# The effects of hormonotherapy administered concurrent radiotherapy and trastuzumab on cardiac toxicity in rats

*Yasemin Benderli Cihan, Vedat Arsav**

Clinic of Radiation Oncology and *Pathology, Kayseri Education and Research Hospital; Kayseri-*Turkey*

## Abstract

**Objective:** This experimental study aims to investigate whether radiotherapy (RT) plus trastuzumab (T) followed by subsequent hormonotherapy increase the cumulative toxic effect on cardiac functions in rats.

**Methods:** A total of 70 Wistar-Albino female rats with a mean weight 213±27 g were randomly divided into equal seven groups. The first group (C) underwent no procedure. The second group (RT) underwent the whole thoracic radiation including heart. The third group (T) was administered T through tail vein alone. The fourth group (RT+T+Tx) was administered T initially and the whole thoracic radiation at two hours, followed by tamoxifen at one week. The fifth group (RT+T+Le) was administered T and then underwent thoracic radiation, followed by letrozole. The sixth group (T+RT+An) was administered T and then underwent thoracic radiation, followed by anastrazole. The seventh group (T+RT+Ex) was administered T and then underwent thoracic radiation, followed by exemestane at one week. Hormonotherapy was administered to the rats in the Group 5, 6 and 7, as indicated in the Group 4. Radiation therapy was administered following T treatment at two hours as a single 12 Gy fraction. After the rats were sedated under anesthesia and sacrificed at 24 weeks, cardiac tissues were removed. Serial sections obtained following paraffin blockage were stained and the ratio of myocardial fibrosis was assessed. According to statistical analyses by the one-way ANOVA test and Tukey HSD test, a significant difference between test components.

**Results:** At the end of the study, no loss and adverse effects were seen in any group. There was a statistically significant difference among atrium, ventricle and aorta ($p<0.001$). The mean value of fibrosis scores increased in the rats which underwent RT. In the assessment of atrium, a significant difference was found between RT group and RT+T+An group and also T group and RT+T+Fe group ($p<0.001$). In the assessment of ventricule, a statistically significant difference was observed among RT, RT+T+An and RT+T+Ex. In the assessment of aorta, the scores of RT group was significantly higher than RT+T+An and RT+T+Ex. A statistically significant difference was observed among these groups ($p<0.001$).

**Conclusion:** Our study results suggested that there was no significant additional cardiotoxicity of adjuvant hormonotherapy following concomitant RT and T treatment, compared to RT in terms of cardiac fibrosis. *(Anadolu Kardiyol Derg 2014; 14: 328-33)*

**Key words:** heart, fibrosis, radiotherapy, trastuzumab, hormonotherapy, cardiac toxicity, rat

## Introduction

Breast cancer is the most common tumor in women in the worldwide. Postoperative chemotherapy, radiotherapy (RT) and endocrinological treatments reduce the recurrence rate and cancer-related death events. Subsequent treatment is typically recommended (1, 2).

Nearly 20-30% of the women with breast cancer have overexpression of HER-2. HER-2/neu, also known as erb-B2, is a tyrosine kinase receptor located in cellular surface. Several studies have shown that overexpression of HER-2 (HR+) is associated with more aggressive and poor prognosis (2-4).

Trastuzumab (T) is a monoclonal antibody against HER-2/neu receptor. It specifically binds to protein. Also, it prevents cellular growth, blocking signal conduction over HER-2. It has been reported that T administration once a week or once every three weeks increases progression-free survival (PFS) and overall survival in patients with HER-2 positive breast cancer (1-5).

The most significant side effect of T is cardiomyopathy. The incidence of cardiomyopathy with T monotherapy is 1.4%, while it significantly increases with T plus anthracyclines, particularly. Although the long-term outcomes of T-related cardiomyopathy are still unknown, it has been reported that T-related cardiomyopathy is relieved over time when the treatment is discontinued (3, 5-7).

**Address for Correspondence:** Dr. Yasemin Benderli Cihan, Kayseri Eğitim ve Araştırma Hastanesi, Radyosyon Onkolojisi Bölümü, 38010, Kocasinan, Kayseri-*Türkiye*
Phone: +90 352 336 88 84-1922 E-mail: cihany@erciyes.edu.tr

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Cardiac side effects may also occur during or after radiotherapy (RT) on breast or chest wall. Radiation-induced heart disease may after radiotherapy whenever all or part of the heart was situated in the radiation field. The pathogenesis of radiation-induced heart disease is largely unknown and a treatment is not available. It presents accelerated atherosclerosis, pericardial and myocardial fibrosis, conduction abnormalities, and injury to cardiac vessels. It may vary from acute pericarditis which has usually a benign nature to severe sclerosis accompanied by cardiac construction. Radiation-related cardiomyopathy results from diffuse fibrosis which occurs gradually and progress over time and lead to dysfunction (4, 8-11).

