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Drug-induced Cardiotoxicity Studied by Longitudinal B-Type Natriuretic Peptide Assays and Radionuclide Ventriculography

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Abstract. *Background: To study the longitudinal variations of plasma B-type natriuretic peptide (BNP) with reference to left ventricular ejection fraction (LVEF) during and after chemotherapy with cardiotoxic drugs. Patients and Methods: We prospectively measured plasma BNP using an immunoradiometric assay in 12 anthracycline-treated breast cancer patients monitored for a mean time of 880±293 days (pilot group). Prior to each cycle and throughout the following year, LVEF and cardiac output were measured by radionuclide ventriculography. Anthracycline pharmacokinetics was studied during the first cycle. Relationships between serial observations were analysed with the general linear mixed effects model. Identical methods were subsequently applied to a test group of 67 anthracycline or trastuzumab-treated patients. Results: Five out of 70 (6.33%) patients developed anthracycline-induced heart failure. BNP concentrations were found to be positively correlated to anthracycline cumulative dose and negatively to LVEF values. Variables entering the mixed models were cumulative anthracycline dose, time and cardiac output. Conclusion: An infra-clinical cardiotoxicity of anthracyclines as defined by BNP elevation is frequent but reversible. Patients who developed heart failure showed a continuous BNP increase and concentrations over 100 ng/ml.*

Anthracyclines are effective drugs for adjuvant or post-recurrence treatment of breast cancer. However, their use can be restricted due to their cumulative cardiac toxicity: the incidence of congestive heart failure was reported to be 0.14% at 400 mg/m^2, 7% over 550 mg/m^2 and up to 18% over 700 mg/m^2 (1). Sensitivity to anthracycline-induced cardiotoxicity is subject to individual differences. Anthracycline-induced cardiac dysfunction can develop early (2, 3), although delayed cardiac damage, even years after treatment, has also been reported (4, 5). Trastuzumab-based therapies are also known to induce cardiac dysfunction in some patients (6).

The reference method for monitoring drug-induced cardiac toxicity is the measure of left ventricular ejection fraction (LVEF) by radionuclide ventriculography. This implies serial LVEF measures, which are expensive. A biological monitoring by serial assays might be a useful method to select patients who require LVEF measures and we studied the value of B-type natriuretic peptide (BNP) in this context. BNP is a member of the cardiac natriuretic hormones, secreted by the pathologic left ventricle in response to myocyte stretch and cardiac overload. Plasma BNP elevation reflects the cardiac response to counteract heart failure. The variations of BNP levels before and after anthracycline-based chemotherapy have been described in cancer patients but, to our knowledge, none of the previous studies included serial BNP assays during chemotherapy (3, 7-12).

In a pilot study, we prospectively evaluated the longitudinal variations of plasma BNP during and after anthracycline therapy with reference to LVEF and anthracycline pharmacokinetics during the first cycle. Our aim was to identify variations of BNP that might predict clinical cardiac damage. We subsequently applied the same statistical methods to analyse the results obtained in an independent group of anthracycline- or trastuzumab-treated breast cancer patients at different stages.

Patients and Methods

Pilot study. A prospective protocol enrolling anthracycline-treated breast cancer patients was implemented. To be included in the protocol, patients needed to fulfil the following criteria:

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histologically confirmed breast cancer, age ≤80 years, life expectancy >3 months, performance status (ECOG) <2, normal liver function tests (ASAT, ALAT, alkaline phosphatase ≤2.5 normal levels, total bilirubin ≤1.5 normal level), normal renal function (creatinin ≤1.5 normal level) and haemoglobin ≥100 g/l, neutrophils $\geq 2.10^9$/l, platelets $\geq 100.10^9$/l and pre-treatment LVEF, determined by radionuclide ventriculography, ≥48 %. Patients who presented with previous congestive heart failure, even if stabilised, myocardial infarction, atrial or ventricular arrhythmia, atrio-ventricular block or chronic obstructive pulmonary disease, were excluded. Our protocol was approved by the regional ethical committee and informed written consent obtained from all patients.

