

Hormone therapy with tamoxifen reduces plasma levels of NT-B-type natriuretic peptide but does not change ventricular ejection fraction after chemotherapy in women with breast cancer

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Abstract

The objective of this study was to evaluate the effect of tamoxifen on the plasma concentration of NT-pro-B-type natriuretic peptide (NT-proBNP) in women undergoing chemotherapy for breast cancer and to correlate changes in NT-proBNP with the left ventricular ejection fraction (LVEF). Over a period of 12 months, we followed 60 women with a diagnosis of breast cancer. The patients were separated into a group that received only chemotherapy (n = 23), a group that received chemotherapy + tamoxifen (n = 21), and a group that received only tamoxifen (n = 16). Plasma levels of NT-proBNP were assessed at 0 (T0), 6 (T6), and 12 (T12) months of treatment, and echocardiography data were assessed at T0 and T12. Plasma NT-proBNP levels were increased in the chemotherapy-only group at T6 and T12, whereas elevated NT-proBNP levels were only found at T6 in the chemotherapy + tamoxifen group. At T12, the chemotherapy + tamoxifen group exhibited a significant reduction in the peptide to levels similar to the group that received tamoxifen alone. The chemotherapy-only group exhibited a significant decrease in LVEF at T12, whereas the chemotherapy + tamoxifen and tamoxifen-only groups maintained levels similar to those at the beginning of treatment. Treatment with tamoxifen for 6 months after chemotherapy significantly reduced the plasma levels of NT-proBNP and did not change LVEF in women with breast cancer.

Key words: Cardiovascular risk profile; Adjuvant chemotherapy; Hormone therapy; NT-B-proBNP; Ecocardiography

Introduction

An increase in the incidence of cardiovascular disease (CVD) in women has been associated with a dramatic decrease in the secretion of ovarian hormones that occurs after menopause, owing to the loss of the cardioprotective action of estrogen (1). Hormone therapy with tamoxifen hydrochloride, a selective estrogen receptor modulator (SERM), interferes with the binding of estrogen to its receptor, thus acting as an estrogen agonist in some tissues, such as the heart, and antagonists in others, such as the breast (2). In women with estrogen receptor (ER)-positive tumors, tamoxifen is recommended as an adjuvant systemic therapy that is typically administered after traditional surgery or chemotherapy treatments (3). Studies have demonstrated that the use of this drug in post-menopausal

women with breast cancer not only reduces the risk of relapse but also has beneficial effects on the lipid profile, thereby exerting a cardioprotective effect by reducing serum levels of total cholesterol and low density lipoprotein (LDL) (4,5).

In addition to hormone treatment, chemotherapy is also recommended in some cases of breast cancer. Studies have shown, however, that some agents, especially anthracyclines (e.g., doxorubicin), have short- and long-term cardiotoxic effects in addition to their cytotoxic and cytostatic effects (6,7). Among those effects, increased production of reactive oxygen species (ROS) is particularly notable. ROS contribute to the development or aggravation of pre-existing CVD and can cause progressive myocardial

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damage in either a dose-dependent or a cumulative fashion (8). Such damage can vary from an asymptomatic decrease in left ventricular ejection fraction (LVEF) to a risk of irreversible chronic cardiac failure (9).

For the detection and prevention of cardiotoxicity at an early stage, the use of NT-pro-B-type natriuretic peptide (NT-proBNP) has also been investigated as a marker of clinical and subclinical cardiac failure. It is secreted by the ventricles in response to an increase in wall stress or ventricular dilatation owing to stretching of myocardial fibers in response to a volume or pressure overload (10).

Thus, although the cardiotoxic effects of chemotherapy are well known, the influence of hormone therapy with tamoxifen on the deleterious effects of chemotherapy on the cardiovascular system have not been demonstrated. The objective of the present study was, therefore, to evaluate the effects of tamoxifen on an important marker of cardiac damage (NT-proBNP) and on ventricular cardiac function, and to examine possible correlations between NT-proBNP plasma levels and LVEF after chemotherapy in women with breast cancer.

