD. This effect is evident also when controlling for observation time, use of anthracyclines and age. The estimation of cardiac dose from manual treatment planning is difficult due to limited dosimetric data. As such, dosimetric data were only available in the 106 patients with CT-planned RT. However, as outlined above, comparison of the two principal techniques by demonstrated that 3D-planning is generally associated with lower cardiac doses (259). The higher risk of DD in the manually planned patient population may therefore be a result of higher cardiac doses rendering them more susceptible to later cardiac dysfunction.

In the 106 patients where dosimetric data on RT were available, we could not identify any correlation between RT doses and echocardiographic parameters. Neither did we find a correlation between RT doses and the prevalence of SD or DD. As mentioned previously, this may be explained by the relatively low cardiac doses received by the BCSs in our cohort.

There was no difference in systolic and diastolic function between patients with left- and right-sided disease. The lack of effect by laterality, also found for CAC-score, has been discussed extensively above.

There are limited long-term data on prevalence of VD following modern BC treatment. However, it is well established that prevalence and severity of VD after RT are dose-dependent, but the mechanism is largely unknown (271). Left-sided VD was uncommon in the current study cohort, and all patients were asymptomatic. Moreover, no association was observed between VD and RT dose, planning technique or laterality, which may be ascribed to the relatively low dose to heart.

2.6 Overall conclusion and future implications

The current thesis evaluates cardiological effects of multimodal treatment for BC, with the main focus on the consequences of RT, in two separate patient cohorts. Both cohorts are examples of the essence of cancer survivorship studies. Only from patients of the past can we learn about long term outcome, and only these studies can guide the follow-up of today's survivors.

Patients in study group 1 were treated 30-40 years ago, and thus, the continued evaluation of their outcome may seem without relevance for today's practice. However, the study of outdated treatment given to these women sheds light on the contemporary efforts to improve RT in BC by hypofractionation. As such; paper 1 found that the most hypofractionated of two RT regimens resulted in an increased risk of death from IHD. Further, the differences between the two treatment groups emerged first after 10 years, indicating the need for long-term observation to evaluate the safety of ongoing trials.

Study group 2 received a more contemporary treatment and the overall risk of cardiac complications a decade after treatment appears low. With the reduction in cardiac doses from RT, exposure to systemic drugs like anthracyclines may become more important for long-term cardiac outcome. This is of relevance when new systemic agents with potential cardiac effects are incorporated into adjuvant regimens. With a larger focus on avoiding adverse effects of treatment, traditional, modifiable risk factors probably add significantly to the risk conferred by BC treatment. Finally, depending on the level of risk of disease, early detection of cardiac damage by noninvasive tools may be relevant to subgroups of cancer survivors, but the studies presented need longer follow-up to address the utility of CT-CAC, biomarkers or refined echocardiographic methods in BCSs.

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