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Cardiovascular Side Effects of Radiotherapy in Breast Cancer

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Abstract
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The aim of the thesis was to study cardiovascular side effects of radiotherapy (RT) in breast cancer (BC).

In a study base of 25,171 women with BC diagnosed 1970-2000, we found a statistically significant 12% increase of stroke, compared to the stroke incidence in the background population.

A case-control study of 282 cases with BC followed by a stroke and 1:1 matched controls with BC but not stroke was performed. In women irradiated to internal mammary chain (IMC) and supraclavicular lymph nodes (SCL) vs. a pooled group of women not irradiated or irradiated to targets other than IMC and SCL, a statistically significant increase of stroke with an odds ratio of 1.8 was observed. There were no associations between BC laterality, targets of RT, and hemisphere location of stroke. The radiation targets IMC and SCL, showed a statistically significant trend for an increased risk of stroke with daily fraction dose.

A study of 199 patients with BC, examined by coronary angiography, detected a four- to seven-fold increase of high grade coronary artery stenosis in mid and distal left anterior descending artery (LAD), including distal diagonal branch, when comparing women with irradiated left-sided BC to those with right-sided. An increase of clinically significant coronary artery stenosis was found in pre-specified hotspot areas for radiation among women irradiated to the left breast/chest wall or to the IMC. Thus, the coronary arteries should be regarded as organs at risk in RT of BC.

In a study of 15 BC patients treated with 3D conformal RT, a marked difference in dose distribution in mid and distal LAD between left- and right-sided BC was demonstrated. Irradiated right-sided BC mainly received low doses of scattered and transmitted radiation to the coronary arteries. On the contrary, tangential RT to the left breast without regional lymph node irradiation yielded coronary artery max doses of approximately 50 Gray to distal LAD, probably not safe concerning late radiation vascular effects.

To conclude, we found cardiovascular side effects in women irradiated for BC, resulting in stroke and coronary artery disease, and showed an association between the targets for RT and the anatomical location of these vascular events.

Keywords: Breast Cancer, Radiotherapy, Cardiovascular, Stroke, Coronary artery stenosis, Coronary artery disease

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To Regina, Felix and Alma

List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I Nilsson G, Holmberg L, Garmo H, Terent A, Blomqvist C. "Increased incidence of stroke in women with breast cancer." *Eur J Cancer*, 2005; 41(3): 423-429.

II Nilsson G, Holmberg L, Garmo H, Terent A, Blomqvist C. "Radiation to supraclavicular and internal mammary lymph nodes in breast cancer increases the risk of stroke." *Br J Cancer*, 2009; 100(5): 811-816.

III Nilsson G, Holmberg L, Garmo H, Duvernoy O, Sjögren I, Lagerqvist B, Blomqvist C. "Distribution of coronary artery stenosis after radiation for breast cancer." *J Clin Oncol*, 2012; 30(4): 380-386.

IV Nilsson G, Witt Nyström P, Isacsson U, Garmo H, Duvernoy O, Sjögren I, Lagerqvist B, Holmberg L, Blomqvist C. "Postoperative radiotherapy for breast cancer and coronary artery stenosis: A dosimetry study of 15 patients examined by coronary angiography after breast cancer treatment." *In manuscript.*

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Abbreviations

BC	Breast cancer
MRI	Magnetic resonance imaging
BCS	Breast conserving surgery
RT	Radiotherapy
SNB	Sentinel node biopsy
OS	Overall survival
DFS	Disease free survival
ER	Estrogen receptor
PR	Progesterone receptor
EBCTCG	Early Breast Cancer Trialists' Collaborative Group
AI	Aromatase inhibitor
CMF	Cyclophosphamide, methotrexate, fluorouracil
FEC	Fluorouracil, epirubicin, cyclophosphamide
HR	Hazard ratio
IMC	Internal mammary chain
SCL	Supraclavicular lymph nodes
IHD	Ischaemic heart disease
CT	Computed tomography
3DCRT	Three-dimensional conformal radiotherapy
OAR	Organs at risk
kV	kilovolt
MV	Megavolt
Gy	Gray
CRE	Cumulative radiation effect
BED	Biologically effective dose
RIHD	Radiation induced heart disease
NF-κB	Nuclear Factor-Kappa B
IL-8	Interleukin-8
LDL	Low-density lipoprotein
SPECT	Single-photon emission computed tomography
LAD	Left anterior descending artery
EORTC	European Organisation for Research and Treatment of Cancer
RR	Relative risk
OR	Odds ratio
CI	Confidence interval

ICD	International classification of diseases
RCA	Right coronary artery
LMCA	Left main coronary artery
LCX	Left circumflex artery
mdLAD+dD	Mid, distal and distal diagonal branch of left anterior descending artery
Prox RCA	Proximal right coronary artery
mdLAD	Mid and distal left anterior descending artery
LSMEANS-estimate	Least square means-estimate
DBCG	Danish Breast Cancer Cooperative Group
NNT	Number needed to treat

Background

Epidemiology

Breast cancer (BC) now represents the most common female malignancy in both the developing and developed world, and is the primary cause of death in women globally [1]. In Sweden, approximately 7,900 new cases of BC were diagnosed in 2010, which constituted 30% of all female cancer [2]. The median age at diagnosis of BC was 64 years [3]. The cumulative probability of developing BC before the age of 75 years is 11% in Swedish women [2]. Breast cancer incidence has increased with 1.3% annually during the last 20 years but the increase in the recent 10-year period is weaker with an annual change of 0.9% (Figure 1) [2].

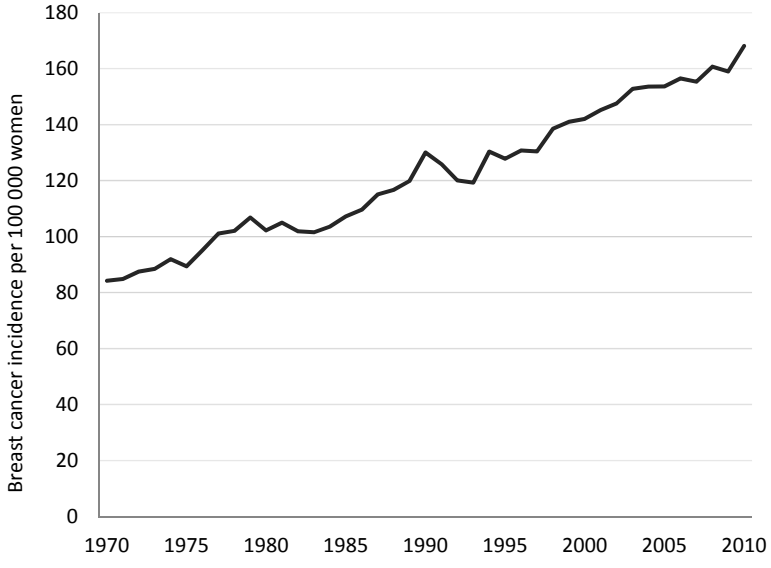

Figure 1. Incidence of female breast cancer in Sweden 1970-2010 (The Swedish National Board of Health and Welfare).

Aetiology

There are several hormone-related risk factors for BC, including low age at menarche, nulliparity, high age at first childbirth, short time from last pregnancy, current and recent oral contraceptive use, and current and long-term hormonal replacement therapy [4]. Ionising radiation, low physical activity, obesity, alcohol intake, and inherited mutations of the genes BRCA1 and BRCA2 are other established risk factors [4].

Prognosis

Breast cancer prognosis has steadily improved over the last decades from a relative ten-year survival of approximately 50% in the 1960s to almost 80% in the year of 2009 in a Swedish population [4-5]. Two major reasons for this substantial gain in survival have been proposed, the use of mammography screening [6-8] and the development of adjuvant treatments [9-12].

Diagnosis

Approximately 50% of breast cancers in Sweden are detected by screening mammography, while the others are clinically detected. The diagnostic procedures include clinical examination, mammography, often supplemented by ultrasound, and fine needle aspiration for cytological examination. In some circumstances a core biopsy for histopathological examination is required. If a malignancy is diagnosed or suspected, the next step is surgical, except for the minority of patients with locally advanced breast cancer, where neoadjuvant systemic therapy is considered. Magnetic resonance imaging (MRI) of the breasts is a sensitive radiologic method shown to be particularly effective in detecting BC in young BRCA-mutation carriers [13] but has not yet been shown to improve the prognosis [14].

Surgery

The surgical treatment of the breast consists of mastectomy or breast conserving surgery (BCS), depending on the relation between tumour size and volume of the breast and patient preference [15]. In clinical trials with BCS plus adjuvant radiotherapy (RT) vs. mastectomy, no difference in survival is shown [11, 16-18]. The axillary lymph nodes may be treated by sentinel node biopsy (SNB) or axillary lymph node dissection. If the SNB is negative, no further axillary dissection is required since local control and

overall survival (OS) are not affected by further axillary surgery [19-20]. On the other hand, if the SNB is positive, an axillary lymph node dissection has been recommended [15]. However, two newly reported studies on SNB positive patients support the role of SNB as a staging procedure [21-22]. No differences in disease free survival (DFS) or OS were detected between women randomised to axillary lymph node dissection or no further surgery. A benefit of SNB over axillary lymph node dissection is the reduction in risk of developing lymph oedema in the arm [23-24]. Accordingly, if possible, the preferred minimally invasive surgical procedure in early breast cancer is BCS with SNB.

Pathology

The pathology report is essential for estimating prognosis and the need for adjuvant treatment. Most malignant tumours of the breast are adeno-carcinomas, divided into ductal carcinomas, lobular cancers and several other uncommon types of tumours. The TNM classification (Table 1), tumour size (T), presence of regional lymph node metastasis (N) or distant metastasis (M), has a strong prognostic impact [25-28]. Based on the TNM classification, the disease is grouped into stages (Table 2). Other prognostic factors are histological grade [29-30], proliferation index [31-32], vascular invasion [30, 33], estrogen (ER) and progesterone (PR) receptors [34], and the Her-2 receptor [35]. ER and Her-2 receptors also serve as predictive factors for response to endocrine therapy and trastuzumab, respectively [9, 36-37].

Table 1. TNM classification according to the American Joint Committee on Cancer (7^{th} edition)

TNM	Clinical staging	pN	Pathological staging
T0	No evidence of primary tumour		
Tis	Cancer in situ		
T1	Primary tumour ≤20 mm		
T2	Primary tumour 21-50 mm		
T3	Primary tumour ≥51 mm		
T4	Infiltration of chest wall, skin ulceration, skin nodules, inflammatory cancer		
N0	No regional lymph nodes		
N1	Movable axillary nodes	pN1	1-3 positive axillary nodes
N2	Fixed axillary or internal mammary nodes clinically apparent	pN2	4-9 positive axillary nodes
N3	Supra/infraclavicular nodes or internal mammary nodes together with axillary nodes	pN3	≥10 positive axillary nodes or positive infraclavicular nodes
M0	No distant metastasis		
M1	Distant metastasis		

Table 2. Breast cancer stage based on TNM classification

Stage	T	N	M
Stage 0	Tis	N0	M0
Stage I	T1	N0	M0
Stage IIA	T0-T1	N1	M0
Stage IIA	T2	N0	M0
Stage IIB	T2	N1	M0
Stage IIB	T3	N0	M0
Stage IIIA	T0-T2	N2	M0
Stage IIIA	T3	N1-N2	M0
Stage IIIB	T4	N0-N2	M0
Stage IIIC	Any	N3	M0
Stage IV	Any	Any	M1

Recently, a new classification of prognostic importance based on gene expression profiles has been proposed [38]. The diversity between the different subtypes of BC is largely driven by the expression of genes related to ER, Her-2 receptor and proliferation.

Subtypes of breast cancer based on gene expression:

- Luminal A: high expression of ER and ER-related genes
- Luminal B: expression of ER-related and proliferation-related genes
- Basal-like: low expression of ER, PR, Her-2 receptor and expression of basal cytokeratins
- Her-2 like: expression of Her-2 receptor and related genes
- Normal-like: expression of genes occurring in adipose and other non-epithelial tissue

Endocrine therapy

Tamoxifen has been used as adjuvant treatment for ER positive breast cancer since the 1980s. An updated meta-analysis by the Early Breast Cancer Trialists' Collaborative Group (EBCTCG) has shown a 9% absolute reduction in 15-year breast cancer mortality from the use of tamoxifen, corresponding to a 30% relative reduction of BC mortality [9]. The recommended treatment duration is five years. In the early 2000s introduction of the aromatase inhibitors (AI), anastrozole, letrozole, and exemestane, for adjuvant treatment for ER positive postmenopausal BC started [39-41]. The AIs are used either for five years or as a sequential treatment with tamoxifen for 2-3 years with the AI upfront or vice versa [39-41]. Compared to tamoxifen for five years, the further gain in DFS is approximately 10-20% with a modest gain in OS, shown in two of three studies [39-41].

Chemotherapy

Adjuvant chemotherapy, given in lymph node positive high risk breast cancer started in the 1970s. The combination of cyclophosphamide, methotrexate, and fluorouracil (CMF) showed superiority compared to no chemotherapy [42]. In the 1990s, the anthracyclines were introduced for adjuvant BC treatment. Polychemotherapy, with an anthracycline included (adriamycin or epirubicin), cyclophosphamide, and fluorouracil (FAC/FEC) further reduced BC mortality [43]. The next step of development was the combination of taxanes and anthracyclines in the 2000s. The use of docetaxel in node positive BC improved survival by approximately 25% in two studies [44-45]. Moreover, paclitaxel increased DFS and OS in combination with anthracyclines [46-47]. In a head-to-head study, weekly paclitaxel and docetaxel every third week seemed to be the optimal schedules for these two drugs [48]. A meta-analysis of 19 adjuvant taxane studies yielded a reduction in the hazard ratio (HR) for DFS and OS of 19% for taxane containing regimens [49]. Two studies have addressed the issue of adding capecitabine to adjuvant chemotherapy regimens containing anthracyclines and taxanes [50-51]. A combined analysis of these two studies showed statistically significant improvement in DFS and OS in the capecitabine containing treatment groups with a HR of 0.83 and 0.71, respectively [52].

In a recent EBCTCG meta-analysis, a comparison of long-term outcome between different polychemotherapy regimens was performed [10]. CMF vs. no chemotherapy reduced BC mortality rate by 20-25%. Anthracycline containing regimens vs. CMF further reduced the BC mortality rate by 15-20%. Multiplying BC mortality risk rates for these findings, $0.775 \times 0.825 = 0.64$, would suggest a 36% rate reduction of BC mortality in comparison to no chemotherapy. A reduction of 36% in death rate in each year would reduce a 10-year risk of BC death from 30% to 20%, corresponding to a reduction by about a third. The absolute gain from a one-third BC mortality reduction depends on the absolute risk without chemotherapy. In trials adding taxanes to a fixed anthracycline-based control regimen, BC mortality was reduced by 14%. However, in trials with extra cycles of taxanes counterbalanced in controls by extra cycles of non-taxane-based chemotherapy, a non-significant difference of 6% BC mortality reduction was seen. The capecitabine trials were not included in this analysis.

