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BNP as a marker for early prediction of anthracycline-induced cardiotoxicity in patients with breast cancer

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Abstract. Anthracycline chemotherapy serves an important role in treating patients with breast cancer but is associated with cardiotoxicity. Although brain natriuretic peptide (BNP) is not the ideal marker for detecting the presence of diseases of the heart, several studies have demonstrated the predictive utility of BNP in the diagnosis of anthracycline-induced cardiotoxicity (AIC). The aim of the present study was to evaluate the role of BNP as a marker for the early prediction of AIC in patients with breast cancer. In the present study, a total of 149 patients with breast cancer who received anthracycline therapy were evaluated. The levels of BNP and echocardiography were detected during the anthracycline-based chemotherapy and patients were followed up after chemotherapy to determine the cardiotoxicity-free survival times. In the patients who received chemotherapy, an increase in the levels of BNP was observed. The concentration of BNP was significantly higher in the cardiotoxicity group during anthracycline chemotherapy (P=0.022) compared with the non-cardiotoxicity group and it was an independent predictor of cardiotoxicity (P=0.028). The optimal diagnostic threshold of BNP after the last anthracycline chemotherapy treatment was 107.9 pg/ml, the diagnostic sensitivity was 0.538, the specificity was 0.794, the Youden index was 0.332, the positive predictive value was 0.583 and the negative predictive value was 0.762. Based on the BNP threshold, a log-rank test was performed and it was determined that the cardiotoxicity-free survival rate of the group with higher levels of BNP was always lower compared with the group with lower levels of BNP. BNP elevation was associated with cardiotoxicity during the anthracycline chemotherapy. Detecting BNP after the final treatment of anthracycline chemotherapy may contribute to the early detection of cardiotoxicity.

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Introduction

Worldwide, breast cancer is the most common cancer and the first leading cause of cancer mortalities in women (1). Anthracycline agents are effective and widely used chemotherapeutic drugs for a number of solid tumors including breast cancer (2). However, the related cardiotoxicity is the leading cause of discontinuation of treatment and may even result in cardiovascular-associated death (3). Furthermore, the cardiotoxicity is usually progressive and irreversible once exhibited, and the treatments available are less effective (4). Therefore, it is important to identify anthracycline-induced cardiotoxicity (AIC) as early as possible in order to adjust or cease treatment prior to irreversible damage of cardiac tissues.

At present, the primarily used methods to detect cardiotoxicity include electrocardiograms and echocardiography, which are widely available and inexpensive. However, the irreversible cardiac cellular damage usually has occurred before detection with electrocardiogram and echocardiography in the majority of cases (5). Previous studies focus on searching for selective biomarkers of cardiotoxicity. So far, several biomarkers such as cardiac troponins (6) and plasma natriuretic peptides (7) were found to have potential to contribute to monitoring chemotherapy-induced cardiotoxicity.

Brain natriuretic peptide (BNP), a neurohormone released by the left ventricular cardiomyocytes in response to cardiac wall stress, is widely used to diagnose and monitor congestive heart failure (CHF) (7). High concentrations of BNP have been described not only in patients with acute myocardial infarction or advanced CHF but also in patients with asymptomatic or minimally symptomatic LV dysfunction (8). Previously,

multiple studies demonstrated that serum BNP may be an independent biomarker in detection of cardiotoxicity, however, relatively little known is about the predictive applicability of BNP in anthracycline-treated patients (9-13). Therefore, the aim of the present study was to assess the potential role of BNP detection in the early prediction of anthracycline-induced cardiotoxicity in patients with breast cancer.

Materials and methods

Population. Between November 2013 and August 2016, patients admitted to the Department of Breast Diseases of The First Hospital of Jiaxing (Jiaxing, China) with breast cancer were prospectively recruited. The patients underwent breast cancer surgery combined with postoperative chemotherapy. Following the surgery, tumor tissues were collected and used for immunohistochemistry (IHC) and fluorescence *in situ* hybridization (FISH) at the Department of Pathology of The First Hospital of Jiaxing. The inclusion criteria were: i) Histologically confirmed primary breast cancer; and ii) indications to suggest treatment with anthracycline-based chemotherapy. The exclusion criteria were: i) Breast cancer *in situ* or at stage IV; ii) prior exposure to anthracyclines; iii) a history of congenital heart disease, coronary heart disease, heart valve disease or a left ventricular ejection fraction (LVEF) <50%; iv) loss to follow-up; v) required treatment with an additional course of chemotherapy or other therapeutic regimen, due to the recurrence or metastasis during the follow-up.