Hormonotherapeutic drugs are used as adjuvant therapy, subsequent therapy or prolonged therapy in women with ER positive breast cancer. Although tamoksifen was once the gold standard endocrine treatment for ER+breast cancer, aromatase inhibitors have emerged as a viable alternative treatment of choice both early and advanced breast cancer in postmenopausal women with ER+disease. Although no clear evidence of their having direct effect on cardiac toxicity has been reported, it is still unknown (12-14).

Another important side effect is treatment-related cardiac sequela. Therefore, studies which shed light into the underlying physiopathology of cardiotoxicity are of utmost importance for applying safer treatments.

In literature review, no previous experimental or clinical studies exist on demonstrating the late side effects on cardiac of hormonotherapy administered concurrent radiotherapy and trastuzumab treatment.

In this experimental study, we aimed to investigate whether T plus RT, followed by subsequent hormonotherapy increase the cumulative toxic effect on cardiac functions.

## Methods

Seventy female Wistar-Albino adult rats weighing 213±27 g were used in the study. They were obtained from Experimental and Clinical Research Center of Erciyes University, Kayseri, Turkey. The animals were kept in a special room at a constant temperature 25±3ºC with 12h light/dark cycles and had free access to diet and tap water. The study protocol was approved by the local Ethics Committee on Animal Experiments. All experimental procedures were conducted in accordance with the Guide to the Care and Use of Laboratory Animals.

Rats were divided into seven groups of ten animals each, including one control group and six experimental groups. The first groups of animals control (C): Rats did not receive irradiation, hormonotherapy or T in this group. The animals were observed with identical conditions of the animals in other groups.

Second group of animals (RT): Every rat had the whole thoracic irradiation including heart under anesthesia with ketamine. One week following radiation, distilled water was administered by oral gavage once daily for 6 months.

Third group of animals (T): This group animals was administered T alone. T was administered IV push through tail vein. One week following T administrated, distilled water was administered by oral gavage once daily for 6 months.

Fourth group of animals (RT+T+Tx): This group animals had T injection first and they received thoracic irradiation, 2 hours after T administration. T was administered IV push through tail vein. Thoracic radiation including the heart the whole thoracic region following T administration was performed under anesthesia with ketamine. Tamoxifen was initiated one week after RT. Tamoxifen was melted in distilled water and administered by oral gavage once daily for 6 months.

Fifth group (RT+T+Le): was administered T and then underwent thoracic radiation, followed by letrozole. Letrozole was administered as indicated in group RT+T+Tx.

Sixth group (T+RT+An): was administered T and then underwent thoracic radiation, followed by anastrazole. Anastrozole was melted in distilled water and administered by oral gavage once daily for 6 months as group RT+T+Tx.

Seventh group (T+RT+Ex): was administered T and then underwent thoracic radiation, followed by exemestane. Exemestane was administered as indicated in group RT+T+Tx.

T was administered initially and thoracic radiation was performed subsequently in Group 5, 6 and 7, as indicated in Group 4. One week following RT, hormonotherapy (tamoxifen, letrozole, anastrazole, exemestane) was initiated. Hormonotherapy was administered as indicated in Group 4.

The rats were weighted before administration. T (Herceptin, Roche) was administered in 6 mg/kg, standard maintenance dose in human for three cycles. T dose which was equivalent to adult dose was calculated based on the weight of each rat. The drug was administered IV push through tail vein.

At two hours following T administration, all rats in groups 2, 4, 5, 6 and 7 were irradiated to the whole thoracic region including heart. Irradiation was performed with a Co-60 (Theratron 780 C, Canada). The rats were sedated under anesthesia with ketamine and four rats at a time were selected. The rats were positioned on the foam mechanism. The arms and legs were fixed in supine position. Simulation was performed including both lungs and the heart. The dose was administered as a single 12 Gy fraction at 2 cm depth and 64 SSD from the anterior region to the site of 5x30 cm.