Trastuzumab

In 2005-2006 four adjuvant trials in Her-2 positive breast cancer were presented demonstrating an approximately 50% reduction in disease

recurrences with trastuzumab containing regimens, in short time follow-up [36-37, 53]. In the joint analysis of the North American studies, CALBG B-31 and NCCTG N9831, a reduction of DFS event rate by 48% and a decrease of mortality by 39% were observed among patients receiving trastuzumab, after 4 years of follow-up [54]. In this study, trastuzumab administered concurrently with chemotherapy showed a higher efficacy in comparison to the sequential administration of chemotherapy and trastuzumab [55]. Due to the positive interim results, the patients in the European HERA trial assigned to the observation group were allowed to cross over to receive trastuzumab. With a median treatment delay of 22.8 months, 52% of the patients in the observation group crossed over to receive trastuzumab. At 4-year follow-up, a 24% reduction was noticed in DFS in the intention to treat population in favour of trastuzumab, but no significant differences in OS were demonstrated. In a non-randomised comparison, patients in the selective-crossover cohort had a 32% reduction of DFS events compared to patients remaining in the observation group [56]. The smaller FinHer trial with a short course of only nine weeks of trastuzumab treatment concurrently with docetaxel showed a similar, but not statistically significant, gain in DFS as in the larger studies [57].

Radiotherapy

Introduction

The effect of ionising radiation is mediated by cell nucleus DNA damage either by double-strand or single-strand breaks. The double-strand breaks may lead to cell death. The single-strand breaks cause sublethal DNA damage and the outcome depends on the cell DNA repair capacity. A fundamental principle in therapeutic use of ionising radiation is the fact that DNA repair mechanisms are superior in normal tissue cells in comparison to cancer cells.

Radiotherapy has been used in the treatment of breast cancer since 1940s. In the first decades it was either used as tangential preoperative treatment of the breast or as postoperative treatment of the chest wall. In the 1980s the use of BCS started and postoperative treatment of the remaining breast tissue became a standard procedure. In lymph node positive BC, the regional lymph nodes in the axilla, internal mammary chain (IMC), and supraclavicular area (SCL) has been a potential target of RT during the whole time period. The first randomised RT trials and meta-analyses by EBCTCG all showed a reduction of local recurrences but no OS advantage among patients allocated RT [58-62]. These early analyses showed a

decrease of breast cancer deaths that were counterbalanced by an increase of cardiovascular deaths [63].

In the late 1990s, three randomised clinical trials on RT of lymph node positive BC demonstrated an approximately 10% survival advantage for the irradiated women [64-68]. In the EBCTCG meta-analysis published in 2005, the 5-year local recurrence risk decreased from 26% to 7% (absolute reduction 19%) and the 15-year breast cancer mortality decreased from 49.5% to 44.6% (absolute reduction 5.0%, risk ratio 0.83), in the irradiated vs. the non-irradiated women [11]. On the other hand, there was an excess mortality from heart disease in women given RT with a rate ratio of 1.27 [11]. In the three most recent studies included in the meta-analysis, no increase in ischaemic heart disease (IHD) was noticed among the irradiated women with a minimum of twelve years follow-up [68-69]. In the EBCTCG meta-analysis published in 2011, restricted to women irradiated after BCS, RT reduced the 10-year risk of any (i.e. locoregional or distant) first recurrence from 35.0% to 19.3% (absolute reduction 15.7%, risk ratio 0.52) and reduced the 15-year risk of BC death from 25.2% to 21.4% (absolute reduction 3.8%, risk ratio 0.82) [12]. Overall, about one breast cancer death was avoided by year 15 for every four recurrences avoided by year 10. Non-breast cancer mortality is not reported in this study [12].

Target definition

In a historical perspective, target definition in breast cancer has undergone revisions, with generally larger target volumes in the earlier periods. This is especially true for the regional lymph node targets, where the IMC and axillary targets extended more inferior in the earlier RT regimens. Few large randomised studies comparing different radiotherapy regimens and targets have been performed.

Modern target definition in BC according to the regional guidelines [70]:

- Breast post BCS: The remaining ipsilateral breast tissue
- Chest wall post mastectomy: The soft tissue including the subcutis and ipsilateral pectoral muscle, corresponding to the extension of the breast
- Regional lymph nodes: The ipsilateral axillary, infraclavicular, SCL, and IMC in the first three intercostal spaces

Organs at risk

In the older RT techniques, the radiation fields were chiefly defined according to anatomical bone structures, with uncertainties regarding the exact position of the underlying visceral organs in the individual patient. In computed tomography (CT) planned RT, the anatomical relation between the

target areas and the organs in the thorax is known. In the process of dose planning in three-dimensional conformal radiotherapy (3DCRT), an essential step is to define organs at risk (OAR) for calculation of the distribution of radiation doses.

Several OARs in close proximity to the targets in RT of BC:

- Heart
- Lung
- Large vessels
- Skin
- Connective tissue
- Bone
- Brachial nerve plexus
- Spinal cord
- Oesophagus
- Thyroid gland

Tolerance doses for different organs, based on clinical studies, have been defined by Emami et al in the beginning of the 1990s [71] and was recently updated by the group of QUANTEC [72]. The process of 3DCRT often requires a compromise between target cover and dose to the OARs. The heart and the lungs are the OARs most difficult to protect from radiation exposure in RT of BC. Hitherto, the whole heart has been considered as *an* OAR and tolerance doses have been established for pericarditis [71] and cardiovascular mortality [73]. However, the different anatomical structures in the heart may have different radiation tolerance, illustrated by the study of McGale et al [74]. In a comparison between women with irradiated left- and right-sided BC, a statistically significant increase of angina pectoris and myocardial infarction was noticed, reflecting coronary artery disease. On the contrary, the same comparison showed no increase in heart failure or myocardial diseases like cardiomyopathy, reflecting myocardial function.

Radiotherapy technique

Postoperative RT in breast cancer has been used since the 1940s. The technique used to treat the chest wall and the breast in the 1950s and 60s was orthovoltage irradiation, 250 kV (kilovolt), with direct anterior fields or tangential pairs. In the 1970s and 80s, wide tangential irradiation using Cobalt-60 was commonly used, changing to 6 MV (Megavolt) tangential beams in the late 1980s and 90s. CT dose planned RT started in the mid 1990s. From the 1970s, the thoracic wall has been treated with low energy electrons. Cobalt chain therapy to treat the IMC and SCL was used in the 1960s and early 70s. From the mid 1970s to the early 90s, the IMC and SCL were treated with a mix of Cobalt-60 photons and electrons, changing to a

mix of MV photons and electrons in the mid 1990s. The lymph nodes in the axilla have been treated with a frontal and a dorsal photon field. CT dose planned RT of regional lymph nodes started in the mid 1990s. Further details regarding the RT delivered during our study period 1970-2003 is presented in the method section on page 33-34.

Radiation dose

The SI-unit of radiation dose is Gray (Gy). 1 Gy is defined as absorbed radiation dose of 1 Joule/kilogram. The radiation doses in breast cancer have changed from fewer and higher radiation fractions (hypofractionation) to the today standard regimen of 2 Gy × 25 = 50 Gy. In general, the biological radiation effect was higher with the older radiotherapy regimens. Different fractionation schemes may be compared using formulas for calculating the biological radiation effect.

- Cumulative radiation effect (CRE), d (fraction radiation dose in Gy), N (number of fractions), T (duration of RT in days)

$$CRE = \frac{dN^{0.65}}{(T/N)^{0.11}}$$

- Biologically effective dose (BED), d (fraction radiation dose in Gy), N (number of fractions), α/β (a ratio describing the cell survival curve when irradiating the particular tumour tissue studied)

$$BED = Nd\left(1 + \frac{d}{\alpha/\beta}\right)$$

Radiation induced heart disease

Introduction

Embryonic morphogenesis of the heart is almost complete by the eighth week of gestation [75]. After six months of age, the proliferation of myocytes is terminated and the adult number of myocytes exists. Further cardiac growth occurs through cell enlargement. It has been believed that any subsequent loss of myocytes would be compensated by fibrosis or myocyte hypertrophy. The view of the heart as a postmitotic organ has been challenged according to studies demonstrating cardiac stem cells with regenerative capacity in the adult heart [76]. Other cells essential for the function of the heart, like endothelial and connective tissue cells, have

extremely low proliferative activity. According to the hypothesis that radiation damage predominantly occurs in highly proliferative tissues, the heart was considered to be relatively resistant to radiation doses in the therapeutic range prior to the 1960s.

Starting in the late 60s, experimental studies by Fajardo and Stewart showed that the heart and vasculature are, in fact, radiosensitive anatomical structures [77]. The most common manifestation was pericarditis, becoming a significant problem when large volumes of the heart (i.e. the pericardium) were exposed to doses >40 Gy [78]. Another finding was fibrosis of the myocardium, causing cardiomyopathy [77, 79]. The first studies revealed very few cases of coronary artery disease that could be attributed to radiation [80-81]. However, later observations, particularly in children irradiated for Hodgkin lymphoma, have showed an unexpected high incidence of coronary artery disease [82-83].

Radiation induced heart disease (RIHD) is a term indicating the clinical and pathological conditions of injuries to the heart and large vessels resulting from therapeutic irradiation of malignancies [75, 84-85]. The phenomenon is mostly studied in Hodgkin lymphoma and breast cancer but may occur in any irradiated thoracic tumor, such as lung cancer, oesophageal carcinoma, and thymoma [75]. The pathophysiology of RIHD includes inflammation and fibrosis [75, 84-85]. All of the anatomical structures in the heart can be affected. In the pericardium, pericarditis with effusion, with or without constrictive pericarditis, have been described [86]. In the myocardium, cardiomyopathy may develop, due to microangiopathy and fibrosis, eventually causing heart failure [87]. Irradiation may also cause valvular heart disease with stenoses and insufficiencies of the valves [88]. Uncommonly, radiation induced damage to the conduction system may lead to bundle branch block and atrioventricular block [88-90]. Irradiation to the arteries, due to macroangiopathy, may accelerate atherosclerosis and cause coronary artery disease and carotid artery disease, leading to an increased risk of IHD and ischaemic stroke, respectively [11, 91-94].

Cardiac doses in breast cancer treatment increased from the 1950s to the 1970s and diminished substantially in the mid 1980s and 1990s [95-96]. Several treatment related factors differed during these decades; the radiation source (250 kV, Cobalt-60, and MV beams), the target definition (largest targets in the 1970s), the fractionation schemes (high fractions in the early decades), and the introduction of CT planned RT, all contributing to the cardiac doses.

Microangiopathy

The radiation effects in the myocardium are characterised by patches or diffuse fibrosis. The fibrosis is made of a network of collagens fibers that separates the myocytes [77]. The myocardial fibrosis, studied in the New Zealand white rabbit model, is caused by injury to the endothelial cells of the myocardial capillaries, i.e. microangiopathy [97]. In light microscopy, the endothelial cell injury manifests as swelling of the cells, microvascular thrombosis, or microvascular rupture, all of which cause obstruction or destruction of the microvascular network of the myocardium [97]. The proliferation capacity of the remaining endothelial cells is insufficient to repair the damage of the microvascular network, inevitably leading to ischaemia of myocytes, replaced by fibrosis [97]. In the late stage of myocyte degeneration, a significant portion of the ventricular wall may be replaced by fibrous tissue [77, 79]. A reduced capillary density has been shown in animal studies [98-99]. Together, these experimental findings suggest radiation injury to the capillary network as the underlying cause of ischaemic myocardial degeneration, occasionally leading to heart failure and cardiomyopathy after heart irradiation [78].

Several pro-inflammatory molecules have been reported to be upregulated when irradiating endothelial cells [78]. E-selectin, an endothelial cell adhesion molecule, is upregulated in 6 hours in mouse lung after 2 Gy irradiation [100]. This process involves activation of Nuclear Factor-Kappa B (NF-κB). In larger blood vessels, P-selectin, another early pro-inflammatory factor, was also increased. Intercellular adhesion molecule (ICAM-1), which is an important mediator of cell arrest, was upregulated at 2-7 days after 8 Gy total body irradiation in mice [101] and this finding has also been associated with activation of NF-κB. Adhesion-molecule PECAM-1 (CD 31), involved in leukocyte transmigration, has been shown to be upregulated 3 days after endothelial cell irradiation in vitro [102]. These pro-inflammatory events are supposed to be the molecular correlate of early radiation-induced changes observed in the microvessels of the myocardium [78].

Upregulation of cytokines has also been observed after endothelial cell irradiation. Interleukin-8 (IL-8), which is a chemo-attractant for leukocytes and induces endothelial cell proliferation, is upregulated, as is IL-6 [102]. In cell proliferation, the radiation damage would be expressed as mitotic endothelial cell death [78]. In addition, endothelial cell apoptosis has been observed after radiation in vivo [103]. Other cytokines shown to be involved are Tumor necrosis factor (TNF), IL-1, IL-18, monocytes chemotactic factor, Platelet-derived growth factor (PDGF), and Transforming growth factor-β (TGF-β) [104]. Furthermore, there is evidence of prothrombotic effects of

radiation. An increase of von Willebrand factor (vWF) has been observed in capillaries and arteries in several species [105-107], covering a time period from 5 hour to 16 months post radiation. Subsequent activation of the coagulation system leads to platelet adherence and thrombus formation through fibrin deposition [104].

Macroangiopathy

Radiation effects in large arteries have been shown in carotid arteries, iliac arteries and aorta, after irradiation for head and neck, rectal, gynecological and testicular cancers [93, 108-110]. Coronary artery disease has been associated with irradiation for particularly breast cancer and Hodgkin lymphoma [92, 111-112]. In essence, the morphology of radiation-induced coronary artery disease does not differ from coronary artery disease resulting from atherosclerosis of other causes. Characteristic findings of intimal proliferation of myofibroblasts and atherosclerotic plaques of lipid-containing macrophages are seen [113]. In this process there is a narrowing of the vessel lumen and the plaques may fissure, causing thrombosis. However, there are findings of increased depletion of media smooth muscle cells and more extensive fibrotic changes in the media as well as in the adventitia, in comparison to non-irradiation coronary artery disease [88, 114].

In a study by Russel et al, 147 patients undergoing reconstructive surgery after radiotherapy for head and neck or breast cancers were examined regarding radiation effects in medium-sized muscular arteries [115]. Irradiated biopsies were compared to biopsies from control arteries. At a mean of 4 years following irradiation, the inflammatory content was increased in the intima of irradiated arteries and the intimal thickness in internal mammary arteries was significantly increased 1.4-fold in the breast cancer group in irradiated vessels compared to control arteries. An increase of the proteoglycan content of the intima in irradiated vessels was also observed. The mechanism and consequence of this finding is not known. In a study by Halle et al, arterial biopsies from 13 patients who underwent reconstructive surgery for head and neck cancer were investigated [116]. Biopsies were harvested from irradiated cervical donor arteries and from the non-irradiated recipient arteries of the transferred tissue. The radiation dose averaged 60 Gy and the median time from radiation to biopsy was 30 weeks (range 4-500 weeks). A gene array analysis showed an increased expression of genes associated with angiogenesis, coagulation, and inflammation when comparing irradiated arteries to non-irradiated arteries. A majority of these genes were related to the NF-κB signaling pathway and were dysregulated even years after radiation suggesting a sustained inflammation, possibly causing atherosclerosis [116].