In total, 149 cases, all female, with a median age of 51 (range, 28-69) years were included. Among the 149 evaluable patients, 129 cases were confirmed as invasive ductal carcinoma and there were 48 cases of stage I, 73 cases of stage II and 28 cases of stage III cancer according to the 7th Edition American Joint Committee on Cancer staging manual (14). According to the IHC or FISH results of breast cancer tissues, tumors were classified as estrogen receptor-positive (ER^+) if >1% of the cells were stained positively. For human epidermal growth factor receptor-2 (HER2), the staining was scored as previously described (15); tumors were classified as $HER2^+$ if the results of IHC were scored 3+, or if the results of IHC were scored 2+ but confirmed positive by the further FISH detection. Otherwise, tumors were classified as ER-negative (ER^-) or HER2-negtive ($HER2^-$). Among the 149 patients, 77 cases were ER^+ but $HER2^-$, 21 cases were ER^+ and $HER2^+$, 29 cases were ER^- and $HER2^-$ and 22 cases were ER^- but $HER2^+$. The details on the patients and the tumor characteristics are summarized in Table I. The design of the present study was approved by The Ethical Committee of The First Hospital of Jiaxing. All the patients signed written informed consent form to participate in the present study.

Chemotherapy treatment. All patients received anthracycline-based chemotherapy. The chemotherapeutic regimen was dependent on the disease status and risk factors, including epirubicin (E) cyclophosphamide (C), EC-docetaxel (T), pirarubicin (A) C and AC-T. The EC regimen was: 90 mg/m^2 E and 600 mg/m^2 C, both used on the first day and administered every 21 days for a total of four courses. The EC-T regimen was similar to the EC regimen, where E and C were used at the same dose during the first four course, after which, T was given at 90 mg/m^2 for four courses (every 21 days). The AC regimen was: 50 mg/m^2 A and 600 mg/m^2 C were both administered every 21 days for four courses in total. The AC-T regimen was similar to the AC treatment regimen, where A and C were used at the same dose in the first four course, after which, T was given at 90 mg/m^2 for four courses (every 21 days).

All the patients underwent baseline plasma BNP and echocardiography examination prior to initiation of chemotherapy. For BNP detection, all the pre-chemotherapy samples were obtained at least 48 h prior to initiation of therapy from fasting resting venous blood; whereas the post-chemotherapy samples were collected 24 h after each course of therapy (16). The blood samples collected were placed in EDTA anticoagulation tubes and the serum was obtained by centrifuging for 15 min at 4˚C at 1,800 x g for detection of BNP levels. The plasma BNP levels were determined by measuring the chemiluminescence in a micro-particle immunoassay. Briefly, the plasma BNP was isolated using an Abbott's B-type natriuretic peptide assay kit (cat. no. 71008M902; Abbott Pharmaceutical Co. Ltd.) and quantified using Architect i1000 automatic immunoassay analyzer. Echocardiography, which measures both LVEF and left ventricular end-systolic dimension (LVDs), was performed during each course of chemotherapy.

Follow-up. After every 3 to 6 months of initiation chemotherapy, the patients underwent a necessary cardiac evaluation, including a detailed physical examination of their symptoms and signs and echocardiography. During this period, the moment when cardiotoxicity was determined to meet the evaluation criteria set as the end point of observation and the cardiotoxicity-free survival time were recorded. The median follow-up duration was 1.5 (0.1 to 3.8) years.

Cardiotoxicity evaluation criteria. In the present study, variations in LVEF and LVDs were used for cardiotoxicity evaluation (17-19). LVEF variation was measured by calculating maximum drop in LVEF (ΔLVEF), calculated using baseline LVEF-monitored minimum LVEF. Maximum rise in LVDs (ΔLVDs) was used to calculate variations in LVDs and it was calculated as the monitored maximum LVDs-baseline LVDs. If any one of these four criteria including symptoms and signs of cardiac insufficiency, LVEF <55%, ΔLVEF ≥10% and ΔLVDs ≥7 mm was observed in the participants, they were moved to the cardiotoxicity group, otherwise, the patients were placed in the non-cardiotoxicity group.

Receiver operating characteristic curves plotting. To evaluate the ability of serum BNP levels to predict anthracycline-induced cardiotoxicity early, a receiver operating characteristic (ROC) curve was plotted. The statistical analysis of the ROC curve was performed using SPSS 17.0. The area under the curve (AUC) was determined using the Wilcoxon Mann-Whitney method. The optimal BNP threshold, diagnostic sensitivity and specificity of BNP in predicting anthracycline-induced cardiotoxicity were determined by the maximum of Youden index, which was calculated as Youden index=sensitivity (Se) + specificity (Sp) -1. The prevalence (p) of cardiotoxicity was calculated as p=number of patients with cardiotoxicity/total number of patients. The positive predictive value (PPV) was

Table I. The association of cardiotoxicity with clinicopathological characteristics.