Patients and Methods

Patients

This study was conducted between February 2008 and December 2011 in a group of 60 women with a confirmed history of breast cancer and who were undergoing chemotherapy and/or hormone therapy with tamoxifen. Patients between the ages of 40 and 60 years with early-stage breast cancer without metastases were eligible for inclusion in the study. Selected patients were clinically monitored by the same oncologist to ensure that there were no differences between the types of treatment. All women agreed to participate in the study by signing a free and informed consent form. Women who were smokers, had hypertension, diabetes or pre-existing CVD, and those with an LVEF <60% on the first echocardiogram (ECO) were excluded from the study. The study was approved by the Human Research Ethics Committee of Universidade Federal do Espírito Santo under registration No. 23/2008

and conducted according to the principles of the Declaration of Helsinki.

Study protocol

At the beginning and the end of the study, blood pressure (BP), heart rate (HR; HEM, 742, automatic, Omron, Japan), body weight and height (Welmy[®] scale, Brazil, with readings of 0.1 kg and 0.5 cm, respectively) were measured. Blood samples were collected at 0 (T0), 6 (T6), and 12 (T12) months of treatment to measure serum levels of NT-proBNP. Initiation of treatment (T0) was defined as the period between the diagnosis of breast cancer and the start of chemotherapy or hormone therapy. The participants were divided into 3 groups (Figure 1): a) women who received chemotherapy treatment for 6 months (n=23), b) women who received chemotherapy for 6 months followed by an additional 6 months of tamoxifen (n=21), and c) women who received tamoxifen only for 12 months (n=16). The daily doses of tamoxifen were 20 mg (tamoxifen hydrochloride, Sanofi-Aventis, France).

Biochemical analysis

All samples included in the first biochemical analysis (T0) were from patients who had not received any treatment for breast cancer or other cancers. After 12 h of fasting, 20 mL blood was collected from the antecubital vein using Vacutainer tubes (Becton Dickinson, USA). Plasma was separated by centrifugation at 2158 g for 10 min at 4°C. Blood for the NT-proBNP assay was collected into EDTA tubes and a validated, commercially available immunoassay (Elecys[®] NT-proBNP, Roche Diagnostics, USA) was used according to the manufacturer's instructions.

Mean arterial pressure and body mass index

After measuring BP at T0 and T12, mean arterial pressure (MAP) was calculated as follows: $MAP = (\text{systolic BP} + 2 \times \text{diastolic BP}) / 3$. Body mass was also assessed at T0 and T12 using the body mass index (BMI), which was calculated as $\text{weight (kg)} \times \text{height (m)}^{-2}$.

Echocardiogram

Cardiac performance was assessed by ECO and LVEF

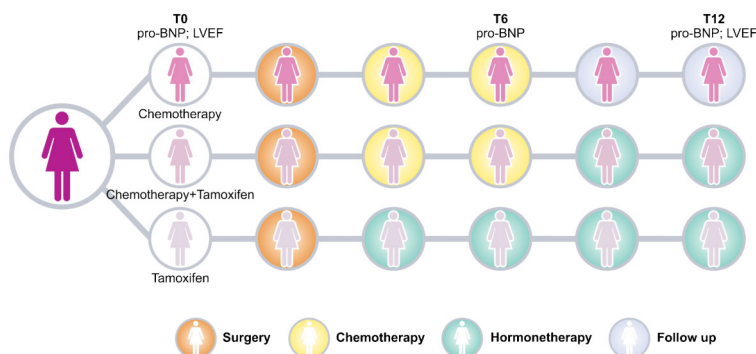


Figure 1. Group division: women who received chemotherapy treatment for 6 months; women who received chemotherapy for 6 months followed by an additional 6 months of tamoxifen; and women who received tamoxifen for 12 months. proBNP: pro-B-type natriuretic peptide; LVEF: left ventricular ejection fraction.

at T0 and T12. The study participants laid on a medical stretcher in the supine position for approximately 10 min in a quiet, temperature-controlled room (20-22°C). All ECOs were performed and evaluated by the same echocardiography physician using a high-resolution device (Toshiba Aplio CV ultrasound system-SSA 790A/CV; Toshiba Corp., Japan). LVEF was obtained from the apical four- and two-chamber views according to Simpson's rule and was considered abnormal if <55%.

Statistical analysis

We used one-way analysis of variance (ANOVA) followed by a *post-hoc* Bonferroni test to compare means, with $P < 0.05$ indicating statistical significance. Data are reported as means \pm SD. The correlation between variables (NT-proBNP and LVEF) was determined by Pearson's correlation coefficient (r), and r values were assigned according to Dancey and Reidy's (2005) classification (11). Statistical analyses were performed using the GB-Stat software (Version 6.5, Dynamic Microsystems Inc., USA).