Seven patients received neoadjuvant therapy and five patients had previously received various doses of anthracycline. Neoadjuvant treatment comprised six courses of doxorubicin (60 mg/m^2) and paclitaxel (200 mg/m^2), followed by loco-regional treatment (surgery-radiotherapy). The previously-treated metastatic patients had received epirubicin (≥360 mg/m^2) or mitoxantrone (≥60 mg/m^2) or doxorubicin (≥240 mg/m^2) and were scheduled to receive a new anthracycline-based chemotherapy until progression. When patients received epirubicin or mitoxantrone, equivalent doxorubicin doses were calculated according to Okada *et al.* (13) and Robert (14).

Test group: patients receiving cardiotoxic treatments. BNP and LVEF results from 67 female breast cancer patients during or after treatment were analysed. Fifty of them received an anthracycline-based treatment, either as adjuvant treatment or after recurrence. Seventeen patients, all pre-treated by anthracyclines, received a combined treatment of trastuzumab and taxanes or vinorelbine. Patients treated by dexrazoxane or with a cumulative anthracycline dose <100 mg/m^2 were excluded. For each concomitant BNP and LVEF determination, the corresponding anthracycline cumulated dose and patient's clinical status were recorded.

Cardiac evaluation. In the pilot study, cardiac evaluation, including electrocardiogram (ECG) and LVEF measure, was performed *prior* to chemotherapy, before each cycle and over 1-3 months after treatment completion. In the case of abnormal BNP or LVEF results, measures were repeated every 3 months until recovery. For the test group, BNP and LVEF measures were performed at the clinician's request.

Radionuclide ventriculography was performed with the patient at rest in the supine position. Red blood cells were labelled *in vivo* with 550-900 MBq of 99^m technetium 30 minutes after an intravenous injection of 10-20 μg/tin/kg "cold" stannous ion (TcK7-Cis Bio International, Gif sur Yvette, France). Acquisition of gated cardiac studies initiated by the R wave of the ECG were collected in frame mode (64x64), using a gamma camera (SP6X General Electric, Milwaukee, MI, USA) fitted with a low energy medium resolution, high sensitivity collimator and oriented to a 45-degree left anterior oblique view with a 15-degree caudal tilt. Cardiac cycles with an R-R interval much shorter or longer than the average R-R interval during acquisition were considered as premature ventricular beats (arrhythmia) and automatically rejected. LVEF, heart rate, diastolic and systolic volumes were automatically obtained. A cardiac output (ml/min) was then calculated on the basis of the difference between diastolic and systolic volumes multiplied by the heart rate (15).

Cardio-pulmonary disorders were classified according to the New York Heart Association (NYHA), and grouped according to the origin of cardio-pulmonary disorders: cardiotoxic drugs, or acute or chronic diseases linked to other identified aetiologies, or of mixed origin.

Pharmacokinetics of anthracyclines (pilot study). Blood samples were collected in EDTA tubes, immediately centrifuged and stored at –20°C until analysis. Doxorubicin or epirubicin and their hydroxylated metabolites, doxorubicinol and epirubicinol, were quantified by high pressure liquid chromatography (HPLC). Extraction was performed on Sep-Pak C18 cartridges (Waters, Milford, MA, USA), conditioned by successive elutions with 3 ml methanol, 3 ml methanol-water (50/50) and 10 ml phosphate buffer (Na_2HPO_4 0.1 M in distilled water). Five hundred μl of plasma were spiked with 50 μl of internal standard (daunorubicin, 5000 μg/l) and passed through the cartridge, followed by 3 ml of phosphate buffer. Elution was performed by 4 ml of chloroform/methanol (2/3:1/3, v/v) in glass tubes. After drying under nitrogen at 45°C, the residue was diluted in 120 μl of mobile phase and injected into the HPLC system. Analysis was performed through a Micro-Bondapak C18 30 cm column (Waters) with a mobile phase of formiate/acetonitrile buffer (68: 32, v/v) at pH 4 with a flow rate of 1.5 ml/min. Fluorescent detection was performed with a spectro-fluorimeter at λex=480 nm and λem=560 nm. The limit of quantification was 5 μg/l for both drugs. The pharmacokinetic parameters were calculated with Micro-Pharm software (S. Urien, Centre René Huguenin, Saint-Cloud, France).