	Total (n=149)		Cardiotoxicity group (n=52)		Non-cardiotoxicity group (n=97)		
Variable	n	%	n	%	n	%	P-value
Age, years							0.991
≤50	66	44	23	44	43	44	
>50	83	56	29	56	54	56	
Menopause status							0.496
Premenopausal	83	56	27	52	56	58	
Menopause	66	44	25	48	41	42	
BSA							0.777
≤1.7 m^2	75	50	27	52	48	49	
>1.7 m^2	74	50	25	48	49	51	
BMI							0.991
≤23.9	83	56	29	56	54	56	
>23.9	66	44	23	44	43	44	
Hypertension							0.247
Yes	37	25	10	19	27	28	
No	112	75	42	81	70	72	
Tumor histology							0.308
Invasive ductal	129	87	43	83	86	89	
Others	20	13	9	17	11	11	
TNM stage							0.657
I	48	32	19	37	29	30	
IIA	26	17	9	17	17	18	
IIB	47	32	14	27	33	34	
IIIA	20	13	6	12	14	14	
IIIB	1	0	1	2	0	0	
IIIC	7	5	3	6	4	4	
ER/HER2 status[a]							0.303
ER^+ $HER2^-$	77	52	26	50	51	53	
ER^+ $HER2^+$	21	14	11	21	10	10	
ER^- $HER2^-$	29	19	8	15	21	22	
ER^- $HER2^+$	22	15	7	13	15	15	
Chemotherapeutic regimen							0.536
EC/AC	44	30	17	33	27	28	
EC-T/AC-T	105	70	35	67	70	72	
Cumulative dose of adriamycin							0.151
≤175 mg/m^2	75	50	22	42	53	55	
>175 mg/m^2	74	50	30	58	44	45	
Herceptin therapy							0.973
Yes	26	38	9	17	17	18	
No	123	62	43	83	80	82	
Tamoxifen therapy							0.871
Yes	56	38	20	38	36	37	
No	93	62	32	62	61	63	

[a]Tumors were classified as ER^+ if >1% of the cells were stained positively; tumors were classified as $HER2^+$ when they were scored 3+ at immunohistochemistry or at fluorescence *in situ* hybridization. ER, estrogen receptor; HER2, human epidermal growth factor receptor-2; BSA, body surface area; BMI, Body mass index; TNM, tumor node metastasis; E, epirubicin; C, cyclophosphamide; T, EC-docetaxel; A, pirarubicin.

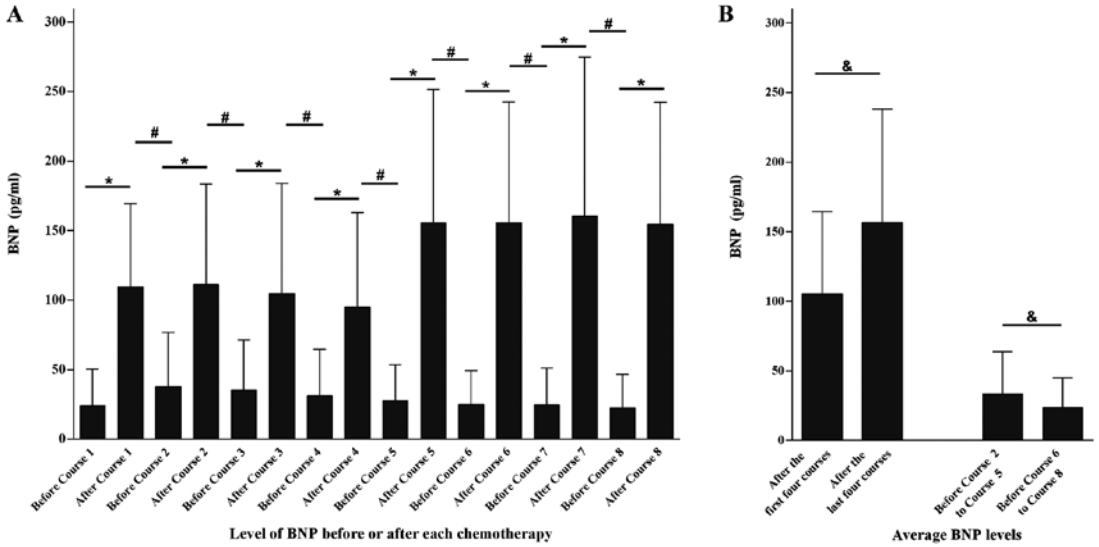

Figure 1. Effect of chemotherapy on the levels of BNP. (A) The level of BNP before or after each treatment. (B) The average levels of BNP related to the anthracycline (after the first four courses or before Course 2 to Course 5) or docetaxel (after the first four courses or before Course 6 to Course 8). The serum BNP concentration after each therapy was always higher compared with before. *P<0.001, paired samples t-test. The levels of BNP prior to the next dose of chemotherapy were lower compared with the measurement taken after the previous dose. #P<0.001, paired samples t-test. The average value after the first four courses was lower compared with last four courses. &P<0.001, paired samples t-test. The average serum BNP levels of the second to the fifth course and the sixth to eighth course prior to each treatment were calculated, the average level of BNP in the former set off measurements was higher compared with the latter set of measurements &P<0.001, paired samples t-test. BNP, brain natriuretic peptide.

calculated as PPV=p x Se/[(p x Se + (1-p) x (1-Sp)]. The negative predictive value (NPV) was calculated as NPV=(1-p) x Sp/[(1-p) x Sp + p x (1-Se)].