Results

Patient characteristics

There were no differences in the average age and BMI among the chemotherapy-only (48 ± 7 years and 25.6 ± 4.17 kg/m²), chemotherapy + tamoxifen (47 ± 7 years and 25.8 ± 3.9 kg/m²), and tamoxifen-only (48 ± 4 and $26.9 \pm$

3.13 kg/m²) groups. Forty-three women (72%) had tumors smaller than 2 cm, 10 (17%) had tumors between 2 and 5 cm, and only 4 (11%) had tumors greater than 5 cm. Most women (65%) had received 5 chemotherapy sessions, and the most frequently used chemotherapy (73%) was a combination of 50-60 mg/m² doxorubicin and 500-600 mg/m² cyclophosphamide (Table 1).

Cardiovascular profile

There were no significant differences in MAP at T0 and T12 in the chemotherapy-only (92 ± 6 and 93 ± 10 mmHg, respectively), chemotherapy + tamoxifen (94 ± 8 and 91 ± 9 mmHg, respectively) and tamoxifen-only (93 ± 9 and 91 ± 6 mmHg, respectively) groups.

Likewise, there were no significant differences in HR between the study groups at T0 and T12 (74 ± 5 and 73 ± 4 bpm, in the chemotherapy group; 73 ± 4 and 72 ± 4 bpm in the chemotherapy + tamoxifen group; 72 ± 4 and 73 ± 5 bpm in the tamoxifen group).

Cardiovascular risk markers

Pro-B-type natriuretic peptide. Figure 2 shows the plasma levels of NT-proBNP at T0, T6 and T12. NT-proBNP levels were significantly increased in the chemotherapy group at T6 and T12 (69.06 ± 11.7 and 42.35 ± 12.9 pg/mL, respectively, $P < 0.01$) relative to T0 (8.98 ± 2.1 pg/mL). NT-proBNP levels were also increased in the chemotherapy + tamoxifen group at T6 (67.35 ± 11.9 pg/mL, $P < 0.01$) relative to T0 (9.48 ± 1.79 pg/mL); however, at T12 the

Table 1. Participants and treatment characteristics.

	TAM (n = 16)	CHEMO + TAM (n = 21)	CHEMO (n = 23)
Age (years)			
T0	49 \pm 4	47 \pm 7	48 \pm 7
BMI (kg/m ²)			
T0	25.4 \pm 2.26	25.7 \pm 3.43	26.8 \pm 4.28
T12	26.9 \pm 3.13	25.8 \pm 3.9	25.6 \pm 4.17
Menopause (n)	15	16	18
Primary tumor size (n)			
≤ 2 cm	11	15	17
2-5 cm	2	4	4
> 5 cm	3	2	2
Type of surgery (n)			
Partial mastectomy	14	17	17
Radical mastectomy	2	4	6
Type of chemotherapy (n)			
Dox + Cyc	NA	21	23
Dox + Cyc + 5Flu	NA	1	0
Paclitaxel	NA	1	2
Radiotherapy (n)			
30 sessions	NA	21	23

Data are reported as means \pm SD or as number (n). TAM: tamoxifen; CHEMO: chemotherapy; T: time; BMI: body mass index; NA: not applicable; Dox: doxorubicin; Cyc: cyclophosphamide; 5Flu: 5-fluorouracil.

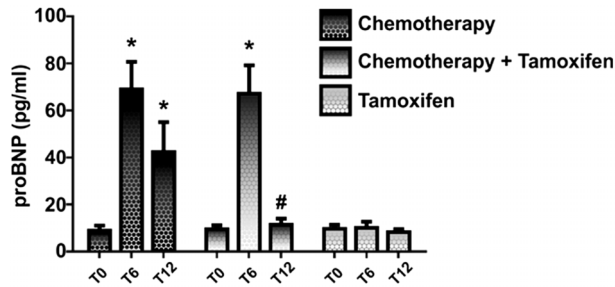


Figure 2. Plasma concentrations of pro-B-type natriuretic peptide (proBNP) in the groups treated with chemotherapy alone, chemotherapy followed by tamoxifen, and tamoxifen alone. Data are reported as means \pm SD. * $P < 0.01$ compared to T0; # $P < 0.01$ compared to T6 (Bonferroni test).

levels in this group were similar to those at T0 (11.39 ± 2.74 and 9.48 ± 1.79 pg/mL, respectively). No differences were detected in the plasma concentrations of NT-proBNP in the women in the tamoxifen-only group.