Brain natriuretic peptide assays. Blood samples were collected after 20 minutes supine rest in EDTA-aprotinin (500 kUI/ml) tubes (Beckton-Dickinson, Plymouth, UK) and placed in ice-cold water. Plasma was immediately separated and stored at –30°C if not immediately assayed. BNP was measured in duplicate by immunoradiometric assay (Shionoria, Cis Bio International, Gif Sur Yvette, France) with a detection limit of 2.0 ng/l. Cross-reaction of monoclonal antibodies with ANP (atrial natriuretic peptide) and CNP (C-type natriuretic peptide) were less than $1x10^{-5}$. Quality control was ensured by assaying two levels of control sera in each series. All results showing a coefficient of variation between duplicates over 10% were re-assayed. The upper level of normal range used was 40 ng/l, to take into account variations due to sex and age over 50 years (16). The widely accepted limit of 100 ng/l for cardiac dysfunction was also considered (17).

In the course of the pilot study, plasma BNP was measured prior to each cycle of chemotherapy and after treatment at each visit throughout the following year. During the first cycle, additional samples were drawn for anthracycline pharmacokinetics. BNP levels were not used for clinical decisions.

Statistical methods. Statistical analyses were performed using the SAS statistical package (SAS Institute, Cary NC, USA). Non parametric methods were used to estimate and plot the relationships between BNP, LVEF and cardiac output *versus* the cumulative doses. This involved classifying the observations into a data set according to a range of cumulative doses, *e.g.*, 0 mg/m^2, 1-100 mg/m^2, 101-200 mg/m^2, 201-300 mg/m^2, 301-400 mg/m^2, 401-500 mg/m^2 and >500 mg/m^2. Mean BNP, LVEF and cardiac output were plotted against the centres of the cumulative doses intervals.

To analyse longitudinal data identifying the relationships between serial observations collected from the same patient, the general linear mixed effects model: $Y_i = X_i .\beta + Z_i .\alpha_i + \varepsilon_i$

Table I. *Summary of the biological and clinical data in the pilot group classified according to cumulative anthracycline dose.*

Patient no.	Age (years)	Baseline BNP (ng/l)	Maximum of BNP (ng/l)	Days from 1st chemotherapy course	Type of variation no. of samples over 40 ng/l /total	FEVG	Anthracycline cumulative	Clinical symptoms dose at entry (mg/m^2)	Pre-existing cardiomyopathy	Risk factors	Endpoint status
1	43	11.88	41.68	+ 143 (C6)	fluctuating 2/13	constantly ≥ 48%	0	none	none	none	disease-free
2	54	5.78	17.91	910	fluctuating 0/21	constantly ≥48%	0	none	none	none	disease-free
3	57	35.07	51.62	805	fluctuating 4/19	constantly ≥48%	0	dyspnea NYHA II (reversible)	none	HT** obesity (BMI*32.5)	disease-free
5	58	6.47	10.96	+ 78 (C5)	fluctuating 0/18	constantly ≥48%	0	none	none	none	disease-free
7	62	11.9	38.41	24 th hour (C1)	fluctuating 4/19	transient decrease (from day 77 to day 483)	0	dyspnea NYHA II (reversible)	cardio-megaly	obesity (BMI* 32.4)	disease-free
8	48	2	18.38	422	fluctuating 0/17	normal except 45% at day 127	0	none	none	none	disease-free
9	53	1.13	18.17	803	fluctuating 0/15	constantly ≥48%	0	none	none	HT	disease-free
6	47	20.9	78.05	176	fluctuating 7/13	constantly ≥48%	140	none	none	none	deceased from cancer
10	52	1.92	13.77	181	fluctuating 4/19	constantly ≥48%	202	none	none	none	disease-free
11	66	19.4	65.94	130	fluctuating 8/17	constantly ≥48%	245	arrhythmia	none	HT	disease-free
4	52	9.14	226.9	396	constant increase from C6 to C8 and after 5/14	drop to 42% at day 393	260	heart failure	none	none	deceased from cancer
12	52	61.57	780.6	+ 183 (C4)	constant increase up to C4 14/14	drop to 32% at day 183	388	dyspnea NYHA II (non reversible) heart failure	none	obesity (BMI* 34.5) liver cirrhosis	cancer in progression

*BMI= body mass index (kg/m^2). **HT=arterial hypertension.