Statistical analysis. Statistical analyses were performed using SPSS 17.0 (SPSS, Inc.). Measurements were presented as the mean ± standard deviation and counts were expressed in absolute value. For assessing the differences between BNP levels before and after each chemotherapy, a paired samples t-test was used, whereas an independent samples t-test was used for the differences in BNP between the cardiotoxicity group and non-cardiotoxicity group. Counting data were compared using a χ^2 test. Repeated measures analysis of variance (ANOVA) method was used to assess the variation of the levels of BNP during each course of chemotherapy. Kaplan-Meier method was used for calculating the survival rates and a log-rank test was used for comparing survival rates and to infer the cardiotoxicity-associated factors, a Cox proportional hazards regression model was used. The optimal diagnostic threshold was determined using ROC and the AUC was determined using the Wilcoxon Mann-Whitney method. P<0.05 was considered to indicate a statistically significant difference. All experiments and statistical analyses were performed twice to ensure accuracy.

Results

Cardiotoxicity and patient characteristics. In the present study, a total of 52 patients who met one of the cardiotoxicity criteria were classified into the cardiotoxicity group and the remaining 97 patients were classified into the non-cardiotoxicity group. Table I summarizes the characteristics of the patients. There was no significant difference in the various clinical and pathological features between the two groups. Chemotherapy regimens and drug dose of these patients are also listed in Table I. Body surface area (BSA) was calculated as=0.73x height (in m) + 0.0127x weight (in kg) -0.2106. The median BSA value in the study was 1.7 (range 1.4-2.0) m^2. Body mass index (BMI) was calculated as=weight (in kg)/height (in m)^2. The cumulative dose of adriamycin refers to the dose administrated per m^2 BSA. In the present study, the median value of the cumulative dose of adriamycin was 175 (152-216) mg/m^2. The dose of epirubicin administered was determined using a 1:2 adriamycin to epirubicin ratio (3) and that of pirarubicin was determined using a 1:1.08 adriamycin to pirarubicin ratio (20). For the group with a cumulative dose of adriamycin ≤175 mg/m^2, the incidence of cardiotoxicity was reduced compared with the group with a cumulative dose >175 mg/m^2. However, the difference was not statistically significant, possibly because the cumulative doses of adriamycin amongst all patients were relatively low in the present study. The incidence of cardiotoxicity in patients who received EC-T/AC-T chemotherapy was slightly higher compared with patients who received EC/AC therapy, indicating that docetaxel may contribute to heart tissue damage, although the difference was not significant, as the temporary-induced damage may be not be permanent (Table I).

Effect of chemotherapy on the levels of BNP. The levels of BNP were measured before and after each treatment in the present study, and the variation was evaluated by a paired t-test (Fig. 1A). The serum BNP concentration after each therapy was significantly increased compared with before (P<0.001), suggesting that a single dose of chemotherapy could lead to elevated levels of BNP. Furthermore, the paired t-test also indicated that levels of BNP prior to the next dose of chemotherapy were significantly decreased compared with the measurement taken after the previous dose (P<0.001), suggesting that the

Table II. The relationship between BNP levels and cardiotoxicity.

	Cardiotoxicity group		Non-cardiotoxicity group		
Chemotherapy	BNP levels, mean ± SD, pg/ml	n	BNP levels, mean ± SD, pg/ml	n	P-value
Before course 1	27.2±31.5	52	22.5±23.3	97	0.306
After course 1	119.0±65.3	52	104.6±56.5	97	0.164
Before course 2	47.8±47.7	52	32.6±32.3	97	0.043[a]
After course 2	121.6±78.1	52	105.7±68.6	97	0.199
Before course 3	42.2±44.2	52	31.7±30.4	97	0.091
After course 3	118.3±85.2	52	97.6±75.0	97	0.127
Before course 4	39.4±44.6	52	27.3±24.5	97	0.072
After course 4	114.2±65.2	52	85.1±66.8	97	0.012[a]
Before course 5	29.3±30.0	35	26.9±23.7	70	0.654
After course 5	161.2±123.0	35	152.8±80.1	70	0.674
Before course 6	24.9±23.1	35	25.2±25.1	70	0.954
After course 6	140.2±62.4	35	163.6±95.9	70	0.136
Before course 7	27.6±31.2	35	23.4±24.0	70	0.456
After course 7	154.9±104.9	35	163.2±119.3	70	0.727
Before course 8	25.1±26.1	35	21.4±23.3	70	0.458
After course 8	146.8±76.5	35	158.6±92.8	70	0.519