When we evaluated whether the different treatments differentially influenced NT-proBNP levels, we found that the chemotherapy and chemotherapy + tamoxifen groups had higher plasma levels of NT-proBNP (69.06 ± 11.7 and 67.35 ± 11.86 pg/mL, respectively, $P < 0.01$) than the tamoxifen-only group (10.21 ± 2.49 pg/mL) at T6. At T12, plasma levels of NT-proBNP remained high in the chemotherapy group (42.35 ± 12.93 pg/mL, $P < 0.01$), but were significantly decreased in the chemotherapy + tamoxifen group (11.39 ± 2.74 pg/mL), in which the levels were similar to those of the tamoxifen-only group (8.35 ± 1.66 pg/mL).

LVEF. Figure 3 shows cardiac performance as assessed by ECO, which was used to assess LVEF at T0 and T12. In the chemotherapy group, LVEF was significantly reduced at T12 ($63.3 \pm 4.3\%$, $P < 0.01$) relative to T0 ($74.7 \pm 4.8\%$). In contrast, the LVEF values for the chemotherapy + tamoxifen and tamoxifen-only groups at T12 (76.2 ± 3.7 and $75.6 \pm 4.1\%$, respectively) were similar to those at T0 (73.7 ± 4.2 and $76.8 \pm 4.82\%$, respectively).

Assessing the effects of treatment on cardiac performance revealed that the LVEF values were similar among the chemotherapy ($74.7 \pm 4.79\%$), chemotherapy + tamoxifen ($73.7 \pm 4.23\%$) and tamoxifen-only ($76.8 \pm 4.8\%$) groups at T0. However, there was a reduction in LVEF in the chemotherapy group ($63.3 \pm 4.3\%$, $P < 0.01$) relative to the chemotherapy + tamoxifen ($76.2 \pm 3.7\%$) and the tamoxifen-only ($75.6 \pm 4.1\%$) groups at T12.

Correlation between NT-proBNP and LVEF. Pearson's correlation analysis showed a strong negative correlation (-0.77 , $P < 0.01$) between LVEF and plasma NT-proBNP levels in the chemotherapy group (Figure 4A) at T12. However, the negative correlation between LVEF and NT-proBNP levels in the chemotherapy + tamoxifen (Figure 4B) and tamoxifen-only (Figure 4C) groups was weak (-0.29 and -0.05 , respectively), which is consistent with the finding that neither LVEF nor NT-proBNP differed significantly in

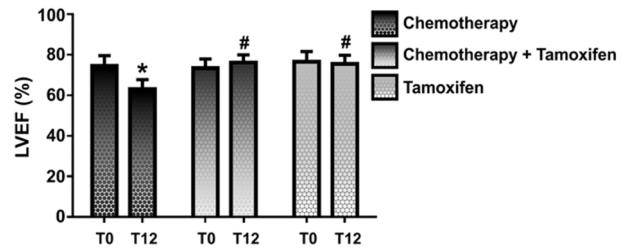


Figure 3. Cardiac performance assessed by left ventricular ejection fraction (LVEF) in the groups treated with chemotherapy alone, chemotherapy followed by tamoxifen, and tamoxifen alone. Data are reported as means \pm SD. * $P < 0.01$ compared to T0; # $P < 0.01$ compared to T12 in the group treated with chemotherapy alone (Bonferroni test).

those groups at T12.

Discussion

This study showed that tamoxifen treatment promotes significant changes both in the plasma concentration of an important marker associated with an increased risk of CVD (NT-proBNP) and in functional parameters of cardiac performance (LVEF) in women with breast cancer who have previously undergone chemotherapy. Previous studies had confirmed the cardiotoxic effects of chemotherapy (8,9), and we are not aware of any data in the literature on the cardiac actions of tamoxifen following chemotherapy.

Broeyer et al. (12) demonstrated that patients who underwent anthracycline-based chemotherapy had higher levels of NT-proBNP combined with a decrease in myocardial contractility relative to the control population. An increase in plasma concentrations of this peptide is a reliable marker of ventricular dysfunction, because it is produced and secreted by the heart in response to wall stress or ventricular dilatation (10). Corroborating those results, our data showed an increase in plasma concentrations of NT-proBNP combined with a reduction in LVEF 6 months after the cessation of chemotherapy. Furthermore, we found a strong negative correlation (-0.8 ; $P < 0.001$) between LVEF and NT-proBNP in this group at T12.