Stewart et al [117] examined apolipoprotein-negative mice, irradiated with a single dose of 14 Gy to the carotid artery and followed the development of atherosclerotic plaques for up to 34 weeks. The onset of initial plaque formation was earlier and the rate of plaque growth faster in irradiated carotid arteries compared to non-irradiated arteries. Histologically, these carotid arteries showed signs of plaque instability such as intraplaque haemorrhage or macrophage accumulation. Stewart concluded that radiation in these animals resulted in chronic inflammation, favouring the development of vulnerable plaques [117].

Atherosclerosis

Coronary artery disease is a multifactorial progressive disease, driven by many genetic and exogenous factors [118]. NF-κB-mediated inflammation in combination with local shear stress and lipid accumulation is supposed to trigger the process, followed by a systemic accumulation of Low-density lipoprotein (LDL) in the vascular intima [119]. The LDL particles are transformed into pro-inflammatory lipids and attract leukocytes, platelets, and monocytes. The monocytes differentiate to macrophages and internalise apoptotic cell fragments or oxidised LDL particles thereby transforming to the characteristic foam cells. The inflammatory cascade develops with contribution from leukocyte adhesion molecules, cytokines, proteases, cytotoxic oxygen and nitrogen radical molecules. The immunity and the coagulation pathways become activated and the final result is local plaque formation and eventually plaque rupture.

Several of these atherosclerotic events can be triggered by radiotherapy and an acceleration of these events by radiotherapy is also conceivable. When examining the role of radiation in the process of atherosclerosis, it appears to be an independent risk factor that acts in concert with other known risk factors [78]. It has been postulated that atherosclerosis is a monoclonal process (like cancer), starting with a single cell mutation, initiated by an exogenous factor, e.g. radiation, giving a possible explanation of the increased risk of cardiovascular events even with low dose radiation [120]. On the other hand, smooth muscle cells in humans appear to be monoclonal in origin, suggesting an expansion of naturally pre-existing clones rather than radiation-induced mutational events [121]. Another possible phenomenon linking radiation damage to atherosclerosis is genomic instability, shown in both conditions, perhaps indicating a common pathogenetic mechanism [104].

Exposition to low radiation dose and cardiovascular diseases

Even lower than therapeutic radiation doses may be harmful, illustrated by the atomic bomb survivor studies. In a population of 86,000 survivors, who received a whole body uniform dose of 0-4 Gy, increases in heart disease mortality of 17% per Gy and stroke mortality of 12% per Gy was shown [122]. In a study by Carr et al, long-term follow-up of 1,859 patients treated with RT for peptic ulcer was performed [123]. The patients were irradiated during the time period 1936-1965 with approximately 5% of the heart in the radiation field to a dose of 7.6-18.4 Gy. In total, an increase of coronary heart disease mortality of 19% was noticed and the risk was dose-dependent. In a study of 90,000 US radiologic technologists who started working prior to 1940, there was an increase of mortality due to circulatory system diseases compared to those starting after 1960 [124].

Radiotherapy trials and cardiovascular diseases

The early randomised radiotherapy trials and the EBCTCG meta-analyses showed a decrease in breast cancer deaths that were counterbalanced by an increase in cardiovascular mortality [63]. Left-sided BC has been associated with a higher mortality due to IHD, compared to right-sided [125-130]. Regarding modern RT, conflicting results exist and the follow-up is probably still too short. Some studies have reported an increase of IHD in women with left-sided BC [131-132] while others have not [133-135]. In two prospective studies using the method of single-photon emission computed tomography (SPECT), myocardial function was investigated before and after RT [136-137]. Regional perfusion defects corresponding to the radiation fields in the anterior part of the left ventricle of the heart were found and this was considered as an indicator of microangiopathy. The perfusion defects may persist or may appear 3-6 years post RT [137]. The clinical significance of this finding is unclear, since the perfusion defects were not associated with changes in regional wall motion or ejection fraction [137].

In the study by Correa et al, the incidence and distribution of coronary artery disease was compared in patients with left- and right-sided BC with a history of RT [92]. Eighty-two patients were examined with cardiac stress tests. A statistically significant higher prevalence of stress test abnormalities was found among left-sided BC patients. These abnormalities were primarily located in the anterior part of the left ventricle of the heart. Fourteen patients were referred for coronary angiography at a median time post RT of 15 years. Thirteen patients had been irradiated to the left breast and 12 of the 13 patients had coronary stenosis of which 11 of them were located in the left anterior descending artery (LAD) in the anterior left ventricle. Wang et al identified 91 Canadian women previously irradiated for BC who underwent

coronary angiography with a median time of 4.2 years from RT [138]. In a comparison between left- and right-sided BC, no significant differences in the degree of stenosis of any part of the coronary arteries were observed.

The IMC is a target area located close to the anterior part of the heart. The risk-benefit of irradiating this area has resulted in a longstanding and ongoing debate among breast cancer physicians. In the use of older radiotherapy techniques, with a wide frontal photon field to cover the IMC, the radiation dose to the heart was high. Modern CT dose planned technique delivers lower doses, but still potentially harmful, to the coronary arteries. RT to the IMC has been associated with increased risk of myocardial infarction in some studies while in other studies no increase of cardiac deaths and IHD were observed [68-69, 139-140]. The large randomised trial 22922/10925 by European Organisation for Research and Treatment of Cancer (EORTC) will elucidate the effect of treating the IMC with modern RT [141].

In adjuvant radiotherapy of the SCL in breast cancer, the proximal part of the carotid artery is included in the irradiation portals. Studies in patients with head and neck cancer have shown increased risk of carotid stenosis and ischaemic stroke post radiotherapy [91, 93-94]. Dorresteijn et al studied 367 patients irradiated for head and neck tumours with a median follow-up after RT of 7.7 years [93]. The relative risk (RR) for ischaemic stroke was 5.6, in comparison to the expected incidence of stroke in the background population. Five of six strokes in patients unilaterally irradiated for a parotid cancer occurred at the ipsilateral side. In the study by Haynes et al, the risk of stroke in 413 patients irradiated for squamous cell carcinoma of the head and neck was compared to the expected rate in a non-irradiated population [94]. The patients had a median radiation dose of 64 Gy and the RR of stroke was significantly increased to 2.1, with a short follow-up of only 25 months. In the study by Moser et al, in 476 long-term survivors after treatment for aggressive non-Hodgkin lymphoma, the RR of stroke was significantly increased to 1.8 in irradiated patients compared to population-based rates [142]. A dose-dependent increase was shown with the highest risk for patients irradiated with doses >40 Gy. These patients had a RR for stroke of 8.6. In the study by Bowers et al of 1,926 childhood Hodgkin survivors, the RR of stroke was significantly increased to 4.3 using a sibling comparison group [143]. In the group of patients treated with a mantle irradiation field, well covering IMC and SCL bilaterally, the RR of stroke was further elevated to 5.6.

Hooning et al have defined a cohort of breast cancer survivors in the Netherlands, “The Late Effects Breast Cancer Cohort”. An analysis of 4,414 women in this cohort, restricted to 10-year survivors of BC, showed a

significantly decreased risk of stroke with an odds ratio (OR) of 0.8 after a median follow-up of 18 years [144]. Patients irradiated to IMC and/or SCL did not experience a higher risk of stroke compared to patients not receiving radiation to these targets. However, an association between tamoxifen use and stroke was found. Jagsi et al studied 820 consecutive stage I or II breast cancer patients in Michigan treated with BCS and RT [145]. After a median follow-up of 6.8 years, a statistically significant increase of stroke with an OR of 1.7 was found. A non-significant increase of stroke was noticed in the group receiving RT to SCL, in which the OR was 1.9 with 95% confidence interval (CI), (0.9-3.7). Woodward et al used >4 positive lymph nodes in the axilla as a surrogate marker for RT to SCL in a comparison with lymph node-negative patients (surrogate for no SCL radiation) to study a U.S. population of 5,752 women with BC using the SEER database [146]. No increase of stroke was detected in the group of women supposed to have been irradiated to SCL. Neither of these two studies reported IMC radiation [145-146].

In a prospective study by Woodward et al, 46 women with breast cancer irradiated to SCL were examined by carotid artery ultrasound [147]. The median follow-up was 14.6 years and no increase of carotid stenosis was found. Stokes et al have studied combined cardiac and cerebrovascular mortality in a population-based Canadian breast cancer cohort of 4,929 women [148]. In a comparison between patients receiving regional lymph node irradiation vs. not, a small but statistically significant increase of fatal cardiovascular events was found, 5% vs. 3.5% respectively. Regional lymph node irradiation included the SCL but the IMC was irradiated in only 9% of the patients. Scott et al performed a pooled analysis on five studies in stroke after RT to the neck with a total of 6,809 patients irradiated for head and neck cancer, non-Hodgkin lymphoma, Hodgkin lymphoma, and breast cancer [93-94, 142-143, 145, 149]. The crude risk of stroke was 2.6% after neck RT vs. 0.29% in the non-irradiated comparison-population, corresponding to a statistically significant increased OR of 9.0. To summarise, an association between RT to the neck and subsequent stroke was shown with a high OR but only a limited absolute risk increase.

Radiation protection of the coronary arteries

In the intricate counterbalance between sufficient radiation dose to the targets and minimal dose to the OARs, i.e. optimisation of radiotherapy, 3DCRT will often produce a dose plan with sufficient target coverage and acceptable doses to the OARs. Occasionally, there is a definite conflict between target coverage and doses to the OARs. In these instances, other considerations with both pros and cons may be taken into account.

Available methods to protect the coronary arteries from radiation:

- Mastectomy instead of BCS plus RT

One obvious method to protect the coronary arteries from radiation is to perform a mastectomy and not irradiate. Some patients may have unfavourable anatomy for radiation and other patients may be at high risk of local recurrences or a new primary tumour in the breast in spite of RT. In these circumstances, mastectomy may be a reasonable alternative. This is an option available mainly for node-negative disease, since postoperative RT reduces mortality after mastectomy in cases with involvement of axillary lymph nodes [11].

- Omitting RT after BCS

Some subsets of BC have an excellent local control after partial breast resection, even without RT [12, 150]. In selected cases an alternative is to omit RT after BCS if the patient is at risk of receiving a substantial cardiac dose, after a careful risk-benefit assessment.

- Partial breast irradiation

When using different methods of partial breast irradiation, the cardiac dose generally is lower, and with some methods negligible, compared to whole breast radiotherapy [151]. The risk of local recurrences with partial breast irradiation may be elevated compared to whole breast radiotherapy [152] but may be acceptable in patients with low baseline risk of local recurrence and/or contraindications (especially cardiac) for whole breast radiotherapy.

- Respiratory gating

In respiratory gating, the concept is to deliver the radiation dose in a part of the breathing cycle where the heart is “out-of-field” and it has been shown to substantially reduce the cardiac and coronary artery dose [153].

- Prone position

Prone position RT can be used when irradiating large pendulous breast, with the intention to separate the radiation target from the chest wall and lowering the lung dose. Prone position does not necessarily lower the cardiac and coronary dose because the heart as well as the breast is displaced anteriorly [154].

- Intensity modulated radiotherapy

3DCRT may lower the heart and coronary artery doses substantially compared to the older techniques. Intensity modulated radiotherapy may further decrease the doses, but the distributed integral radiation dose over a larger volume of the heart and the coronary arteries will be higher [155]. Since tolerance dose for coronary arteries is still uncertain this higher integral dose may theoretically eliminate any beneficial effect of lowering

hot-spot doses. Thus, intensity modulated radiotherapy may not be the ideal method of reducing cardiac toxicity of breast irradiation.

- Proton RT

Proton RT reduces cardiac doses compared to photon therapy [156]. However, no randomised trials with cardiac endpoints have been performed. This is a promising method, becoming available at more radiotherapy centres in the future.

Thus, several methods to lower the heart and coronary artery doses exist but some of these methods may be expensive, time-consuming and not available for this potentially large group of women with BC. Studies with a risk-benefit (and cost-benefit) approach, selecting patients for treatments other than 3DCRT, are needed.

Aim

Overall aim

To study cardiovascular side effects of radiotherapy in breast cancer.

Specific aims

I To elucidate a possible association between breast cancer and stroke.

II To assess whether adjuvant radiotherapy in breast cancer affects the risk of stroke and to explore radiotherapy targets and doses regarding risk and location of stroke.

III To examine the distribution of coronary artery stenosis in women with breast cancer and to study the correlation between radiotherapy targets and location of stenosis.

IV To describe the distribution of radiation dose in segments of coronary arteries in women with left- and right-sided BC who have received 3D conformal radiotherapy and to study the relation of the dose to the postulated radiation hotspot areas of the ventral segments of the coronary arteries defined in Paper III.

Material and methods

Study subjects

The studies were confined to women with breast cancer in the Uppsala-Örebro health care region, a central part of Sweden with both urban and rural areas with a population of nearly 2.0 million. As data sources, we used the Swedish Cancer Register, the nationwide Hospital Discharge Register, and two regional coronary angiography registers. In Papers II-IV the medical records and radiotherapy charts were reviewed.

In Paper I, the study base of breast cancer diagnosed 1970-2000 in the Uppsala-Örebro health care region consisted of 25,171 women. In this study base, we defined in our linkage women who after a BC were recorded with a diagnosis of stroke during the study period 1970-2000, and 1,766 such women were identified.

Paper II consisted of a case-control study of stroke after a breast cancer diagnosis nested in a cohort of women with BC. The cohort of 4,689 women with BC diagnosed 1970-2003, residing in Uppsala County at the time of diagnosis, was linked to the Swedish Hospital Discharge Register, to find cases with a stroke after breast cancer diagnosis. We identified 316 eligible women and their medical records were reviewed. Thirty-four women were excluded, leaving 282 cases for analysis. For each case, one control was chosen by incidence density sampling at random among those in the BC cohort who were alive and without a history of stroke at the date of the stroke, for the corresponding case [157].

In Paper III, we conducted a study of the distribution of coronary artery stenosis, determined by coronary angiography, in a cohort of women with BC diagnosed 1970-2003, residing in the Uppsala-Örebro health care region. The cohort was linked to the registers of coronary angiography in the hospitals of Uppsala and Falun, covering the time period 1990-2004. By linking we found 252 women, with an invasive breast cancer or a ductal carcinoma in situ who subsequently underwent coronary angiography, of whom 53 were excluded after medical record review. This left 199 eligible women for analysis. To obtain “a baseline distribution” of coronary artery stenosis, we defined a population of reference women. From the registers of

coronary angiography we sampled at random 1:1 matched reference women not treated for BC. The angiograms of these 199 women were reviewed and eleven women were excluded, leaving in total 188 reference women to analyse.