[a]P<0.05, independent samples t-test. SD, standard deviation; BNP, brain natriuretic protein.

increase in BNP was transient and following clearance of chemotherapeutic agents *in vivo*, the levels of BNP gradually decreased (Fig. 1A). For patients who underwent EC-T/AC-T chemotherapy, the average levels of BNP in each patient after the first four and the last four course were compared (Fig. 1B). The results showed that the average value after the first four courses was significantly decreased compared with last four courses (P<0.001), indicating the ability of anthracycline to increase serum BNP levels was lower compared with docetaxel. To compare the lowest possible measurements of BNP throughout the recovery period after treatment with anthracyclines and docetaxel, the average serum BNP levels of the second to the fifth course and the sixth to eighth course prior to each treatment were calculated. The average level of BNP in the former set off measurements was significantly higher compared with the latter set of measurements (P<0.001), suggesting a slower clearance of BNP levels following treatment with anthracyclines compared with docetaxel chemotherapy (Fig. 1B).

BNP and cardiotoxicity. In the cardiotoxicity group and non-cardiotoxicity group, an independent t-test was performed to compare the serum BNP levels before and after each dose of chemotherapy. Prior to the second dose (Course 2) of chemotherapy, the serum levels of BNP were significantly increased in the cardiotoxicity group compared with the non-cardiotoxicity group (P=0.043; Table II), suggesting that the initial dose of anthracyclines resulted in a slower decrease of BNP levels in the cardiotoxicity group. The underlying reason might be that once myocardial damage has been established, BNP would last longer due to sustained release. However, following the fourth dose of chemotherapy, the BNP levels were significantly increased in the cardiotoxicity group compared with the non-cardiotoxicity group (P=0.012; Table II), suggesting that at this dose the administered anthracyclines had resulted in the maximal amount of damage to cardiac tissue. These results showed that the elevation of BNP levels induced by anthracyclines was associated with cardiotoxicity. Furthermore, there was no significant difference in the levels of BNP found between the cardiotoxicity group and non-cardiotoxicity group during docetaxel chemotherapy (Table II).

ANOVA was used to assess the variation of the levels of BNP during the chemotherapy. The results above demonstrated that BNP levels were associated with anthracyclines rather than docetaxel, BNP levels from the first and fourth dose of chemotherapy were included in this comparison. A significant difference in the levels of BNP was determined between the two groups, with the levels of BNP consistently being higher in the cardiotoxicity group compared with the non-cardiotoxicity group in each treatment with anthracycline (P=0.024). Therefore, the increase in the levels of BNP was associated with anthracyclines-induced cardiotoxicity (Fig. 2).

Cox proportional hazards regression model. To plot the survival curves, the factors which may have influenced the disease-free time, including serum BNP levels after the fourth course of chemotherapy, cumulative dose of adriamycin, hypertension, age, height and BSA were analyzed. These factors were included in a multivariate analysis of Cox proportional hazards regression model and the specific effect of the factor and therefore its involvement in the regression model was termed as 'entry'. Serum BNP levels following the fourth course of chemotherapy was an independent predictor for cardiotoxicity (P=0.047); whereas the other factors were not (Table III).