Based on the half-time of T (an interval of one week that has been shown to be the half life of T in animals was given), hormonotherapy was initiated one week after RT. Equivalent doses of tamoxifen (20 mg/60 kg; Tamoksifen-Teva tablet, Med-İlaç, Turkey), anastrozole (1 mg/60 kg; Arimidex film tablet, AstraZeneca, Turkey), letrozole (2.5 mg/60 kg; Femera film tablet, Novartis, Turkey) and exemestane (25 mg/60 kg, Aromasin tablet, Pfizer, Turkey) were calculated for rats with a mean weight of 200 gr. Drugs were melted in distilled water and administered by oral gavage once daily. Drugs were not administrated to rats in control group, irradiated and T groups.

The study was discontinued at 24 weeks. The rats were sedated under anesthesia and sacrificed. Thoracic site was dissected and cardiac tissues were removed. Tissue samples were washed in iced isotonic saline solution and stored in a sterilized plate containing 10% formaldehyde for 24 hours. Serial sections obtained following paraffin blockage were stained with hematoxylin and eosin and Masson's trichrome. Atrial, ventricular and vascular structures of the tissue samples were assessed using light microscope.

Histopathological examination was performed using scoring system proposed by Kruse et al. (9). As a quantititave end point, fibrosis score graded by a histopathologist. The severity and score of fibrosis for the left ventricle were assessed in the pathological examination. The scoring range was 0 to 3 (0: normal, 1: injury of the minor site, 2: <10% injury, 3: >20% injury; or the assessment of the severity of fibrosis 0.5: moderate fibrosis, 1: severe fibrosis). For atrium, 0: normal, 1: fibrosis involving endocardial layer, 2: fibrosis involving epi- and myocardial layer, 3: fibrosis involving endo-, myo- and epicardial layer. For the histopathological examination of aorta and cardiac vessels, the scoring range was 0 to 3 (0: normal, 1: mild fibrosis, 2: moderate fibrosis, 3: severe fibrosis). After examining the whole section for each rat, the average value was taken as the fibrosis (ventricle, atrium, aorta and coronary vessels) score and mean values of the group were calculated.

The SPSS 15.0 statistical software package programme for Windows and SigmaStat v.3.5 (SPSS Inc., Chicago, Ill, USA) software were used for statistical calculations. Kolmogorov-Simirnov was used to analyze the normal distribution of the data. It was seen that all variables for each group were normally distibuted.One-way ANOVA test was used for intergroup analysis, while Tukey HSD posthoc test was performed for pairwise differences among the groups. A p value of <0.05 was considered statistically significant.

## Results

At the end of the study, none of the group had any lost and adverse side effects. The mean values of fibrosis scores in the ventricles, atria and aorta/coronary vessels and p values are shown in Table 1.

A significant difference in atrial assessment was found among the groups. Results indicated that radiation caused a significant increase in mean value of fibrosis score as compared to the control group. Thus, it was found that radiation causes fibrosis in the atria. The mean values of fibrosis scores of T were higher than control group ($p<0.001$). Statistically significant differences were found between the radiotherapy group and RT+T+An group and also T and RT+T+Fe groups ($p<0.001$). RT+T+Fe group had higher mean value of scores compared to RT+T+An and RT+T+Ex groups. On the other hand, significant difference was determined to exist between RT+T+Fe group and RT+T+An group ($p<0.001$). It was found that anastrozole and exemestane do not increase fibrosis in the atrium.

In the assessment of ventricles, mean value of fibrosis score increased in the groups exposing to radiation. Results revelaed that radiation caused a significant increase in mean value of fibrosis score as compared to the control group as well as T group ($p<0.001$). The fibrosis score of the group that was administered T alone was determined to be significiantly increased, when the compared to control group. The group exposing to T+RT+Le had a higher total fibrosis score when compared to the C, T, T+RT+An, T+RT+Tx, RT, and T+RT+Exe groups. Statistically significant differences were found between the radiotherapy group and RT+T+An group and also T and RT+T+Fe groups ($p<0.001$). The difference between the group given T and T+RT+Tx, T+RT+Le groups was statistically significant. RT+T+Fe group had higher fibrosis scores compared to RT+T+Ex and RT+T+An groups ($p<0.001$).

There was also a significant difference in the assessment of aorta and cardiac vessels among the groups. When the RT group was compared to the RT+T+An and RT+T+Ex groups, radiotherapy was found to increase the total score, this increase was statistically significant ($p<0.001$). Statistically significant differences were found between the T+RT+Tx group and RT+T+An group and also RT+T+Ex groups.