In Paper IV, a subgroup of women from Paper III was studied. The women who had received 3DCRT for breast cancer and thereafter been examined by a coronary angiography were included. Twenty-three women in the study base had received that kind of RT. Due to missing information and mismatch between RT and angiography; eight women were excluded, leaving in total 15 patients eligible for analysis.

Stroke

The definition of stroke was broad and based on the ICD (International Classification of Diseases) codes, and the ICD codes were grouped into the following subtypes of stroke: cerebral infarction, cerebral haemorrhage and ill defined cerebrovascular lesion (Table 3). For the stroke classification in Paper II, we used all available information in the medical records: medical history including clinical presentation, results of CT scan and MRI, angiography, surgery reports, and autopsy records. We reviewed the medical records to define the stroke as a vertebrobasilar or carotid stroke [158]. For the carotid strokes we also registered left or right hemisphere location.

Table 3. ICD (International Classification of Diseases) codes and definition of subtypes of stroke

ICD 8 (1970-1986)		ICD 9 (1987-1996)		ICD 10 (1997-2000)	
Cerebral infarction					
432	Occlusion of precerebral arteries	433	Occlusion and stenosis of precerebral arteries	G45	Transient cerebral ischaemic attacks and related syndromes
433	Cerebral thrombosis	434	Occlusion of cerebral arteries	G46	Vascular syndromes of brain in cerebrovascular diseases
434	Cerebral embolism	435	Transient cerebral ischaemia	I63	Cerebral infarction
435	Transient cerebral ischaemia				
437	Generalised ischaemic cerebrovascular disease				

Cerebral haemorrhage					
430	Subarachnoid haemorrhage	430	Subarachnoid haemorrhage	I60	Subarachnoid haemorrhage
431	Cerebral haemorrhage	431	Intracerebral haemorrhage	I61	Intracerebral haemorrhage
		432	Other and unspecified intracranial haemorrhage	I62	Other nontraumatic intracranial haemorrhage
Ill-defined cerebrovascular lesion					
344	Other cerebral paralysis	344	Other paralytic syndromes	I64	Stroke, not specified as haemorrhage or infarction
436	Acute, but ill-defined cerebrovascular disease	436	Acute, but ill-defined cerebrovascular disease	I65	Occlusion and stenosis of precerebral arteries, not resulting in cerebral infarction
438	Other and ill-defined cerebrovascular disease	437	Other and ill-defined cerebrovascular disease	I66	Occlusion and stenosis of cerebral arteries, not resulting in cerebral infarction
		438	Late effects of cerebrovascular disease	I67	Other cerebrovascular diseases
				I69	Sequelae of cerebrovascular disease

Coronary angiography

The coronary angiographies were reviewed by two radiologists. The coronary arteries; the Right Coronary Artery (RCA), the Left Main Coronary Artery (LMCA), the Left Anterior Descending artery (LAD), and the Left Circumflex artery (LCX), were further divided into 18 segments (Figure 2) [159]. The segments were graded according to a five-grade scale of stenosis, where grade 0 indicated a normal segment without any atheromatosis or stenosis; grade 1, light atheromatosis; grade 2 to 4, increasing grade of stenosis; and grade 5, occlusion of the segment of the vessel [159]. Grade 3 to 5 stenosis was considered clinically significant, occasionally referred to as significant stenosis. Because of anatomic reasons, segment 15 was combined with segment four, segment 17 with 12, and segment 16 with 13.

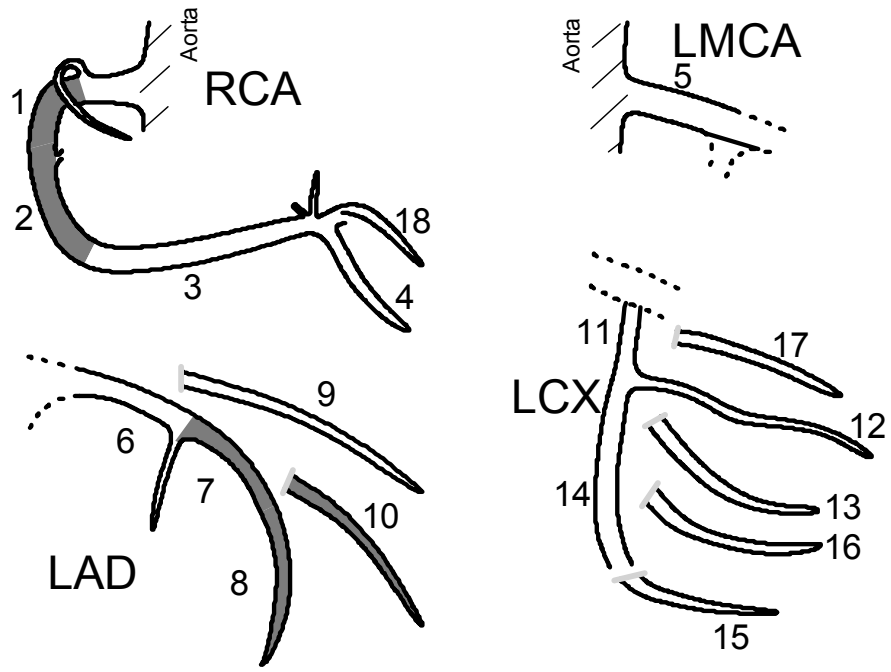

Figure 2. Segments of coronary arteries with hotspot areas for radiation highlighted. RCA, right coronary artery; LMCA, left main coronary artery; LAD, left anterior descending artery; LCX, left circumflex artery.

Radiotherapy

Radiotherapy regimens used 1970-2003

During the study period (1970-2003), several different radiotherapy regimens have been used. The thoracic wall has been treated with low energy electrons during the whole period. The fraction schemes were 3 Gy × 15 = 45 Gy (1970-85), 2.3 Gy × 20 = 46 Gy (1986-96), and thereafter 2 Gy × 25 = 50 Gy. The use of BCS started in 1982. The remaining breast tissue after BCS was treated with two opposed tangential photon fields 2 Gy × 27 = 54 Gy. From 1997 and further on the fraction scheme was 2 Gy × 25 = 50 Gy. The regional lymph nodes have been treated with different techniques and fractionation during this period. In 1970-72 small size frontal Cobalt-60 photon fields to cover the SCL and IMC were given. One such small field of 7 Gy was given each day and in consecutive days a chain of fields were given to cover the targets. The axilla was treated with photons (Cobalt-60), 4 Gy × 7 = 28 Gy in a frontal field and 4 Gy × 6 = 24 Gy in a dorsal field. In 1973-76 the IMC, SCL, and axilla were treated with a frontal Cobalt-60 photon field of 4 Gy × 10 = 40 Gy. The IMC was simultaneously treated

with a frontal field of electrons 3 Gy × 5 = 15 Gy, and the axilla was given 4 Gy × 4-5 = 16-20 Gy in a dorsal photon field.

In 1977-85 the IMC, SCL, and axilla were treated with a frontal Cobalt-60 photon field of 3.5 Gy × 9 = 31.5 Gy. The IMC and SCL were simultaneously treated with a frontal field of electrons 3 Gy × 5 = 15 Gy, and the axilla was given 4 Gy × 6 = 24 Gy in a dorsal photon field. In 1986-93 the IMC, SCL, and axilla were treated with a frontal Cobalt-60 photon field of 2.5 Gy × 12 = 30 Gy. The IMC and SCL were simultaneously treated with a frontal field of electrons 2.5 Gy × 8 = 20 Gy, and the axilla was given 3.2 Gy × 8 = 25.6 Gy in a dorsal photon field. In 1994 and further on the treatment of regional lymph nodes was CT dose planned with a mix of MV photons and electrons, and lymph nodes in the target were given 2 Gy × 27 = 54 Gy. In 1997 and further on regional lymph nodes received 2 Gy × 25 = 50 Gy.

Radiotherapy classification in Paper II

From the radiotherapy charts information was extracted regarding target areas: remaining breast tissue after BCS, chest wall after mastectomy, regional lymph nodes in the axilla, the IMC, and the SCL. Radiation fraction doses and total radiation doses were registered. With respect to radiation exposure, patients were separated into three groups: 1. no RT, 2. RT to breast/chest wall/axilla, *but not* to IMC/SCL, 3. RT to IMC/SCL irrespective of RT to other targets. In the following we refer to these groups as: 1. “No RT”, 2. “RT except IMC/SCL”, and 3. “RT to IMC/SCL”.

Radiotherapy classification in Paper III

As in Paper II, detailed information about target areas and radiation techniques were abstracted from the radiotherapy charts.

Postulated hotspot areas for stenosis after radiation:
Two hotspot areas, most likely to receive radiation dose (Figure 2-4), located in the most anterior part of the heart, were defined before any analyses were performed [95]. The first area was the coronary artery segments 7, 8 and 10, located to the left of the sternum, corresponding to the mid, distal and distal diagonal branch of the LAD, hereafter referred as mdLAD+dD. The second area was the segments 1 and 2, located retrosternal, close to the midline in the superior part and then running inferior to the right of the sternum, corresponding to the proximal RCA, hereafter referred as prox RCA.

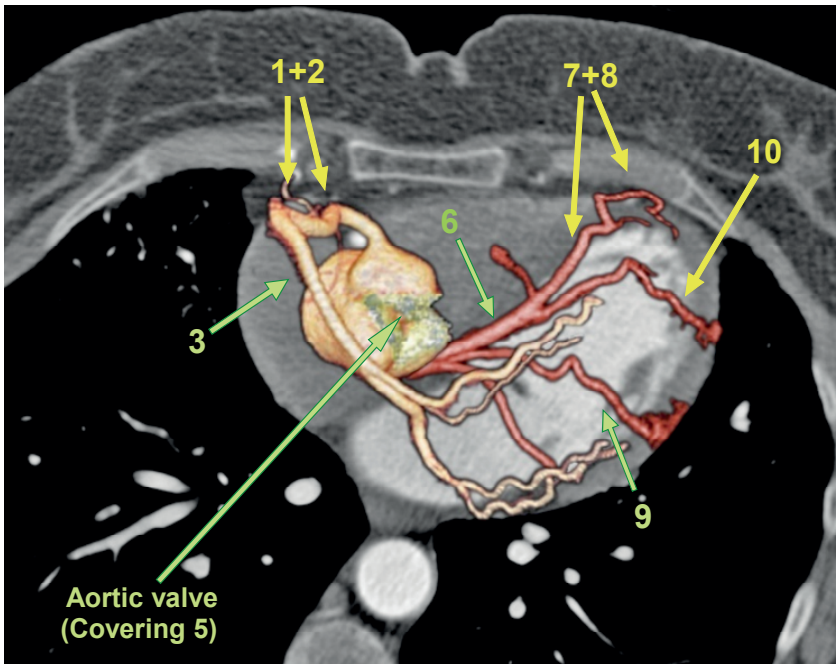

Figure 3. Coronary angiogram superimposed on computed tomography of heart illustrating anatomy of coronary arteries with branches of right coronary artery (orange) and left circumflex and left anterior descending arteries (red); numbered arrows indicate segments.

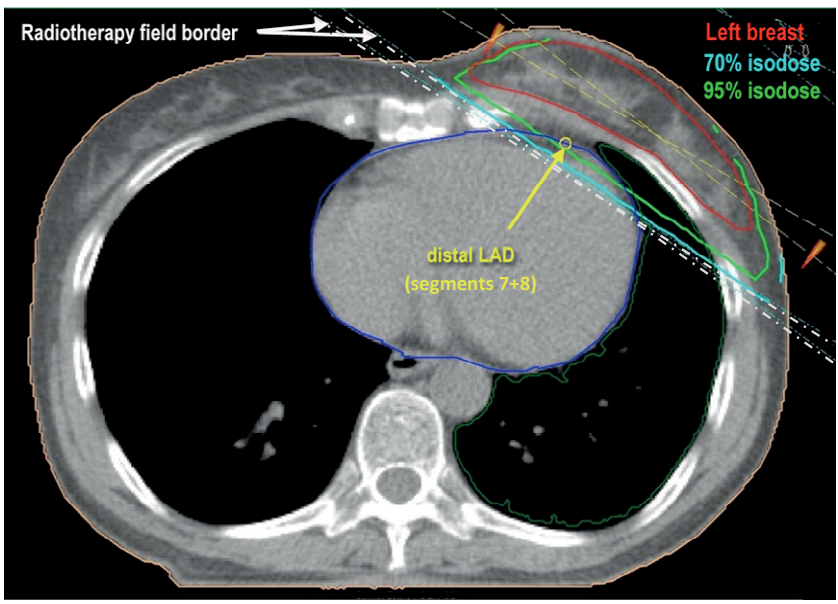

Figure 4. Computed tomography dose planned left tangential breast irradiation showing distal left anterior descending artery (LAD), yellow circle, and radiation fields.

Definition of high-risk and low-risk RT:
The different radiotherapy regimens used during the study period imply varying risk of receiving radiation dose to the coronary arteries [95-96]. Therefore, the radiotherapy targets and regimens were categorised into high risk or low risk, regarding radiation to the hotspot areas, prox RCA and mdLAD+dD. Left-sided RT to the chest wall or breast was considered high risk for mdLAD+dD. RT to the left IMC before 1995 included a frontal photon field and was regarded as high risk for prox RCA and mdLAD+dD. In 1995 and further on the left IMC were treated with tangential RT, still implying high risk for mdLAD+dD, but not prox RCA. RT to the right IMC was considered high risk for prox RCA. All the remaining RT targets: the right chest wall, the right breast, the axillas, and the SCL, as well as no RT, were considered low risk of receiving radiation to the hotspot areas.

Radiotherapy classification in Paper IV

The individual CT study of each patient was retrieved in the treatment planning system Helax-TMS®. The original target definition from the treatment session was used and no target re-evaluation was performed. Eleven patients received tangential RT with opposed photon beams to cover the targets. The treatment plans were individually optimised with beam angles, wedges, and collimator angles. One patient was treated with "the double angle technique", a tangential radiation technique with a steeper gantry angle in the superior part of the target to cover the SCL and IMC and a flatter gantry angle in the inferior part of the target to cover the breast and simultaneously minimise the radiation dose to the defined OARs, i.e. the lung and the heart. Three patients were irradiated with a technique developed in the department, using mixed electron-photon beams conformed with a multileaf collimator, described in detail by Jansson et al [160].

The coronary artery segments 1, 2, 3, 5, 6, 7, and 8 were defined as separate OARs (Figure 2-3) [159]. Segments 7+8, corresponding to the mid and distal LAD, are hereafter referred as mdLAD. The coronary arteries were visible in the majority of the patients due to calcifications of the arteries. In difficult cases, reliable anatomical cardiac landmarks were used to define the coronary artery positions. A margin of 2-3 mm was added to each OAR to allow for uncertainties regarding the exact position of the coronary artery and to assure that the coronary artery were within the OAR. No extra margins for internal movements, i.e. respiratory movement and heart beating movement, were added. The whole heart was also defined as an OAR. The previously individually optimised treatment plans were used and dose-volume histograms were generated for the defined OARs. For each OAR, mean and max radiation doses were assessed for individual patients. For the

heart as an OAR, the volume receiving 40 Gy ($V_{heart\ 40\ Gy}$) and 20 Gy ($V_{heart\ 20\ Gy}$) were calculated.