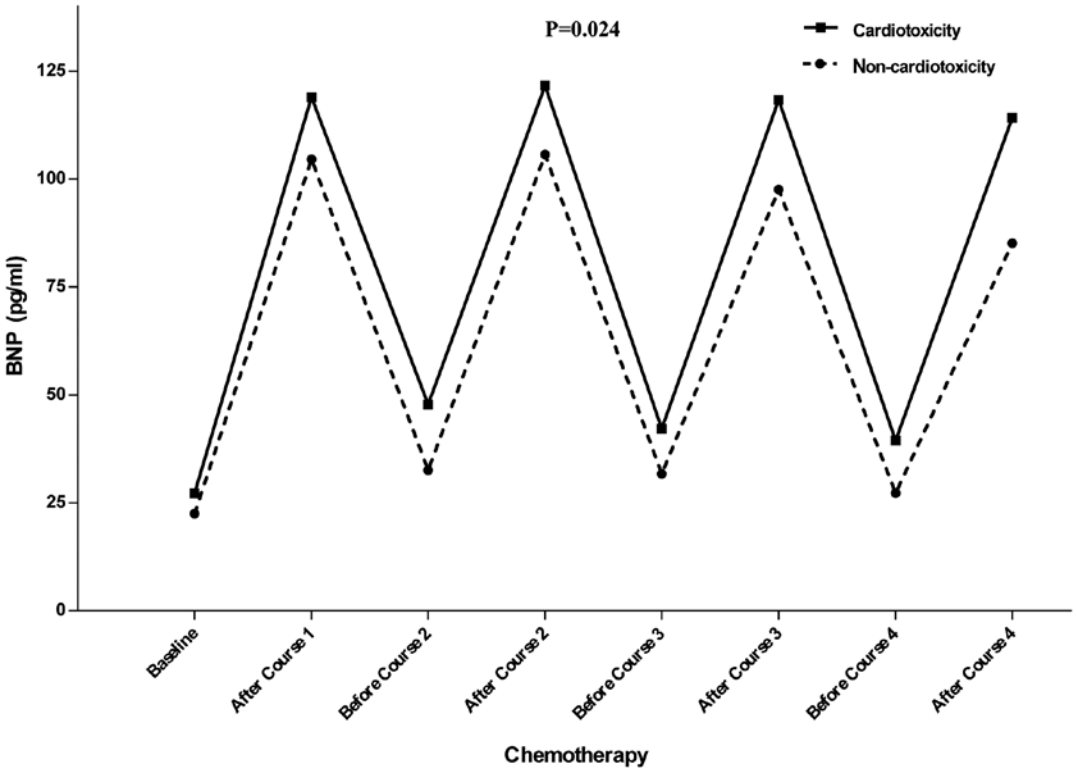

Figure 2. Repeated measures analysis of variance to assess the variation of the levels of BNP during the chemotherapy. Results showed that the levels of BNP were consistently higher in the cardiotoxicity group compared with the non-cardiotoxicity group in each treatment with anthracycline. P=0.024. BNP, brain natriuretic peptide.

Ability of serum BNP levels in predicting cardiotoxicity early. The ROC curve of BNP levels after the fourth course of chemotherapy against cardiotoxicity is shown in Fig. 3. The statistical analysis of the ROC curve was performed using SPSS 17.0. The area under the cure (AUC) was 0.673 [95% confidence interval (CI), 0.584-0.763], suggesting that the BNP level after the fourth dose of chemotherapy had a role in diagnosing cardiotoxicity. ROC curve analysis demonstrated that the optimal BNP threshold was 107.9 pg/ml at the maximum Youden index of 0.332. The prevalence of cardiotoxicity was 34.9%. The diagnostic sensitivity of anthracycline-induced cardiotoxicity was 0.538, the specificity was 0.794, the positive predictive value (PPV) was 0.583 and the negative predictive value (NPV) was 0.762.

Based on this BNP threshold, patients who were followed up were divided into two groups, the low BNP level group (BNP <107.9 pg/ml) and the high BNP level group (BNP ≥107.9 pg/ml). According to their cardiotoxicity-free survival time, a Log-rank test was performed (Fig. 4) and it was shown that the cardiotoxicity-free survival rate of the high BNP level group was consistently lower compared with the low BNP level group (P<0.001). Therefore, it was suggested that there was significant difference in the cardiotoxicity-free survival rate of cardiotoxicity-free between the low BNP level group and high BNP level group between the low BNP level group and the high BNP level group. When patients received the final dose of anthracycline during chemotherapy 9 weeks after the initial chemotherapy course, the detected BNP levels after the fourth dose of chemotherapy could predict the cardiotoxicity for a median time period of 1.5 years, suggesting that the serum BNP levels after the final dose of anthracycline during chemotherapy may contribute to the detecting cardiotoxicity early.

Discussion

Anthracyclines are commonly used to treat patients with breast cancer. However, the acute, chronic and delayed cardiotoxicity may occur during the anthracycline therapy and may possibly occur immediately after the initiation of chemotherapy (21). Furthermore, aside from clinical dilated cardiomyopathy and heart failure, ventricular dysfunction with no symptoms was more commonly observed in the patients receiving anthracycline based chemotherapy and the damage was often irreversible when diagnosed (22). Therefore, to prevent irreversible cardiotoxicity, breast cancer patients who received anthracycline, should be monitored during treatment and early diagnosis of AIC is necessary. A number of biomarkers, such as cardiac troponin T (6), fluorouracil (23) and circulating microRNAs (24) have been reported to have a predictive role in identifying AIC. Serum BNP elevation during chemotherapy has also been well investigated (13). The present study focused on monitoring the dynamic variation in serum BNP levels during anthracycline chemotherapy.

In the present study, 52 of the 149 patients who received anthracycline therapy exhibited cardiovascular events during the observation period. The follow-up duration was to 0.1 to 3.8 years, with acute, chronic and some delayed cardiotoxicity events observed. Since ECG detection is not highly specific, echocardiography and clinical symptoms of patients were used to aid in diagnosis of cardiotoxicity in the present study. Previous

Table III. Multivariate analysis of factors that may have influenced the disease-free time.