Histopathological examination revealed that letrosole increased fibrosis in atrium and ventricles structures (Fig. 1A-C). In the group exposing to RT alone, RT increased fibrosis in atrium, ventricule and aorta/cardiac vessels (Fig. 2A-C). Anastrazole and exemestane reduced the fibrosis severity, reaching normal tissue (Fig. 3A-C).

## Discussion

Our study results showed that tamoxifen following concomitant use of T and RT increased cardiac fibrosis slightly, while anastrazole and exemestane reduced radiation-induced cardiac fibrosis. Letrozole following concomitant use of T with RT increased fibrosis in the extra-aortic and cardiac structures. We observed that no data including the cardiotoxic effect of hormonotherapies are available.

The high prevalence of breast cancer has led to better understanding of the structure, behavior, clinical presentation and treatment protocols. In addition to this concrete advantage, the need for individual treatment, literature review and recent development has been continued for the patients with HER-2 positive breast cancer, particularly (1, 3, 7).

Although the disadvantages of concomitant chemotherapy and RT as an adjuvant therapy have been proposed, their possible benefits on specific patient populations are also on debate. Thanks to high response rate of T in patients with HER-2 positive breast cancer, its concomitant use with RT or subsequent use have been widely investigated (3, 5, 7). However, treatment-related cardiac sequelae are critical to discuss. Therefore, studies which shed light into the underlying physiopathology of cardiotoxicity are of utmost importance for applying safer treatments.

**Table 1. The mean values of fibrosis scores in the ventricles, atrium and aorta/heart vessels**

| | Group 1 | Group 2 | Group 3 | Group 4 | Group 5 | Group 6 | Group 7 | F value | *P* (<0.05) |
|---|---|---|---|---|---|---|---|---|---|
| Ventricular (Mean±SD) | 0.2±0.42 | 1.8±0.63 | 1.1±0.56 | 1.6±0.51 | 2.0±0.47 | 1.0±0.47 | 1.3±0.82 | 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 2-3, 2-6, 2-7, 3-4, 3-5, 5-6, 5-7 | 0.001 |
| Atrium (Mean±SD) | 1.0±0.31 | 1.9±0.56 | 0.9±0.31 | 1.7±0.67 | 2.2±0.42 | 1.0±0.66 | 1.1±0.56 | 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 2-6, 3-5, 5-6 | 0.001 |
| Aortic/heart vessel (Mean±SD) | 0.2±0.42 | 2.0±0.0 | 1.3±0.48 | 2.0±0.66 | 1.3±0.48 | 0.6±0.69 | 1.1±0.56 | 1-2, 1-3, 1-4, 1-5, 1-7, 2-6, 2-7, 4-6, 4-7 | 0.001 |

Values are expressed as mean±SD. Groups comprised 10 rats in each. F value: The groups in the same column with different numbers are statistically significant (p<0.05). Group 1, C: Control; Group 2, RT: The radiotherapy-only group; Group 3, T: The trastuzumab-only group; Group 4, RT+T+Tx: Radiotherapy+ trastuzumab+tamoxifen; Group 5, RT+T+Le: Radiotherapy+trastuzumab+letrozole; Group 6, RT+T+An: Radiotherapy+trastuzumab+ anastrozole; Group 7, RT+T+Ex: Radiotherapy+ trastuzumab+ exemestane.