Statistical methods

In Paper I, the observed number of women in the study base of breast cancer who suffered a stroke was compared with the expected numbers of cerebrovascular events in the background population. Comparisons are expressed in a quotient between the observed and expected number of cases to produce a RR (i.e. standardised incidence ratio) with a 95% CI [161]. The expected number of strokes was calculated by combining the person-time at risk in the study base of BC with the number of strokes recorded in the Hospital Discharge Register per person-time unit, stratifying on five-year age groups and calendar year of observation.

In Paper II, the ORs with 95% CI, in the 1:1 matched case control study, were calculated using conditional logistic regression adjusting for age in four categories (<60 years, 60-69 years, 70-79 years, 80+ years) at breast cancer diagnosis [162]. Tests of independence between laterality of BC and stroke for various RT regimens were performed using Fishers exact test.

In Paper III, segment-wise comparisons in left BC vs. right BC, OR with 95% CI were calculated using conditional logistic regression conditioned on age (in five-year groups), calendar period (in five-year groups), and site of coronary angiography (Uppsala or Falun) [162]. In order to take the distribution of stenosis within each woman into account, in the analysis of high-risk RT, generalised linear mixed model was used. In these models the intra- and inter-individual variation of stenosis is taken into account. The degree of stenosis in high-risk RT segments was compared to low-risk RT/no RT segments. Each woman was considered to be the random subject and the models were adjusted for age (in five-year groups), calendar period (in five-year groups), site of coronary angiography (Uppsala or Falun), segment site, and study subject category (RT treated BC, BC without RT treatment, and Reference subjects). OR with 95% CI based on least square means-estimate (LSMEANS-estimate) was calculated. The LSMEANS-estimate reflects the risk of having a stenosis for an "average subject", considering the intra- and inter-individual variation.

All statistical analyses were performed using the statistical program package R [163], except the modelling of generalised linear mixed models, which was performed in the procedure GLIMMIX in SAS.

Results

Paper I

In adjuvant breast cancer radiotherapy to the regional lymph nodes, a part of the proximal carotid artery is included in the SCL radiation field and studies on RT of head and neck cancer had shown an increase of ischaemic stroke post RT [93-94]. Furthermore, increased cardiovascular mortality after RT for BC was shown in the EBCTCG meta-analysis [62]. In the present study, the stroke incidence in a large Swedish breast cancer study base was compared to the expected in the background population.

In the study base of 25,171 women, mean age at diagnosis of breast cancer was 63.6 years and median follow-up period was 5.4 years. Among the 1,766 women with a stroke, mean age at diagnosis of BC was 71.4 years and mean age at diagnosis of stroke was 78.5 years. The results are summarised in Table 4. We detected a statistically significant 12% increase of stroke in the study base of women with BC. The increased risk was confined to cerebral infarction, while there were no significant increases in risk for cerebral haemorrhage or ill defined cerebrovascular lesions. In women aged 55-69 years and 70 years and older, the risk of stroke was significantly increased by 11% and 14% respectively, while there was no increase of stroke in women below 55 years of age at BC diagnosis.

We further analysed the incidence of stroke in relation to the length of follow-up. In the first year after BC diagnosis, there was a 22% increased risk of stroke. This increase was confined exclusively to the women 70 years or older, RR = 1.26; 95% CI = 1.08-1.46, data not shown. Between 1-5 years after BC diagnosis there was no statistically significant increase in the incidence. However, with longer follow-up, an increase of stroke was seen with 17% in follow-up 5-10 years and 14% in follow-up >10 years after BC diagnosis. We also divided the study base in two subgroups (1970-1985 and 1986-2000) to explore if differences in treatment policies for BC may have influenced risk. In both subgroups the RR was statistically significantly increased, being 1.22 in the period 1970-85 and 1.08 in the period 1986-2000. A trend towards higher risk in the earlier period vs. the later was seen but the difference was not statistically significant.

Table 4. Relative risk of stroke by age at diagnosis of breast cancer and time of follow-up after breast cancer

	Stroke		Cerebral infarction		Ill-defined cerebrovascular lesion		Cerebral haemorrhage	
	RR (95% CI)	Cases/Expected	RR (95% CI)	Cases/Expected	RR (95% CI)	Cases/Expected	RR (95% CI)	Cases/Expected
BC study base	1.12 (1.07-1.17)	1766/1576	1.12 (1.05-1.19)	977/874	1.06 (0.98-1.15)	606/571	1.05 (0.90-1.21)	183/175
Age at breast cancer diagnosis (years)								
<55	1.01 (0.83-1.21)	114/113	1.07 (0.84-1.35)	72/67	1.00 (0.61-1.55)	20/20	0.81 (0.51-1.23)	22/27
55-69	1.11 (1.02-1.21)	566/509	1.13 (1.01-1.25)	350/311	1.04 (0.88-1.22)	149/143	1.01 (0.78-1.28)	67/67
>69	1.14 (1.07-1.21)	1086/954	1.12 (1.03-1.22)	555/496	1.07 (0.97-1.18)	437/408	1.16 (0.94-1.42)	94/81
Follow-up after breast cancer diagnosis (years)								
<1	1.22 (1.06-1.39)	216/177	1.18 (0.97-1.44)	104/88	1.22 (0.97-1.51)	85/70	1.32 (0.87-1.92)	27/20
1-5	1.04 (0.96-1.13)	577/555	1.05 (0.93-1.17)	299/286	1.05 (0.92-1.20)	227/216	0.82 (0.61-1.07)	51/63
5-10	1.17 (1.07-1.27)	515/441	1.13 (1.00-1.27)	277/245	1.05 (0.90-1.22)	170/162	1.42 (1.10-1.80)	68/48
>10	1.14 (1.04-1.25)	458/402	1.16 (1.04-1.31)	297/255	1.01 (0.84-1.20)	124/123	0.84 (0.59-1.16)	37/44
Breast cancer diagnosis and stroke								
1970-1985*	1.22 (1.10-1.34)	388/319	1.18 (0.99-1.39)	138/117	1.17 (1.01-1.34)	204/175	1.36 (0.99-1.81)	46/34
1986-2000	1.08 (1.01-1.17)	742/3684	1.07 (0.98-1.17)	479/447	1.09 (0.94-1.25)	185/170	0.97 (0.76-1.20)	78/81

Abbreviations: RR, relative risk; CI, confidence interval; BC, breast cancer. The expected number of cases in the various subgroups does not sum to be the expected number of cases of stroke due to censoring effects.

* Follow-up ended on 12-31-1985.

Paper II

Paper I showed an increased risk of stroke in a breast cancer cohort. The aetiology of this increased risk could not be elucidated in the study [164]. One may speculate about possible mechanisms, a therapy-related factor or a confounding factor? In this case-control study of cases with BC and a subsequent stroke and controls with BC without a history of stroke, BC treatment-related factors were investigated.

The 282 cases were significantly older than the 1:1 matched controls. Mean age at diagnosis of BC was 71.5 years for the cases and 58.7 years for the controls. Median follow-up period was 6.2 years for the cases and 5.5 years for the controls. Almost 80% of the strokes originated in the carotid arteries and 20% in the vertebrobasilar system. Sixty-two percent of the cases had ischaemic stroke and 10% had haemorrhagic stroke. Twenty-eight percent of the cases had ill-defined stroke, mostly diagnosed in "the pre-CT era". We evaluated the relationship between stroke after BC diagnosis and adjuvant treatments (Table 5). Since the cases were significantly older than the controls, the ORs were age adjusted. The overall OR for RT did not differ statistically significantly from unity. However, as the analyses were stratified on type of radiotherapy, differences emerged. RT except IMC/SCL compared to no RT was associated with a lower risk of stroke. RT to IMC/SCL vs. no RT was associated with a higher, although not statistically significant, risk of stroke (OR=1.3; CI=0.8–2.2). When pooling the groups no RT and RT except IMC/SCL, a post hoc comparison of RT to IMC/SCL vs. the pooled group showed an increase of stroke with an age adjusted OR=1.8; CI=1.1– 2.8.

We repeated the analyses stratifying for cerebral haemorrhage in one group (n=29) and ischaemic stroke plus ill-defined cerebrovascular lesion in one group (n=253). The same pattern as for all types of stroke was seen in the group ischaemic plus ill defined stroke, an increased risk of stroke among the subjects receiving RT to IMC/SCL vs. not (OR=1.9; CI=1.2–3.2). No statistically significant association between radiotherapy and haemorrhagic stroke was seen, although the confidence intervals were wide. Adjuvant chemotherapy and tamoxifen were seldom used in the time period of the study and due to the small numbers in risk sets, the results were not informative. We proceeded to investigate the laterality of the breast cancer, and thus the radiotherapy, in relation to the laterality of the stroke (Table 6). This analysis was restricted to cases with right or left carotid strokes. We did not find any statistically significant association between the BC laterality, targets of RT, and the location of the stroke.

Table 5. Odds ratios and 95% confidence intervals for stroke and subtypes of stroke associated with adjuvant therapy

	Cases		Controls		Stroke, age adjusted		Cerebral haemorrhage, age adjusted		Ischaemic stroke and ill-defined cerebrovascular lesion, age adjusted	
	N	(%)	N	(%)	OR	95% CI	OR	95% CI	OR	95% CI
Radiotherapy										
No	125	(44.3)	91	(32.3)	Ref		Ref		Ref	
Yes	157	(55.7)	191	(67.7)	0.85	(0.56-1.30)	1.39	(0.38-5.07)	0.79	(0.50- 1.24)
Radiotherapy										
No	127	(45.0)	92	(32.6)	Ref		Ref		Ref	
RT except IMC/SCL	58	(20.6)	97	(34.4)	0.45	(0.25 -0.79)	1.52	(0.35 -6.59)	0.34	(0.18 -0.65)
RT to IMC/SCL	97	(34.4)	93	(33.0)	1.32	(0.80 -2.19)	1.23	(0.25 -5.96)	1.33	(0.77 -2.28)
Radiotherapy										
No RT and RT except IMC/SCL	185	(65.6)	189	(67.0)	Ref		Ref		Ref	
RT to IMC/SCL	97	(34.4)	93	(33.0)	1.78	(1.13-2.82)	1.00	(0.25-4.01)	1.93	(1.18-3.17)
Chemotherapy										
No	276	(97.9)	261	(92.6)	Ref		Ref		Ref	
Yes	6	(2.1)	21	(7.4)	0.69	(0.23-2.05)	3.58	(0.32-40.6)	0.26	(0.06-1.18)
Tamoxifen										
No	236	(83.7)	239	(84.8)	Ref		Ref		Ref	
Yes	46	(16.3)	43	(15.2)	0.69	(0.37-1.29)	0.16	(0.02-1.35)	0.94	(0.48-1.86)

Abbreviations: N, numbers; OR, odds ratio; CI, confidence interval; Ref, reference; RT, radiotherapy; IMC, internal mammary chain; SCL, supraclavicular lymph nodes.

Table 6. Associations between laterality of breast cancer, radiotherapy, and hemisphere location of stroke among patients with carotid strokes

BC laterality	**Carotis right**		**Carotis left**		**p-value**
	N	(%)	N	(%)	
All carotid strokes					
Right	49	(43.4)	64	(56.6)	
Left	56	(52.3)	51	(47.7)	0.22
RT					
Right	25	(43.9)	32	(56.1)	
Left	31	(51.7)	29	(48.3)	0.46
RT except IMC/SCL					
Right	9	(45.0)	11	(55.0)	
Left	10	(50.0)	10	(50.0)	1.00
RT to IMC/SCL					
Right	16	(43.2)	21	(56.8)	
Left	21	(53.8)	18	(46.2)	0.37

Abbreviations: p, probability; N, numbers; RT, radiotherapy; IMC, internal mammary chain; SCL, supraclavicular lymph nodes.

We further investigated dosage effects of radiotherapy, measured by the daily fraction dose, to different targets and risk of stroke (Table 7). The dosage for RT to the remaining breast after BCS was standardised with very similar doses for all patients treated and as such, a dose-response relation could not be evaluated. However, for IMC and SCL there was a statistically significant trend for higher risk of stroke with increasing daily fraction dose, especially marked for high doses (≥ 4 Gy).

Table 7. Odds ratios and 95 % confidence intervals for stroke in association with daily fraction radiation doses

	Cases	Controls	OR, age adjusted	CI
Daily fraction radiation dose to IMC				
No RT	184	189	Ref	
≤2.5 Gy	20	34	0.56	(0.24 , 1.31)
2.6-3.9 Gy	64	49	2.61	(1.48 , 4.60)
≥4 Gy	11	10	3.05	(0.97 , 9.58)
Unknown dose	3	0	-	-
Daily fraction radiation dose to SCL				
No RT	183	190	Ref	
≤2.5 Gy	19	33	0.54	(0.23 , 1.29)
2.6-3.9 Gy	45	36	2.14	(1.15 , 3.99)
≥4 Gy	32	23	4.06	(1.85 , 8.94)
Unknown dose	3	0	-	-

Abbreviations: OR, odds ratio; CI, confidence interval; IMC, internal mammary chain; RT, radiotherapy; Ref, reference; Gy, Gray; SCL, supraclavicular lymph nodes.

Paper III

RIHD is a recognised medical condition for many years and the whole heart as an organ has been defined as an OAR [71-72, 84]. Studies have shown an increase of cardiovascular mortality, when comparing left- and right-sided breast cancer [125-127]. Symptomatic coronary artery disease is an early, and appropriate, vascular event. In this study we investigated the correlation between the geometric projection of the radiation fields and the locations of coronary artery stenosis, detected by coronary angiography.

In the study, left-sided BC was slightly more common than right-sided (55% vs. 45%), among the 199 women who had been referred for coronary angiography after a BC. No major differences, regarding age, calendar period for BC, follow-up, stage, RT, adjuvant systemic therapy, and distant recurrences, between women with left- and right-sided BC were noticed. Mean age at diagnosis of BC was 58.2 years and median follow-up period between BC and coronary angiography was 10.3 years. Sixty-two percent of the women received RT. Twenty-nine percent were irradiated to the IMC. Adjuvant chemotherapy and endocrine therapy were used in a minority of the patients, only 9% and 17%, respectively.