Covariate	Comparison	Hazard ratio	95% CI	P-value
Age	Continuous	1.010	0.971-1.052	0.612
Height	Continuous	2.677	0.001-5,937.427	0.802
BSA	Continuous	1.219	0.066-22.354	0.894
BNP levels after course 4	Continuous	1.003	1.000-1.007	0.047[a]
Hypertension	No vs. Yes	1.598	0.747-3.417	0.227
Cumulative dose of adriamycin	Continuous	1.007	0.996-1.018	0.212

[a]P<0.05. 95% CI, 95% confidence interval; BSA, body surface area; BNP, brain natriuretic protein.

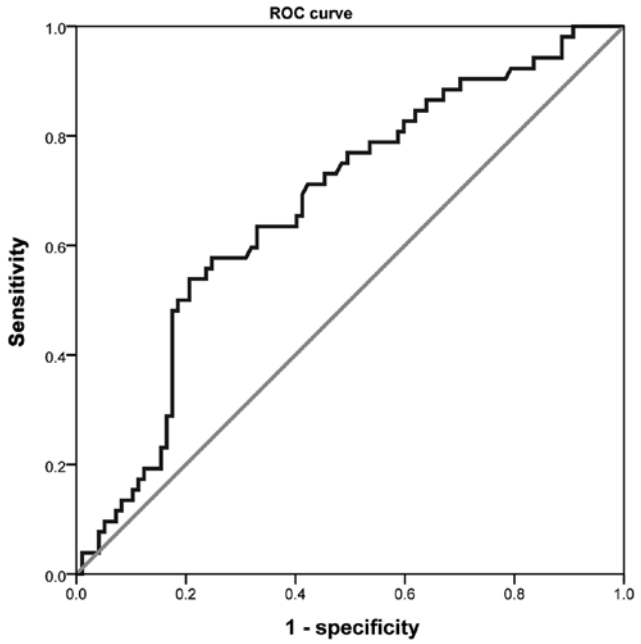

Figure 3. ROC curve of BNP levels after the fourth course of chemotherapy. The area under the cure was 0.673 (95% confidence interval, 0.584-0.763). The optimal diagnostic threshold of BNP after the last anthracycline chemotherapy treatment was 107.9 pg/ml, the diagnostic sensitivity was 0.538, the specificity was 0.794, the Youden index was 0.332, the positive predictive value was 0.583 and the negative predictive value was 0.762. BNP, brain natriuretic peptide; ROC, receiver operating characteristic.

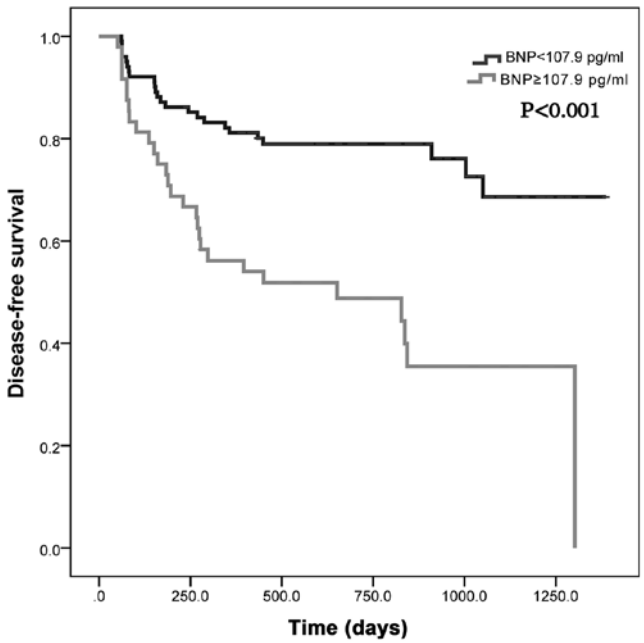

Figure 4. The Log-rank test of the cardiotoxicity-free survival rates of two groups. The cardiotoxicity-free survival rate of the high BNP level group (BNP ≥107.9 pg/ml) was consistently lower compared with the low BNP level group (BNP <107.9 pg/ml; P<0.001). BNP, brain natriuretic peptide.

studies have shown a direct association between the occurrence of heart failure and the cumulative anthracycline dose (25,26). It has been reported that when the cumulative dose of adriamycin is 400 mg/m^2, the incidence of heart failure is 3% increasing to 7.5% at 550 mg/m^2 and to 18.0% at 700 mg/m^2. However, as there was a lower cumulative dose of adriamycin in the present study, there were fewer symptomatic events of cardiotoxicity observed. Therefore, subclinical events of cardiotoxicity like LVDs, which indicates a decrease in cardiac systolic function, were used as one of the characteristics of cardiotoxicity (27-30). In the present study, 50% of the patients received a cumulative dose of ≤175 mg/m^2 anthracycline, whereas the remaining 50% of patients received a cumulative dose of >175 mg/m^2. No significant changes in the ejection fraction and measurements of cardiac systolic function were observed between the two groups during the follow-up period.

