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

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

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

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

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

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

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

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

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

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

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

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

Patients and Methods

This single-Center, prospective, observational clinical study included 60 eligible female patients with operable early stage left-sided breast cancer or ductal carcinoma *in situ*. All patients received adjuvant conformal 3D RT after local breast tumor resection (n=59) or mastectomy (n=1) without axillary or supraclavicular lymph node RT. Patients treated with adjuvant chemotherapy were excluded.

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

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

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

Other exclusion criteria were age over 80 years, dialysis, recent acute myocardial infarction, symptomatic heart failure, chronic atrial fibrillation, pacemaker therapy and severe lung disease. The local Ethical Committee approved the protocol (ETL R10160), and all participants signed informed consent before study enrollment. The study was conducted from June 2011 to May 2013.

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

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

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

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

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

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

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

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

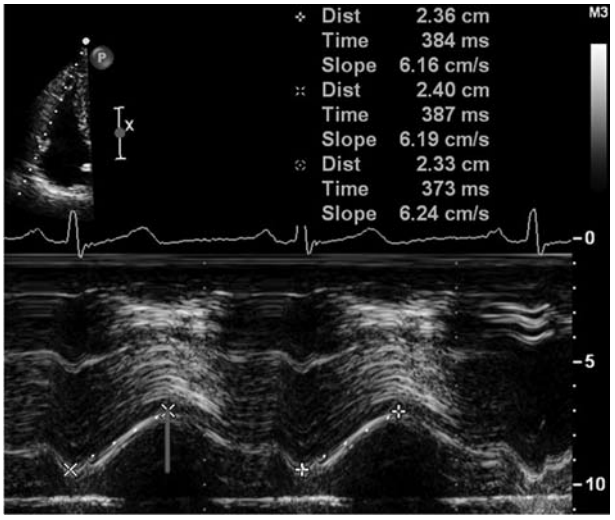

Figure 1. *Measurement of tricuspid annular plane systolic excursion (TAPSE). TAPSE was acquired with the M-Mode cursor placed between the junction of the tricuspid valve and the lateral free wall annulus of the right ventricle. TAPSE was measured as total displacement of the tricuspid annulus from end-diastole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

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

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

Results

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

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

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

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

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

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

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

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

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

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

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

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

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

Discussion

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Conclusion

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

Conflict of Interest

None to declare.

Acknowledgements

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

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