Table 8. Odds ratios for grades of coronary artery stenosis in left- versus right-sided breast cancer

	All patients		Non-irradiated patients		Irradiated patients	
Stenosis Grade	OR	95% CI	OR	95% CI	OR	95% CI
All segments						
1-5	1.18	(0.83 , 1.67)	1.11	(0.61 , 2.05)	1.42	(0.90 , 2.23)
3-5	1.26	(0.83 , 1.92)	1.30	(0.67 , 2.52)	1.38	(0.77 , 2.46)
4-5	1.20	(0.81 , 1.78)	1.06	(0.56 , 2.03)	1.45	(0.85 , 2.46)
Proximal RCA						
1-5	0.97	(0.65 , 1.44)	1.12	(0.55 , 2.28)	0.89	(0.53 , 1.48)
3-5	0.67	(0.36 , 1.24)	0.78	(0.29 , 2.14)	0.88	(0.37 , 2.08)
4-5	0.74	(0.37 , 1.45)	1.09	(0.36 , 3.27)	0.78	(0.29 , 2.07)
mdLAD+dD						
1-5	1.44	(0.95 , 2.18)	0.84	(0.41 , 1.73)	2.04	(1.18 , 3.55)
3-5	2.18	(1.16 , 4.09)	0.98	(0.39 , 2.46)	4.38	(1.64 , 11.7)
4-5	2.91	(1.24 , 6.83)	1.16	(0.36 , 3.76)	7.22	(1.64 , 31.8)

Abbreviations: OR, odds ratio; CI, confidence interval; RCA, right coronary artery; mdLAD+dD, mid, distal and distal diagonal branch of left anterior descending artery.

We first compared women with left-sided BC to those with right-sided (Table 8). Women with left-sided BC showed a trend towards increased incidence of stenosis of all segments and all grades of stenosis. In particular, women with left-sided BC had more often stenosis in mdLAD+dD. The OR increased in relation to the severity of the stenosis, i.e. higher OR with more

severe stenosis. Next, we split the left and right comparison into irradiated and non-irradiated BC. In the non-irradiated group the ORs were close to unity, whilst in the irradiated group evident differences appeared. A statistically significant increase in stenosis of the mdLAD+dD emerged: grade 1-5 (OR=2.04; CI=1.18-3.55), grade 3-5 (OR=4.38; CI=1.64-11.7), and grade 4-5 (OR=7.22; CI=1.64-31.8). In prox RCA no statistically significant differences in stenosis were observed between irradiated left- and right-sided BC, although the ORs were numerically below unity for all grades of stenosis.

We then proceeded to compare women with BC who had received high-risk RT, to BC women who had received low-risk RT/no RT (Table 9). Since the LSMEANS-estimate depends on the segments included in the analyses, two different analyses were performed: one with all segments and one with hotspot areas only. All the odds ratios were above unity. In the analyses of women with BC and hotspot areas, the OR for grade 3-5 stenosis was 1.90 (CI=1.11-3.24) and in grade 4-5 stenosis the OR was 1.87 (CI=1.14-3.09).

Table 9. Odds ratios from generalised linear mixed models for grades of coronary artery stenosis in high- versus low-risk RT or no RT

	All Segments			Hotspot Areas		
Stenosis Grade	LSMEANS Estimate	OR	95% CI	LSMEANS Estimate	OR	95% CI
1-5						
Low-risk RT/no RT	0.18	Ref		0.25	Ref	
High-risk RT	0.27	1.68	(1.10-2.57)	0.38	1.85	(1.17-2.93)
2-5						
Low-risk RT/no RT	0.08	Ref		0.12	Ref	
High-risk RT	0.10	1.25	(0.80-1.94)	0.16	1.33	(0.83-2.13)
3-5						
Low-risk RT/no RT	0.06	Ref		0.07	Ref	
High-risk RT	0.09	1.61	(1.00-2.59)	0.12	1.90	(1.11-3.24)
4-5						
Low-risk RT/no RT	0.04	Ref		0.04	Ref	
High-risk RT	0.08	2.06	(1.21-3.51)	0.08	1.87	(1.14-3.09)

Abbreviations: LSMEANS, least-square means; OR, odds ratio; CI, confidence interval; RT, radiotherapy; Ref, reference.

Among women with BC with at least one segment with significant stenosis, the distribution of such stenoses is shown in Figure 5. The pattern is similar in the groups of reference women and women with low-risk RT/no RT but seems to differ in the groups of high-risk RT, with an increase of stenosis in prox RCA and mdLAD+dD in right and left BC, respectively.

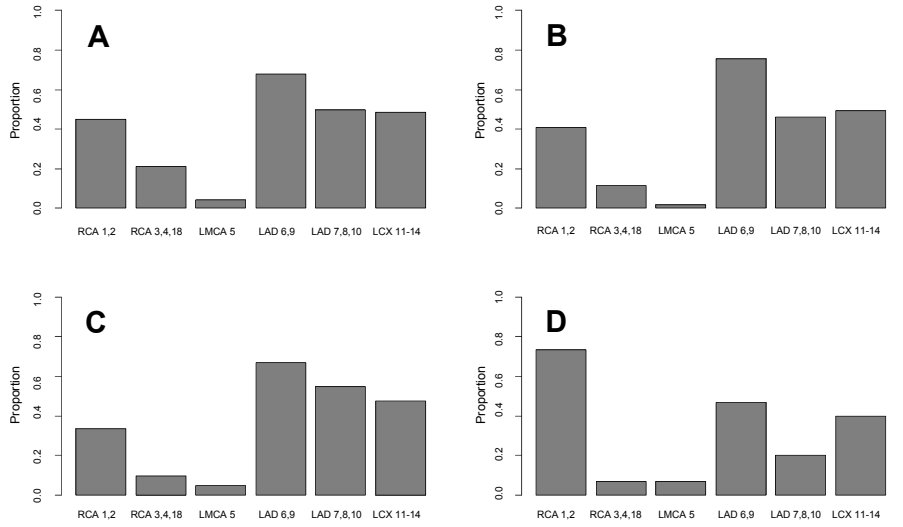

Figure 5. Distribution of significant stenoses, presented as proportions of patients with significant stenosis in each category, in segment groups among women with at least one segment with significant stenosis. (A) Reference women (n = 122 of 188); (B) low-risk radiotherapy (RT) or no RT (n = 61 of 106); (C) left-sided breast cancer (BC), high-risk RT (n = 42 of 67); (D) right-sided BC, high-risk RT (n = 15 of 26). RCA, right coronary artery; LMCA, left main coronary artery; LAD, left anterior descending artery; LCX, left circumflex artery.

Paper IV

The heart is composed of different anatomical structures, which may have different radiation tolerance, supported by the study of McGale et al [74]. Moreover, the cardiac radiation dose has an inhomogeneous distribution in the heart volume and even below the cardiac tolerance dose, the hotspot doses may be high [165]. In the present study, in women with left- and right-sided BC who have received 3DCRT and been examined by coronary angiography, we described the distribution of radiation dose in segments of coronary arteries and reported the distribution of coronary artery stenoses.

Among the 15 patients in this study diagnosed between 1993 and 2002, there were seven cases of left- and eight cases of right-sided BC, respectively (Table 10). Twelve patients had BC stage I-II and three patients had no axillary staging performed. Mean age at diagnosis of BC was 58.9 years (range 46-70 years) and mean follow-up period between termination of RT and coronary angiography was 3.8 years (range 0.3-8.2 years). Eight women received RT to the breast and regional lymph nodes, including the axilla, SCL, and IMC. Two women were irradiated to the breast and axilla, and five women received RT to the breast without regional lymph nodes. RT was

administered with 25-28 radiation fractions of 2.0 Gy to the targets 5 days each week, to a total dose of 50-56 Gy. Adjuvant chemotherapy was received by five women in our study, of whom two women received FEC and three women received CMF. Six women used adjuvant endocrine therapy during the study, all of them treated with tamoxifen. One woman received a combination of chemotherapy and endocrine therapy and five women received no adjuvant systemic therapy. No local or distant recurrences were detected during the follow-up.

All women with right-sided BC had low mean doses to the heart, in the range of 1-3 Gy. Women with left-sided BC had higher mean doses to the heart, in the range of 3-13 Gy. Mean doses to mdLAD and in particular segment 8 (corresponding to distal LAD) were considerably higher than mean heart doses in women with left-sided BC, reflecting high doses in small volumes. In three of the women, the mean doses to prox RCA and in particular segment 2 were substantially higher than the mean doses to the heart. In general, the radiation doses were higher in the ventral located segments 1+2 and segments 7+8, in comparison to the doses of the dorsal adjacent segment 3 and segment 6, respectively (Table 10 and Figure 6).

Three of the women with left-sided BC receiving RT to the breast and regional lymph nodes had significant volumes of the heart (>10%) irradiated with doses exceeding 20 Gy. Two of these women were treated with the mixed radiation technique of electrons-photons and one woman with tangential irradiation. The only woman who received RT to the left breast and regional lymph nodes with "the double angle technique" had low $V_{heart\ 20\ Gy}$ and $V_{heart\ 40\ Gy}$, in the same order of magnitude as women with left-sided BC irradiated to the breast only.

The distribution of radiation dose in the segments of the coronary arteries differed markedly between left- and right-sided BC (Table 10 and Figure 6). Irradiation of left-sided BC gave a substantially higher radiation dose in mdLAD compared to right-sided BC, with doses in segment 8 approaching the target dose of 50-56 Gy. The two women with left-sided BC receiving RT to the breast without regional lymph node irradiation had doses in segment 8 as high as the women irradiated to the regional lymph nodes as well.

Women with right-sided BC generally received low coronary artery mean doses in the range of 1-5 Gy, mainly by transmitted dose through collimators and other field shaping devices. The only exception was a woman where the irradiation of the right IMC contributed to the max dose of 45 Gy in segment 2. Two of the women with left-sided BC also received substantial max doses to the prox RCA. Both these patients were irradiated to the left IMC. These

three women were irradiated with the mixed electron-photon radiation technique in the early period of the study, when the IMC target included the ipsilateral intercostal spaces 1-5. After 1995, the target definition of IMC changed to include only intercostal spaces 1-3 with the purpose of lowering the heart dose, and thereafter all women irradiated to the IMC had low radiation doses to the prox RCA.

Table 10. Patient characteristics, radiation doses, and coronary artery stenosis

Patient number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
BC laterality	L	L	L	L	L	L	L	R	R	R	R	R	R	R	R
Stage	II	I	II	II	I	-	II	-	II	II	II	-	I	II	I
BC year	-94	-94	-95	-95	-96	-98	-01	-93	-93	-94	-96	-98	-99	-01	-02
BC age (years)	53	51	54	65	52	69	59	46	61	57	69	54	63	61	70
FU (years)	4.2	3.5	7.7	8.2	1.3	4.0	0.3	2.4	5.1	3.6	7.3	5.3	1.5	1.5	1.5
Targets	B+ RN	B	B+ RN	B+ RN	B+ Ax	B	B+ RN	B+ Ax	B+ RN	B+ RN	B+ RN	B	B	B+ RN	B
Dose (Gy)	52	54	54	56	54	50	50	54	53	56	56	50	50	52	50
RT technique	Mx	Tg	Mx	Tg	Tg	Tg	Dt	Tg	Mx	Tg	Tg	Tg	Tg	Tg	Tg
Systemic treatm	E	-	C	E	C	-	EC	-	E	C	E	-	-	C	E
Heart															
Max dose (Gy)	46	52	50	58	55	48	47	12	49	12	7.3	1.6	4.9	3.2	3.5
Mean dose (Gy)	12	3.2	8.7	13	5.7	2.9	4.5	0.9	3.3	1.8	2.7	0.8	1.4	1.2	1.1
$V_{heart\ 20\ Gy}$ (%)	22	3	13	20	8	2	5	0	3	0	0	0	0	0	0
$V_{heart\ 40\ Gy}$ (%)	4	2	3	16	4	1	2	0	1	0	0	0	0	0	0
Max dose (Gy)															
Segment 1	35	2.7	36	7.5	3.3	2.5	3.8	2.6	6.3	6.0	5.5	1.2	2.2	3.0	3.5
Segment 2	39	2.8	38	6.1	3.1	2.3	3.6	4.4	45	6.1	5.5	1.3	3.0	3.1	3.2
Segment 3	17	1.8	18	3.4	1.8	1.6	3.0	2.5	4.4	3.7	4.4	1.1	4.5	1.9	2.4
Segment 5	7.5	1.8	6.5	4.3	3.0	1.6	2.8	1.4	3.0	2.6	3.9	1.0	1.6	1.8	3.5
Segment 6	11	2.6	6.5	18	4.2	1.7	3.0	0.7	2.1	2.2	3.6	1.0	1.2	1.5	1.0
Segment 7	38	5.1	25	55	28	37	11	0.8	2.6	2.5	3.1	1.0	1.4	1.5	1.0
Segment 8	46	52	27	57	52	48	46	0.8	3.2	2.8	3.7	1.0	1.8	1.5	1.4
Mean dose (Gy)															
Segment 1	26	1.9	26	5.8	2.9	2.1	3.5	2.2	4.6	4.2	4.7	1.1	1.8	2.5	2.3
Segment 2	33	2.3	31	4.1	2.3	2.0	3.3	3.3	21	4.6	5.0	1.2	2.4	2.8	2.4
Segment 3	7.9	1.5	4.2	2.9	1.5	1.6	2.3	1.5	3.4	2.3	3.6	1.1	3.6	1.4	1.6
Segment 5	6.8	1.7	4.5	3.9	2.7	1.6	2.6	1.1	2.5	2.1	3.8	1.0	1.4	1.6	2.0
Segment 6	9.7	2.2	6.2	8.2	3.7	1.6	2.8	0.7	2.0	2.0	2.6	0.9	1.1	1.4	0.9
Segment 7	25	4.0	15	50	11	6.0	4.1	0.5	2.3	2.2	2.2	0.9	1.2	1.3	0.9
Segment 8	42	31	18	55	40	38	31	0.6	2.8	2.3	3.6	0.9	1.6	1.1	1.2
Stenosis Grade															
Segment 1	0	0	1	1	**3**	**4**	0	0	0	0	0	0	0	0	1
Segment 2	0	0	0	1	**3**	**5**	0	0	0	0	0	0	0	0	1
Segment 3	0	0	0	1	0	0	0	0	0	0	**4**	0	0	0	1
Segment 5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Segment 6	0	0	0	0	1	1	0	0	0	0	1	0	0	0	1
Segment 7	0	0	1	**5**	1	**3**	0	0	0	0	1	0	0	0	1
Segment 8	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0

Abbreviations: BC, breast cancer; L, left; R, right; FU, follow-up; B, breast; RN, regional lymph nodes; Ax, axillary lymph nodes; Gy, Gray; RT, radiotherapy; Mx, mixed photon-electron; Tg, tangential; Dt, double tangential; treatm, treatment; E, endocrine therapy; C, chemotherapy; $V_{heart\ 20\ Gy}$, volume of heart receiving ≥20 Gy; $V_{heart\ 40\ Gy}$, volume of heart receiving ≥40 Gy. Significant stenosis highlighted in bold numbers.

The majority of the coronary segments studied had no stenoses. In four patients, seven segments had clinical significant stenoses. In two patients, significant stenoses were located in hotspot areas of radiation dose, whereas in the other two patients the significant stenoses were located in areas with low exposition of radiation. The two patients with significant stenoses in high-dose areas both had relatively long latency between RT and angiography (4.0 and 8.2 years).