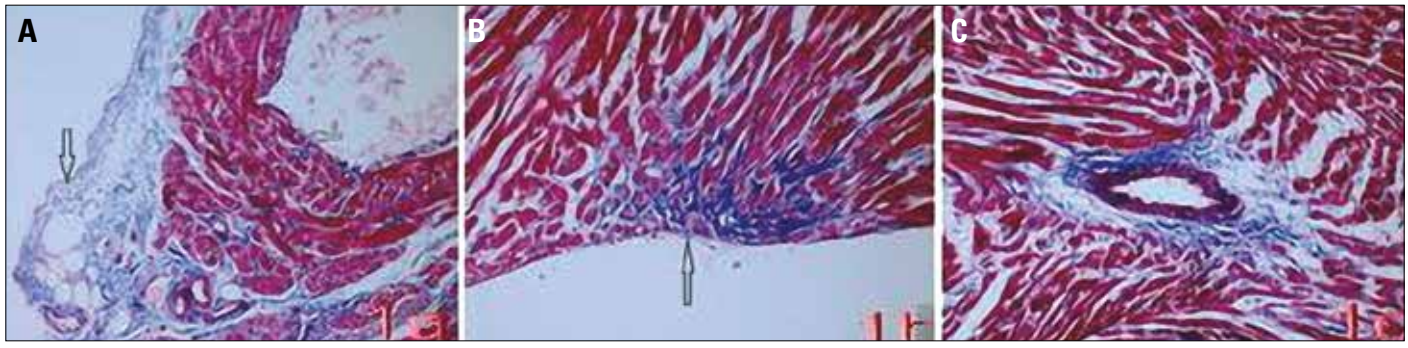


**Figure 1 A-C. RT+T+Le (Radiotherapy+trastuzumab+letrozole) group heart tissue. Letrosole increased fibrosis in atrium (B) and ventricles (A) structures (arrows; Masson's trichrome, x200)**


**Figure 2 A-C. RT group heart tissue. Radiation caused fibrosis in ventricule (A), atrium (B) and aorta/cardiac vessels (C) (arrows, Masson's trichrome, x200)**

The factors which result in cardiotoxicity as an adjuvant therapy can be classified based on the use of RT, use of chemotherapy regimens including anthracyclines or trastuzumab and the characteristics of the patient (6, 15-17). Cardiovascular complication related to RT is undeniable. Radiotherapy may lead to increased atherosclerosis, peri- and myocardial fibrosis and heart valve diseases. Factors leading to cardiac complications and increased severity include radiated side, radiated cardiac volume, radiation energy, fractionation, total radiation dose, concomitant chemotherapy administration and the age of the patient. Although cardiovascular morbidity and mortality have been reduced in the patients who underwent RT, selecting the appropriate RT technique and restricting unnecessary radiation in the last 30 years, cardiac complications are still the leading side effects of RT (4, 8-10, 15, 16).

Trastuzumab can be used concomitantly with other anticancer drugs and RT, while T monotherapy is allowed for prolonged time without any severe side effect (2, 3, 5). The most critical side effect of the treatment is cardiomyopathy, which can be clinically apparent. It may result in cardiotoxic effects, ranging from mild ejection fraction decrease to apparent heart failure. The underlying physiopathological mechanism of cardiac toxicity of T is still unknown. Although the long-term outcomes of T-related cardiomyopathy are still unknown, it has

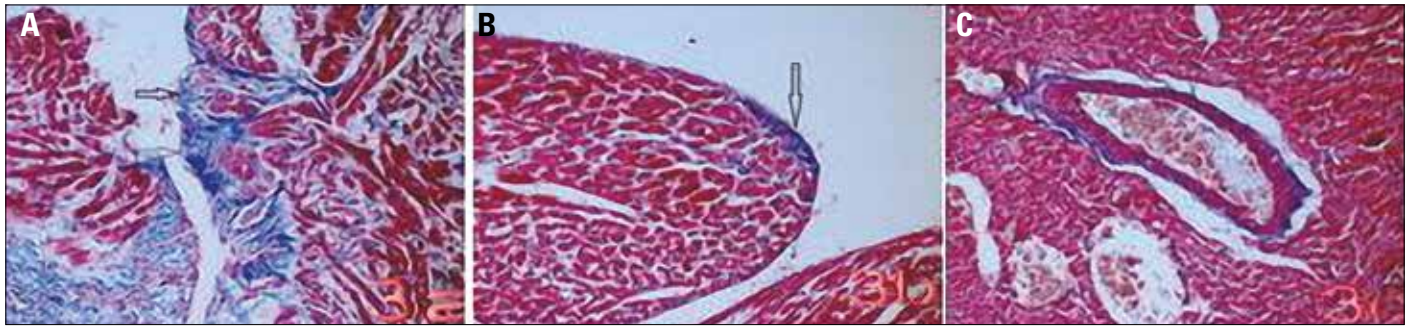


**Figure 3 A-C. RT+T+Ex: Radiotherapy+trastuzumab+exemestane, RT+T+An: Radiotherapy+trastuzumab+anastrozole groups heart tissue. Anastrazole and exemestane reduced the fibrosis severity, reaching normal tissue (arrows, Masson's trichrome, x200)**

been reported that T-related cardiomyopathy is relieved over time when the treatment is discontinued (6, 13, 17-20).