In the present study, patients with breast cancer who were positive for ER or HER2 may have additionally received tamoxifen and Herceptin treatment. Previous studies have suggested that tamoxifen has a protective effect on cardiac function (31), whereas Herceptin treatment may be cardiotoxic (32). However, there was no such evidence of either tamoxifen-mediated cardiac protection or Herceptin-mediated cardiotoxicity in the study, in agreement with other previous studies (33,34). It may be the case that compared with the chemotherapy, both the protective effects of tamoxifen and the cardiotoxic effects of Herceptin were too weak to discern in these patients (35). In the follow-up period, if a case was determined to require an additional course of chemotherapy due to recurrence or metastasis, they were removed from the present study.

The difference observed in the incidence of cardiotoxicity between the respective EC/AC treatments regimens compared with the EC-T/AC-T treatment regimens were not significant, suggesting that the chemotherapy programs containing docetaxel

did not result in additional cardiotoxicity. Increased concentrations of BNP were observed following docetaxel chemotherapy and lower BNP concentrations following docetaxel chemotherapy recovery. Together, the data suggest that cardiotoxicity induced by docetaxel was self-limiting and could be reversed if withdrawn (36). The reversible nature of docetaxel-induced chemotherapy may explain why there was a correlation between BNP after the fourth dose of chemotherapy (subsequent to completion of treatment with anthracyclines) and cardiotoxicity, instead of a correlation between serum BNP levels following the eighth dose of chemotherapy (subsequent to completion of treatment with docetaxel) and cardiotoxicity. These data suggest that increase in the serum levels of BNP was induced primarily by the anthracycline treatment rather than docetaxel treatment during chemotherapy. Furthermore, repeated measures ANOVA analysis suggested that the serum BNP levels from the first to the fourth dose of chemotherapy in the cardiotoxicity group were higher compared with the non-cardiotoxicity group.

To evaluate whether BNP elevation was an effective indicator in diagnosing cardiotoxicity, the ROC curve of serum BNP levels following the final dose of anthracycline was plotted and the AUC was 0.673 (95% CI, 0.584-0.763). It was also concluded that the optimal BNP threshold was 107.9 pg/ml, the diagnostic sensitivity of cardiotoxicity was 0.538, the specificity was 0.794, the Youden index was 0.332, the positive predictive value was 0.583 and the negative predictive value was 0.762. The results showed that elevation of serum BNP levels in cardiotoxicity diagnosis was not very effective, with considerable misdiagnosis or missed diagnoses. However, it should be noted that based on the BNP diagnostic threshold, the Log-rank test showed that the cardiotoxicity-free survival rate of the high BNP level group was consistently lower than that of the low BNP level group, indicating that serum BNP levels following the fourth dose of chemotherapy (9 weeks after the initial dose) predicted the cardiotoxicity with a median length of 1.5 years, suggesting that elevated serum BNP levels may assist in the detection of potential myocardial damage prior to the occurrence of clinical symptoms and possibly prior to detection with traditional echocardiography analysis. The baseline serum BNP levels of the patients did not predict cardiotoxicity, in agreement with previous studies (37-40), although this may have been the result of the exclusion of patients with a previous history of heart diseases.

The present study had several limitations. The research was conducted and patients recruited at a single center and the sample size was small. After from hypertension, other risk factors of heart diseases such as diabetes and hyperlipidemia were not considered. Based on the standard treatment of breast cancer, the cumulative dose of anthracycline the patients received in the study was not very high, potentially resulting in the lower incidence of cardiotoxicity. In addition, blood used for serum BNP detection in the present study was collected only once prior to or after each therapy. As a number of previous studies (40-43) suggested that chemotherapy could cause three types of BNP variation, including no BNP increase, transient increase before decrease and sustained increase. The incidence of cardiotoxicity was associated with these types of BNP variation, as the incidence of cardiotoxicity in the sustained BNP increase group was higher compared with the other two groups, and subsequent impairment of LV function only occurred in patients with persistent BNP elevation (41). Therefore, a single change in serum BNP levels may not be considered convincing, instead multiple measurements of BNP should be performed during the therapy.

In conclusion, BNP can be elevated in patients who have received anthracyclines, measuring the serum level of BNP 24 h after final dose of anthracyclines during chemotherapy contributes to the early detection of cardiotoxicity. As obtaining blood samples is convenient, inexpensive and allows for more continuous monitoring and management of patients with breast cancer, serum BNP levels should be considered as a suitable method for diagnosing and predicting anthracycline-induced cardiotoxicity.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

XL, YYZ, CPC and CH conceived and designed the experiments. XL, LX and DX performed the experiments. YYZ, CPC and DX analyzed and interpreted the data, XL and YYZ wrote the manuscript. MT and OH designed and performed the experiments. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by The Ethical Committee of The First Hospital of Jiaxing (Jiaxing, China). All patients signed written informed consent form to participate in the present study.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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