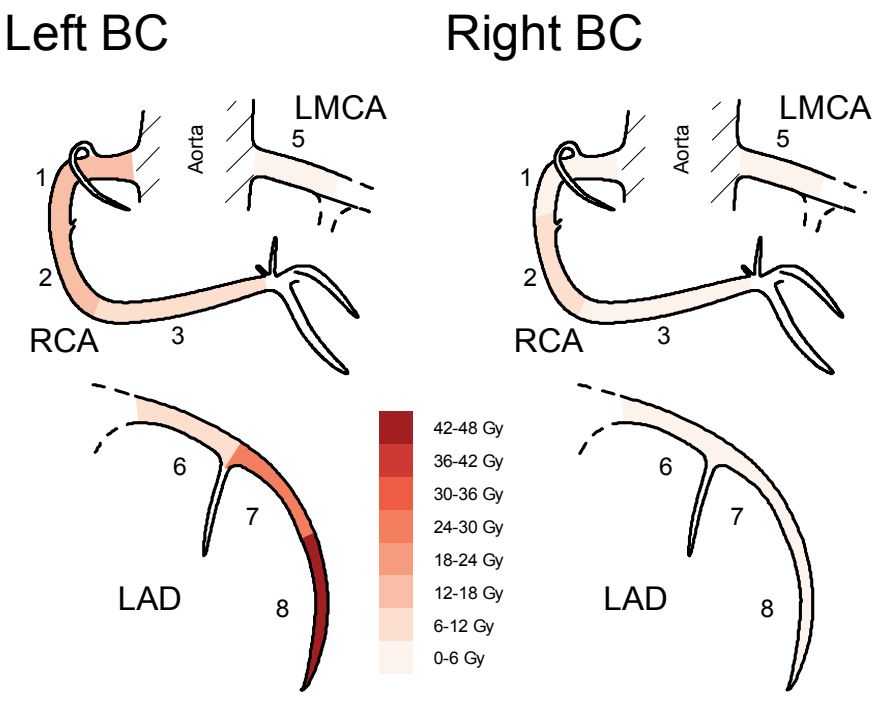

Figure 6. Segment-wise distribution of mean maximal radiation doses, of seven left- and eight right-sided women with breast cancer (BC); RCA, right coronary artery; LMCA, left main coronary artery; LAD, left anterior descending artery; Gy, Gray.

General discussion

Radiotherapy in breast cancer and stroke

Paper I was the first study, to our knowledge, to describe an association between breast cancer and stroke [164]. We found a statistically significant 12% increase of stroke in a study base of breast cancer compared to the stroke incidence in the background population. The strength of the study was the large study base of 25,171 women with BC of whom 1,766 had subsequently suffered a stroke during follow-up. The study was population-based and the registers had good coverage and high quality. In this study, no information regarding adjuvant treatment for breast cancer and cardiovascular risk factors of the individual patients was available. To elucidate the pathogenesis behind the association between BC and stroke we proceeded to perform a case-control study with cases and controls recruited from the study base of breast cancer.

In Paper II, the selection of controls followed standard incidence density based sampling nested within a well-defined cohort [157]. We deliberately avoided matching for age, stage of disease, and time period of diagnosis, since indications and methods for radiotherapy are associated with those factors. Indeed, our findings indicate that such a matching would have entailed over-matching and the risk of stroke may have been underestimated. The overall stroke risk for irradiated women was not increased [166]. However, when the analyses were stratified for type of radiotherapy, differences emerged. A nearly doubled risk of stroke was detected among women who received RT to the targets IMC and SCL compared to women without RT to these targets.

In this study, we had no information on established cardiovascular risk factors such as hypertension, smoking and hyperlipidaemia at the time of radiotherapy. However, if anything, it is likely that these factors are associated with more severe co-morbidity and thus may be associated with a relative contraindication for RT. This suggests that our estimates may be conservative. RT that was not delivered to the targets IMC and SCL was associated with a lower risk of stroke, which probably is due to a selection phenomenon. The majority of women in this group had RT to the remaining breast tissue after BCS and reasons for avoiding RT after BCS during this

period of time was a low performance status and serious cardiovascular disease.

We found no association between BC laterality and the corresponding hemisphere location of the carotid stroke, in contrast to the study by Dorresteijn et al in head and neck cancer [93]. In the three breast cancer studies on RT and stroke besides ours [144-146], only the study by Jagsi et al showed an increased risk of stroke among the irradiated women [145]. In fact, the study by Hooning et al showed a statistically significant decrease of stroke in irradiated women [144], a finding similar to the result of our study in the group of patients irradiated to targets other than IMC and SCL [166]. There is no plausible biological explanation for RT to have a protective effect on the risk of stroke and the findings may be interpreted as selection phenomena since neither of the studies was randomised. Patients were selected by the surgeon for BCS or mastectomy and then selected by the oncologist to receive RT or not, and if RT was suggested, which targets to irradiate. Of course, the breast cancer treatment guidelines have a strong impact in the RT decision but individual circumstances such as performance status, co-morbidity, and cardiovascular risk factors may strongly influence the decision making concerning RT. Thus, there is a possibility that subgroups of women with BC *per se* may have had a lower risk of stroke than the background female population, making the comparison inadequate. Actually, the study by McGale et al on heart disease post RT in BC showed a statistically significant 10% decrease in cardiac mortality in the BC population compared to controls [74]. This suggests that women with BC may have a lower risk of cardiovascular disease than the general population. Moreover, in the McGale study, a probable selection phenomenon was shown [74]. Patients allocated to BCS plus RT had a significantly lower cardiac mortality than patients allocated to BCS without RT.

Including our study, there are four publications looking at the impact of RT to SCL in BC and the incidence of stroke [144-146, 166]. Our report stands out that it is the only study showing an increased risk for stroke in this population [166]. Nearly all the patients in our study receiving RT to regional lymph nodes were irradiated to the combined target of IMC and SCL. The only other study reporting IMC radiation was the work of Hooning et al [144]. In the carotid ultrasound study by Woodward et al in women with BC irradiated to SCL, there was no increase in carotid stenosis [147]. Thus, if there is a direct causal link between radiotherapy and stroke, we speculate that it is the dose to the superior part of the heart and the aortic arch (corresponding to the IMC radiation field) that is detrimental rather than the dose to the proximal part of the carotid artery (SCL radiation field). In that way IMC radiation could increase the risk of atherosclerosis and subsequently thrombo-embolic stroke. The embolus may follow the blood

stream to either the right or left carotid artery, irrespective of the BC laterality and SCL irradiation. However, we could not analyse the targets IMC or SCL as separate risk factors, since the majority of the patients received RT to both targets combined. Our result is consistent with the lymphoma studies by Bowers and Moser [142-143]. In accordance with our speculation that IMC radiation is detrimental, 50% of childhood Hodgkin survivors with stroke had concomitant heart or valve problems, predisposing to cardio-embolic stroke [143]. All of the patients with stroke had received mantle irradiation to a dose of 30-44 Gy, well covering IMC and SCL bilaterally [143].

High daily radiation doses increase the risk of late toxicity, like myocardial infarction [167], lymph oedema [168], and brachial plexopathy [168]. Correspondingly, we found a dose dependent relationship between daily radiation dose to IMC and SCL and stroke. High daily radiation doses is a characteristic of older RT techniques that did not use modern CT dose planning, not calculating doses to the OARs, and irradiated larger targets to a higher biologically effective radiation dose. We did not find any association between adjuvant tamoxifen or chemotherapy and stroke, but adjuvant systemic therapy was seldom used during the time period of our study and the numbers of patients was too small to be informative. The effect of tamoxifen on large arteries is ambiguous. Tamoxifen has been linked to decreased development of atherosclerosis [169], protection from coronary heart disease [170] but increased incidence of stroke [171]. Our results are in contrast to the study by Hooning [144], where they found an association between tamoxifen and stroke.

Radiotherapy in breast cancer and coronary artery disease

To our knowledge, Paper III is the largest study of coronary angiography findings in women with breast cancer [111]. When women irradiated for left-sided BC were compared to those with right-sided, a significant increase of stenosis in mdLAD+dD was seen and the risk was higher for more severe stenosis. An increase of clinically significant coronary artery stenosis was found in hotspot areas for radiation among women with BC irradiated to the left breast/chest wall or IMC, as compared to women with BC who had not received RT to these target areas. When separating the women with BC in defined groups according to type of RT delivered, as shown in Figure 5 on page 45, differences in distribution of coronary stenoses in accordance to radiation exposition emerged.

In the study by Correa et al, 14 patients with a history of radiation for BC were referred to coronary angiography and an association between RT to the left breast and stenosis in LAD was found [92]. On the other hand, Wang et al did not find any association between the laterality of breast cancer and location of coronary stenosis in 91 women previously irradiated for BC who underwent coronary angiography [138]. A major difference between these three studies is the time between RT and coronary angiography [92, 111, 138], an important and perhaps crucial factor. Radiation-induced vascular toxicity is a late effect, not evident in the first years post RT and augmented with longer follow-up, as illustrated by the study of Darby et al [127]. In the two angiography studies with long-term follow-up of 15 years [92] and 10.3 years [111] respectively, an association between the radiation target and the location of stenosis was found, whilst in the study with shorter follow-up of 4.2 years [138] no such association was found. Studies in left-sided irradiated BC patients examined by cardiac SPECT [137] and stress echocardiograms [92] have shown perfusion defects in the anterior part of the left ventricle, in conformity to the hotspot areas in our study.

The majority of the women in Paper III with BC had not received CT dose planned RT. Therefore it was not possible to calculate the exact radiation dose to the heart and the coronary arteries for every woman with BC and the RT targets and regimens were divided into high-risk and low-risk. We used an a priori definition of high-risk RT and of hot spots for coronary changes based on heart anatomy and known distribution of radiation fields. In tangential left-sided RT the irradiation portals could be close to, or even include, the anterior part of the left ventricle and the LAD, as illustrated in Figure 4 on page 35 [165, 172]. Patients with unfavourable anatomy may receive radiation doses >30 Gray to parts of the distal LAD [165] and there is an increased risk of developing IHD, shown in some studies [131-132], but not in all [135]. In our study, radiation to the left breast/chest wall was considered as high-risk RT and was associated with an increased risk of coronary artery stenosis in mdLAD+dD.

The internal mammary chain is a target area located close to the anterior part of the heart. With older radiation techniques and a wide frontal photon field to cover the IMC, the radiation dose to the heart was high. Modern CT planned techniques deliver lower, but still potentially harmful, doses to the coronary arteries. RT to the IMC has been associated with increased risk of myocardial infarction [139-140] in some studies, whereas in other reports, no increase in cardiac deaths [68] and IHD [69] were noted. Hopefully, the large randomised EORTC trial 22922/10925 will elucidate the effect of irradiating the IMC [141]. In our study, radiation to the IMC was considered as high-risk RT and was associated with an increased risk of coronary artery stenosis in the hotspots areas.

In our study, no information was available on established cardiovascular risk factors such as hypertension, smoking, and hyperlipidaemia at the time of radiotherapy. We have no reason to believe that women with left-sided BC differed from those with right-sided BC in that respect or that these factors are strongly related to the decision to irradiate. If anything, established IHD has been considered to be a relative contraindication for RT, especially in women with left-sided BC, thus strengthening our results further. A possible confounder would be if women with BC, and especially those with a left-sided BC, were more often referred for angiography due to the known risk of IHD after breast cancer irradiation, to that they have frequent health care contacts or due to post surgery and irradiation breast pain. The women with BC may have been referred on wider indications. However, this pattern was similar for women with left- and right-sided BC, suggesting that our comparison between left- and right-sided BC is unbiased.

In Paper IV, only patients treated with 3DCRT were included, giving us the opportunity to review a detailed dose distribution in the heart and coronary arteries for each patient. We found a marked difference of radiation dose distribution in mid and distal LAD between women with left- and right-sided breast cancer. The majority of women with left-sided BC had high mean radiation doses in the distal LAD, in the range of 30-55 Gy, whereas women with right-sided BC mostly were exposed to low doses of scattered and transmitted radiation. Max doses close to full target doses in the distal LAD were noticed in patients with left-sided tangential RT of the breast even without regional lymph node irradiation. A radiation dose gradient with higher doses in the most ventral coronary segments and lower doses in segments located more dorsally could be observed, as expected.

In contrast to previous studies employing older radiotherapy techniques [95-96], requiring dose estimations of representative average patients, our study is based on detailed information of the individual patient radiation dose in different parts of the heart and coronary arteries, as all the patients were treated with 3DCRT. In Paper III, hotspot areas for radiation dose to the prox RCA and mdLAD was postulated [111]. This was confirmed in Paper IV. Our results are in accordance with the study by Taylor et al, showing that women with left-sided BC who received tangential RT to the breast were exposed to an average maximum radiation dose of 35.2 Gy to the LAD [165]. In this study, the LAD average mean dose was considerably lower, reflecting the anatomy of the LAD in relation to the radiation fields and the fact that small segments of the artery may receive high doses although the mean dose to the entire artery may be within tolerance limits. To our knowledge, the present study is the first to describe coronary artery doses in segments of the coronary arteries.

The study was initially designed to also explore eventual strong relationship between radiation dose and coronary artery stenosis in specific coronary segments with known dose. However, we found considerably fewer women than we expected that had both angiograms and were irradiated with 3DCRT and the interval between RT and the angiogram was generally short in terms of looking for radiation-induced lesions. Coronary artery disease is a multifactorial and common disease, and also in an irradiated BC population the majority of coronary artery stenoses will be unrelated to RT; a strong relationship between dose and stenosis would be needed for a positive finding. It has been proposed that radiation acts in concert, and perhaps in synergy, with other cardiovascular risk factors, such as hypertension, hypercholesterolaemia, obesity, and diabetes [78]. One patient in our study had an occlusion of segment 7, corresponding to mid LAD, approximately eight years after RT. The mean radiation dose was 50 Gy to the segment. In the same patient, no significant stenoses were detected in other segments of the coronary arteries. This may be a random finding but might illustrate a late radiation vascular effect.

The radiation tolerance dose for the heart is based on studies concerning radiation induced pericarditis [71] and cardiovascular mortality [73] and in these circumstances the whole heart is the OAR. The Danish Breast Cancer Cooperative Group (DBCG) guidelines [173], based on the study by Gagliardi et al [73], has suggested the following criteria for heart irradiation in BC: less than 5% of the heart volume should receive 40 Gy ($V_{heart\ 40\ Gy}$ = 5%) and less than 10 % of the heart volume should receive 20 Gy ($V_{heart\ 20\ Gy}$ = 10%). All the patients with right-sided BC but only 4/7 patients with left-sided BC in the study fulfilled these dose constraints. However, the four patients with left-sided BC who did fulfill the DBCG dose constraints still had high hotspot doses of 46-52 Gy in mdLAD, which we believe is a critical vascular structure for developing late radiation effects. Thus, restricting heart doses according to DBCG criteria does not exclude even quite high point doses in the coronary arteries.