In our study, we investigated whether T plus RT, followed by subsequent hormonotherapy increased the cumulative toxic effect on cardiac functions. We obtained experimental cardiotoxicity using whole thoracic region radiation. The rat model of local heart irradiation has been used succesfully in pre-clinical studies of radiation-induced heart diseases. Localised heart irradiation in rats, both a single dose and fractionated irradiation protocol, leads to histopathological changes inculuding myocardial degeneration and fibrosis. Radiation fibrosis is commonly described after exposures with relatively high doses of radiation. The incidence of these changes after lower dose is not yet known. Several data sets suggest, however, that doses as low as 4-5 Gy might contribute to cardiac toxicity (10). These epidemiologic findings are largely compatible with radiobiologic data on the pathogenesis of radiation-induced heart disease, as comprehensively reviewed by Schultz-Hector and Trott (11).

In our study, in the assessment of ventricles, statistically significant differences was found between the radiotherapy group and T group. Although there is increasing use of concomitant therapies, the extent to RT+T therapies enhance the effects of radiation on normal tissues is not yet known. Bese et al. (20) reported that concomitant use of T with RT (12 Gy) or subsequent use did not increase pulmonary fibrosis in rats. In another animal study, it was found that concomitant use of T with high-dose RT (15 Gy) resulted in might lead to vascular damage that seemed at least additive damage on thoracic aorta (21). In both studies, T was administered in 6 mg/kg dose, in consistent with our study. In their review, Horton et al. (7), Belkacerni et al. (16) and Perez et al. (3) reported that concomitant use of T with RT did not increase the early complications. Horton et al. (7) stressed that concomitant use of T with RT did not increase the side effects. Belkacerni et al. (16) reported that concomitant use of T with RT increased skin and esophagus and decrease the left vetricular ejection fraction, compared to T monotherapy for three weeks. Perez et al. (3) explained the NCCTG N9831 cardiac safety data and reported no significant difference between the right and left-sided radiation. According to these study results, the authors reported that concomitant use of T with RT was safe for cardiac events. The majority of these studies have long-term follow-up for late side effects. Although the complete results have not been explained yet, these findings are consistent with our results, suggesting that concomitant use of T with RT have no significant cardiotoxic effect.

In our study, we observed that tamoxifen following concomitant use of T with RT increased cardiac fibrosis slightly, whereas anastrozole and exemestane decreased the severity of fibrosis. In addition, letrozole increased fibrosis in the extra-aortic and cardiac structures. This result suggested that anastrozole and exemestane had positive effects in reducing cardiac side effects of T and RT. On the other hand, no data including the cardiotoxic effect of hormonotherapies are available. Adjuvant hormonotherapeutic drugs may present with several toxicities (13, 14, 22). In a controlled Swedish study, (23) the incidence of heart disease reduced by tamoxifen in postmenopausal patients. The authors reported that the risk for fatal myocardial infarction was lower in patients who received tamoxifen for 5 years, compared to those who underwent surgery alone. In the analysis of Early Breast Cancer Trialists' Colloborative Group (24) tamoxifen reduced noncancer-related death events (mostly cardiovascular events) by 12%. Tamoxifen has antiestrogenic effects on breast cells and proestrogenic effects on the endometrium and bone, but it has not been shown to protect against ischemic heart disease in large placebo-controlled trials (25) despite earlier metaanalysis which raised this possibility (26). One large phase III trial of the aromatase inhibitor letrozole versus tamoxifen reported increased cardiac events in the letrozole arm at a median follow-up of 26 months (27), which persisted in subgroup analysis at 51 months (28). This observation has not been confirmed in trials of other aromatase inhibitors (29). Further studies including aromatase inhibitors are required to establish the long-term side effects of RT. It is still unknown whether hormonotherapuetic drugs increases cardiotoxicity of RT or T.

## Study limitations

This study is to investigate whether or not the consecutive application of hormonal therapy following the simultaneous application of radiotherapy and trastuzumab may increase the cumulative toxic effects in the heart. We obtained cardiotoxicity using thoracic radiation. We observed that the application of hormonal therapy following the simultaneous radiotherapy and the T treatment added no conspicuous cardiac toxicity to

the expectable side effects of radiation in terms of cardiac fibrosis.

## Conclusion

Our study results suggested that there was no significant additional cardiotoxicity of adjuvant hormonotherapy following concomitant RT and T treatment, compared to RT in terms of cardiac fibrosis. Further clinical studies are required to gain new perspectives on the timing of administration of the drug.

**Conflict of interest:** None declared.

**Peer-review:** Partially peer-reviewed.

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