No correlation was found between LVEF and NT-proBNP at T12 in the chemotherapy + tamoxifen and tamoxifen groups, where levels were similar to those at the beginning of treatment. We have shown that although chemotherapy promoted significant changes in plasma concentrations of NT-proBNP, tamoxifen treatment resulted in a decrease in plasma concentrations of this peptide and an improvement in LVEF 12 months after treatment (T12), thus lowering important risk factors for CVD after chemotherapy. In a recent study, we showed the effects of tamoxifen on other important markers of cardiovascular risk, such as an increase in apolipoprotein-A1 and a decrease in apolipoprotein-B100 and C-reactive protein in women with breast cancer after treatment with chemotherapy (13).

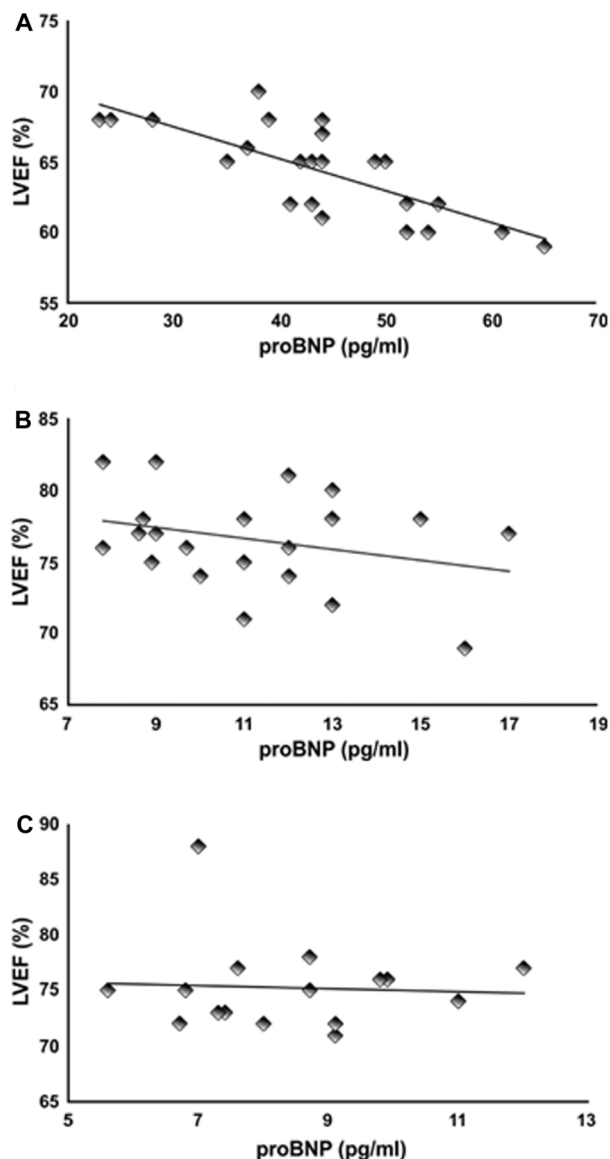


Figure 4. Pearson's correlation (r) between measures of left ventricular ejection fraction (LVEF) and pro-B-type natriuretic peptide (proBNP) in the groups treated with chemotherapy alone (A), chemotherapy followed by tamoxifen (B), and tamoxifen alone (C).

Cardiotoxicity induced by chemotherapy has been demonstrated by experimental studies in which a single dose of anthracycline induced the apoptosis of cardiac myocytes (14). Moreover, increased plasma levels of natriuretic peptides and a subsequent reduction in myocardial contractility were observed in women with breast cancer given anthracycline-based chemotherapy followed by thoracic wall radiotherapy (15,16). Studies have shown that 20-51% of patients who undergo anthracycline-based chemotherapy develop some type of subclinical cardiac dysfunction (17).

No changes in left ventricular mass were observed in any of the groups in our study during the entire treatment period, most likely because the 12-month duration of the study was not long enough to allow for the expression of structural changes that could be detected by echocardiography.