Long-term follow-up of patients treated with RT for testicular seminoma [110] or Hodgkin lymphoma [112] indicates that total doses in the range of 20-40 Gy may cause significant adverse effect on vascular morbidity and mortality. Moreover, several epidemiological studies indicate that even lower doses may be significant and that radiation doses even lower than therapeutic ones may be harmful to the vasculature, as illustrated by the atomic bomb survivor studies [122] and the peptic ulcer study [123]. Thus, presently no safe radiation threshold dose for the coronary arteries can be set, and every effort should be made to eliminate radiation dose to the coronary arteries, if possible.

Our findings are supported in several aspects by the recent study by McGale et al [74]. In a comparison of irradiated left- and right-sided BC, the study showed an increased risk of IHD during the study period of 1977-2001. No differences in IHD incidence was seen in patients receiving RT after 1990 compared to patients irradiated before the 90s. This make sense if the coronary dose rather than the heart (i.e. myocardial) dose is the crucial point in the development of IHD, as the coronary doses were estimated to be at the same level during the whole study period whereas the heart doses were lower after 1990. According to Paper IV, a considerable radiation dose is still delivered to parts of the coronary arteries despite modern radiation technique. The risk of myocardial infarction and angina pectoris were substantially higher in left-sided BC than in right-sided with an OR of 1.22 and 1.25 respectively, in agreement with our findings in Paper III. No differences in the incidence of heart failure or myocardial diseases like cardiomyopathy, between left- and right-sided BC was found in the McGale study [74]. The results are supporting macroangiopathy rather than micro-angiopathy being the most detrimental pathogenetic mechanism of radiation-induced IHD. A synergy between RT and pre-existing IHD was found. For left- and right-sided BC, the OR was significantly elevated to 4.80 and 3.37, respectively, for a new heart disease event in patients with pre-existing IHD compared to patients with no prior IHD. In irradiated as well as non-irradiated patients with coronary artery disease, the majority of stenoses are located in the LAD territory. An equal increase of the relative risk of stenosis in different parts of the coronary arteries would mean a higher absolute risk of stenosis in the LAD compared to other locations of the coronary arteries. Thus, the gain from protecting the LAD from radiation would be substantial. This supports the idea of defining the LAD as a separate OAR and of making an effort to minimise the radiation dose to that region.

Risk-benefit of adjuvant radiotherapy in breast cancer

Adjuvant radiotherapy in breast cancer is without doubt an effective well-documented therapy to prevent local recurrences and to decrease breast cancer mortality [11-12]. In RT trials, an increase in cardiovascular deaths with a delay of >10 years has been shown in patients receiving RT [127]. With modern CT dose planned RT; the cardiac doses are lower but still potentially harmful. Due to the natural history of late effects in RT, long-term follow-up of current RT is needed. The heart is a complex organ with different vital structures, presumably with different radiation tolerance [74]. Furthermore, the dose distribution in the heart is inhomogeneous in irradiated BC, as illustrated in our work. At present, the entire heart is considered as an organ at risk with suggested dose constraints [71-72]. This

strategy may be the right one to prevent microangiopathy in the myocardium. However, macroangiopathy of the coronary arteries could be a more serious hazard since localised hot spot doses of radiation may be detrimental even though the dose constraints regarding the entire heart as an OAR are fulfilled. We propose that the coronary arteries should be regarded as separate OARs and that every effort should be made to avoid radiation dose in these areas.

To conclude, an increased risk of stroke when irradiating the regional lymph nodes of IMC and SCL and an increased risk of coronary artery disease when irradiating left breast/chest wall or the IMCs has to be considered in the decision making for adjuvant RT in BC. The risk-benefit analysis would favour RT in most circumstances, and indeed, when the patient is at a substantial risk of recurrence. On the contrary, in a low-risk patient or a patient with pre-existing IHD, only minimal radiation dose to the coronary arteries should be allowed. Available radiation techniques can often lower or eliminate radiation doses to the OARs. If this is not possible, the indication for RT should be reconsidered.

General conclusions

Paper I: In a study base of breast cancer, we found a statistically significant 12% increase of stroke, in comparison to the stroke incidence in the background population. The mechanisms of the association between breast cancer and stroke were unknown and could not be further explored in the study.

Paper II: Among women with breast cancer, radiotherapy to IMC and SCL almost doubles the risk of stroke, compared to women not irradiated to these targets. A dose-response relation for daily fraction radiation dose and a suggestion of an increased risk of ischaemic stroke, indicate a possible causal link between RT to IMC and SCL and stroke.

Paper III: A four- to seven-fold increase in high grade coronary artery stenosis in mid and distal LAD, including the distal diagonal branch, was detected in a study of women irradiated for breast cancer when comparing women with left-sided to those with right-sided BC. An increase in clinically significant coronary artery stenosis was found in pre-specified hotspot areas for radiation among women irradiated to the left breast/chest wall or to the IMCs, the explanation for which could be the target area for the RT.

Paper IV: In breast cancer patients treated with 3D conformal RT, a marked difference in dose distribution in mid and distal LAD between left- and right-sided BC was noted. Irradiated patients with right-sided BC mainly received low doses of scattered and transmitted radiation to the coronary arteries. On the contrary, tangential RT to the left breast without regional lymph node irradiation yielded coronary artery max doses of approximately 50 Gy to the distal LAD. These doses are probably not safe with regards to late radiation vascular effects.

Future perspectives

Adjuvant treatments in breast cancer have substantially improved the prognosis of the disease [9-12]. Unfortunately, the treatments are associated with hazards and serious side effects [111, 164, 166]. Clinical studies enable us to take into account the potential benefits and risks using different therapeutic strategies. The benefit of a given treatment in a group of patients may be expressed as the number needed to treat (NNT). This refers to the average number of patients required to receive a given treatment in order to produce the desired outcome in one patient. In the EBCTCG radiotherapy meta-analyses, NNTs for local recurrence is approximately 1:5 and for breast cancer death 1:20 [11-12]. Thus, for every fifth patient irradiated, one local recurrence is avoided, and for every four local recurrences avoided, one breast cancer death is avoided.

The prognosis factors: TNM stage, ER, PR, Her-2 receptor, histological grade and proliferation index, modulates the risk for local and distant recurrences. After BCS, patient age below 50 years, presence of DCIS, histological grade III and positive margins after surgery, have been reported as risk factors for local recurrence [174]. After mastectomy, lymph node status and tumour size are dominant risk factors for local recurrence [174]. Today, nearly all patients treated with BCS are offered adjuvant RT, in accordance with the treatment guidelines [15, 70]. After mastectomy, the TNM stage has a strong impact on the decision to irradiate or not [15, 70].

Considering the NNT and the potential hazards with RT, there is a lack of predictive factors to select patients for RT. One possibility is gene array analysis. Gene expression profiles, like MammaPrint and Oncotype DX, are developed as predictive tools for systemic treatment but no such instrument is available for RT as yet [174]. Studies on radiosensitivity are ongoing and in the study by Nimeus-Malmström at al, a gene expression profile that predicted local recurrence after BCS despite RT was found [175].

Technical development within RT is an ongoing process, mainly driven by RT in head and neck cancer. New methods to diminish the dose to the OARs are available. Respiratory-gated RT seems a promising and convenient method to lower the radiation dose to the heart and lungs [153]. Another promising method to lower the dose to the OARs is proton RT [156], soon

becoming available in our department. Further studies are needed to quantify the cardiovascular risks of RT in BC, with relevant clinical endpoints, when using modern CT dose planned RT. The next ideal step would be a study establishing dose-response relation between radiation dose and vascular event for the coronary arteries; a useful tool in the process of RT dose planning not only for BC.

Sammanfattning (Brief summary in Swedish)

Strålbehandling efter operation av bröstcancer minskar effektivt risken för återfall och har i moderna studier även visat förbättrad överlevnad. På lång sikt, har dock tyvärr strålbehandling vid bröstcancer varit förknippad med ökad risk för hjärt-kärlsjukdomar. I tidigare studier inom området, som då fram för allt är utförda på äldre strålbehandlingsteknik, har de ökade hjärt-kärlkomplikationerna uppträtt först efter en uppföljningstid av 10-15 år. Huruvida denna problematik även gäller modern strålbehandling är omtvistat. I samtliga fyra ingående delarbeten i avhandlingen studeras sambandet mellan strålbehandling vid bröstcancer och senare hjärt-kärlsjukdom.

Delarbete I:
Studiebasen utgjordes av 25171 kvinnor i Uppsala-Örebro sjukvårdsregion som diagnosticerats med bröstcancer under åren 1970-2000. Vid en länkning mot Patientregistret, fann vi att 1766 av dessa kvinnor hade haft stroke efter tidpunkten för sin bröstcancer. Det var fler än förväntat, vid en jämförelse med den så kallade bakgrundspopulationen. Vi fann alltså en ökad risk för stroke hos kvinnor som tidigare hade haft bröstcancer och riskökningen uppgick till 12 % i relativa tal. Ökningen utgjordes av stroke som orsakats av blodpropp i hjärnan och inte av hjärnblödning. Detta stämde väl överens med hypotesen att strålbehandling vid bröstcancer mot lymfkörtlarna ovanför nyckelbenet skulle kunna leda till förträngning av halsens blodkärl och öka risken för stroke till följd av blodpropp, vilket tidigare noterats vid uppföljning av patienter strålbehandlade för cancer i huvud-halsområdet. Då vi i denna studie inte hade detaljerade data för individuella patienter, kunde vi inte närmare undersöka orsaker till stroke.

Delarbete 2:
I detta arbete undersöktes huruvida behandlingen av bröstcancer påverkade risken för stroke och ifall det fanns ett samband mellan område (target) för strålbehandling och risk för stroke. Studien var en så kallad fall-kontrollstudie med patienter ur studiebasen från delarbete I. Fallen utgjordes av 282 kvinnor från Uppsala län som först haft bröstcancer och sedan insjuknat i stroke under tidsperioden 1970-2003. Kontrollerna var lika många till antalet, boendes i Uppsala län vid bröstcancerdiagnos, och

slumpmässigt valda ur studiebasen av kvinnor med bröstcancer men *ej* stroke under uppföljningstiden.

Då man delade upp de strålbehandlade kvinnorna efter targets för strålbehandling, sågs tydliga skillnader. Kvinnor som strålbehandlats mot lymfkörtlar ovanför nyckelbenet och längs bröstbenet jämfördes med kvinnor som inte fått denna typ av strålbehandling. Analysen visade en tydligt ökad, nästintill fördubblad, risk för stroke. Inget samband mellan sida för bröstcancer (vä/hö) och sida för stroke (vä/hö) noterades, vilket talar mot hypotesen att strålbehandling mot lymfkörtlar ovanför nyckelbenet skulle öka risken för samsidig stroke. Vi diskuterar istället möjligheten av strålpåverkan av strålfältet över lymfkörtlarna längs bröstbenet, då dessa är belägna centralt i kroppen, i närheten av övre delen av hjärtat och stora kroppspulsådern, vilket skulle kunna förklara det uteblivna sambandet för sida mellan bröstcancer och stroke. Nästintill samtliga patienter som fick strålbehandling mot lymfkörtlar ovanför nyckelbenet fick också strålbehandling mot lymfkörtlar längs bröstbenet, varför det inte fanns möjlighet att undersöka effekten av strålbehandling för vardera target separat. Slutligen, såg vi ett tydligt samband mellan högre daglig stråldos och risk för stroke, vilket förutom stråldos även avspeglar äldre strålbehandlingsteknik jämfört med modern.

Delarbete III:
I denna studie undersöktes ifall det fanns ett samband mellan target för strålbehandling och lokalisation av förträngningar i hjärtats kranskärl. Patienterna rekryterades från samma studiebas av kvinnor med bröstcancer och här ingick 199 kvinnor som hade haft bröstcancer och sedan blivit undersökta med kranskärlsröntgen. Vid en jämförelse mellan strålbehandlad vänstersidig och högersidig bröstcancer, sågs en fyrfaldig ökning av kliniskt betydelsefulla förträngningar i den främre nedåtstigande grenen av vänster kranskärl (även benämnd LAD). Vid jämförelse mellan icke-strålbehandlad vänstersidig och högersidig bröstcancer noterades inga skillnader. En jämförelse mellan ”hög-riskstrålning” och ”låg-riskstrålning” visade en närmast fördubblad risk för kliniskt betydelsefulla förträngningar i de avsnitt av kranskärlen där stråldosen förmodas ha varit som högst.

Vi kunde alltså visa att fördelningen av förträngningar i hjärtats kranskärl påverkades av tidigare strålbehandling, med en klar skillnad mellan vänstersidig och högersidig bröstcancer och en tydlig ökning av förträngningar i de främre avsnitten av kranskärlen med högre risk för strålbelastning. Detta var helt i överensstämmelse med hypotesen att strålbehandling kan leda till kärlpåverkan som visar sig som förträngningar av kranskärlen.

Delarbete IV:
I denna studie ingick 15 kvinnor ur delarbete III som hade erhållit den modernaste formen av strålbehandling, så kallad CT dosplanerad strålbehandling, vilket började användas vid strålbehandling av bröstcancer i mitten på 90-talet. CT dosplanerad strålbehandling möjliggör en noggrann kartläggning av stråldos i olika delar av hjärtat. Huvudfyndet var en tydlig skillnad av fördelning av stråldos i hjärtat och dess kranskärl mellan vänstersidig och högersidig bröstcancer. Patienter med vänstersidig bröstcancer erhöll generellt en hög stråldos, med maxdos i samma nivå som behandlingsdosen på 50 Gray, i mellersta och nedre delen av kranskärlet LAD. Kvinnorna med vänstersidig bröstcancer hade betydlig högre stråldos i mellersta och nedre delen av LAD än i resten av hjärtat. Vid högersidig bröstcancer erhölls i huvudsak låg stråldos i hjärtat och dess kranskärl.

Det var få av dessa 15 patienter som hade förträngningar i kranskärlen och antalet var för litet för att kunna fastställa något samband mellan stråldos och grad av förträngning av kranskärl. Dessutom var uppföljningstiden mellan strålbehandling och kranskärlsröntgen relativt kort för att studera långtids-effekter av strålning på kranskärlen. Men, den patient i studien som hade längst uppföljningstid på drygt åtta år, hade en grav förträngning som helt stoppade blodflödet i kranskärlet LAD samtidigt som övriga avsnitt av kranskärlen var utan betydelsefulla förträngningar. Förträngningen i LAD var belägen i ett högdos-område för strålning med medeldos 50 Gray. Det skulle kunna vara ett slumpfynd men skulle alternativt kunna illustrera strålorsakad åderförkalkning i kranskärl. Sammanfattningsvis, visades att man vid modern CT dosplanerad strålbehandling av vänstersidig bröstcancer erhöll höga stråldoser i kranskärlet LAD, i närheten av den ordinerade behandlingsdosen av 50 Gray, samtidigt som medeldosen i hjärtat var betydligt lägre.

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