One hypothesis to explain the cardiovascular changes that occur with the use of chemotherapeutics is that their metabolism by hepatic mixed-function monooxygenases (cytochrome P-450 oxidase/reductase) leads to the generation of free radicals. The hydrogen peroxide (H_2O_2) produced binds to stable adriamycin-iron complexes, which are then degraded by catalases. Catalase, also known as hydroperoxidase, is stored in peroxisomes, which are present at low levels in human myocardial cells but not in other tissues, such as the kidney and liver (18). Additionally, the myocardium does not express glycoprotein P-170, which is active in the presence of a Ca/Mg-dependent ATPase (modulated by calmodulin) and is responsible for the rapid cellular elimination of chemotherapy drugs. The absence of such mechanisms contributes to myocardial injury following chemotherapy (19). The initial subcellular damage induces the remodeling of cell structures such as the sarcolemma, sarcoplasmic reticulum, extracellular matrix, myofibrils, and mitochondria. The subsequent contractile dysfunction and cardiac arrhythmia are strongly associated with cardiac remodeling and hypertrophy (19-21). Thus, the heart becomes vulnerable to lesions caused by ROS, with significant damage to myocardial cells and subsequent cardiac dysfunction during the process of cardiac injury (20).

Another mechanism that might be responsible for reduced LVEF in the group treated with chemotherapy alone is a change in intracellular Ca^{2+} , which regulates cardiomyocyte contractility. The generation of ROS and H_2O_2 in ischemic cardiomyocytes in the presence of intracellular iron and impaired intracellular Ca^{2+} homeostasis leads to a decrease in myocardial function due to Ca^{2+} uptake through L-type voltage-dependent Ca^{2+} channels and the release of large amounts of Ca^{2+} by the sarcoplasmic reticulum through ryanodine receptors (22). The resulting high cytoplasmic concentrations of Ca^{2+} in the cardiomyocytes contribute to ventricular dysfunction caused by chemotherapy (6).

Our study shows that the use of tamoxifen in the therapeutic regimen promoted normalization of LVEF at T12, and that it remained similar to the LVEF in the group using only tamoxifen for the treatment of breast cancer. This drug may be acting through estrogen receptor beta ($ER\beta$) to stimulate endothelial nitric oxide synthase (eNOS) activity (23) and promote antioxidant effects by increasing catalase activity, which is responsible for the degradation of H_2O_2 into H_2O and O_2 (24). Tamoxifen treatment also promotes a significant increase in the antioxidant activity of glutathione and glutathione peroxidase, which are two cellular antioxidants involved in defense after cellular

damage due to H₂O₂ and lipid peroxidation during myocardial injury (25). These antioxidants are most likely the greatest contributors to the cardioprotective effects of tamoxifen in humans. Tamoxifen may also mimic the action of estrogen through ER β present mainly in the mitochondria of cardiomyocytes, and mediate antihypertrophic effects through the inhibition of calcineurin activity, stimulation of MCIP1 via PI3 kinase, and inhibition of interstitial fibrosis (26). The evaluation of NT-proBNP levels and LVEF before and after chemotherapy would be of great clinical relevance because it would reveal subclinical cardiac damage at an early stage (27). The cardiotoxic potential of drugs used for breast cancer treatment is indicated by increases in plasma NT-proBNP peptide and prolonged QT intervals that have been found 24 h after the administration of anthracycline chemotherapy (12).

Experimental studies in our laboratory have also demonstrated increased adenosine-induced vasodilatation in the coronary beds of spontaneously hypertensive female rats treated with tamoxifen, which was later associated with an improvement in cardiac performance (28).

In this clinical study, we have shown a clear correlation between high NT-proBNP levels and reduced cardiac performance due to the action of chemotherapeutic agents, a result that was reversed by the action of tamoxifen. Our results corroborate data from other studies showing that

plasma levels of NT-proBNP are also elevated in patients with asymptomatic ventricular dysfunction (29), reduced LVEF, ventricular dysfunction, or coronary artery disease (30). Meinardi et al. (31) also reported that plasma levels of NT-proBNP in breast cancer patients were elevated relative to the pre-treatment period when measured 1 year after anthracycline chemotherapy and thoracic wall radiotherapy.

This study demonstrated that the chronic use of tamoxifen can significantly reduce plasma concentrations of a biomarker of cardiac lesions (NT-proBNP) and does not change cardiac function (LVEF) after chemotherapy in women with breast cancer. Although structural changes were not detected during the study period, the positive correlation between elevated NT-proBNP levels and reduced LVEF indicates the presence of significant functional changes in cardiac performance. We suggest careful monitoring and clinical evaluation of cardiac function in women receiving anthracycline chemotherapy for breast cancer to reduce morbidity and mortality from cardiovascular complications.

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