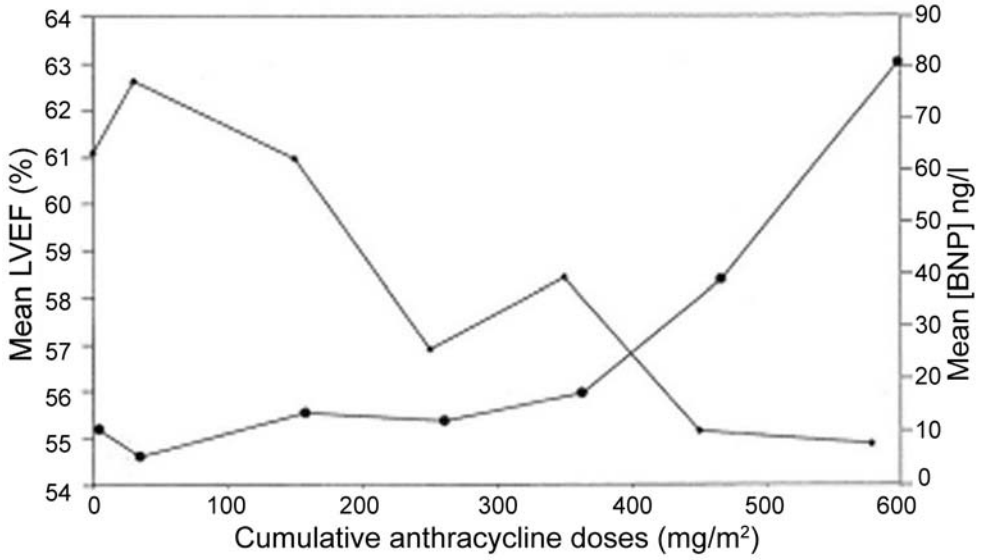

Figure 1. *Mean concentrations of BNP (●) and mean LVEF (■) as a function of anthracycline cumulative dose in the pilot group.*

was used, where: Y_i is the n_i x 1 response vector of the serial log-transformed plasma BNP concentrations for the patient; β denotes the p x 1 vector of unknown population fixed-effects parameters associated with the known n_i x p design matrix X_i containing the covariates which can either be time, or cumulative doses, or cardiac outputs, or all possible interactions between these covariates; α_i denotes the n_i x q vector of unknown individual random-effect parameters associated with the known n_i x q design matrix Z_i containing covariates, just like X_i, which is sometimes reduced to a null matrix, sometimes to the n_i x 1 unit vector; ε_i is an unobserved n_i x 1 vector of error term.

This model allows parameter estimation and inference in situations with considerable variation between patients, both in number and timing of observations, and in the structure of the covariance matrix. Moreover, population averaged curves can be directly estimated, in addition to subject-specific curves. In the subsequent analyses, mixed models were used with the patient included as a random effect, and with or without an intercept as random variable depending on whether the regression coefficient β describes the average population or an individual response to changing X, respectively. The choices of the best models and of the appropriate covariance structures were made according to the Akaike Information Criterion (AIC) (18). Statistical inferences concerning the fixed- and random- effects parameters were obtained by testing a Fischer F statistic. Main and interaction effects were considered statistically significant if $p<0.05$.

Results

Pilot study. Twelve consecutive breast cancer patients (mean age 54±6 years) were investigated during and after treatment (median follow-up time from inclusion 1208 days, range 269-1528 days). Five of them, previously treated by 1 to 4 lines of an anthracycline-containing chemotherapy, had a mean±SD cumulated anthracycline dose before the first cycle of 242±95 mg/m^2. The results are summarised in Table I. Ten patients had previously received left chestwall radiotherapy. There were no statistically significant differences for age or risk factors between neoadjuvant and pre-treated patients. Electrocardiogram profiles before chemotherapy and before each course remained constantly normal. At the end of chemotherapy, cumulated anthracycline doses were 342±21 mg for neoadjuvant patients and 568±90 mg for pre-treated patients. Clinical symptoms of cardio-pulmonary dysfunction were observed for four patients (Table I), leading to treatment discontinuation because of LVEF decrease for two of them. Heart failure related to anthracyclines cardiac toxicity was recorded for two patients (16.7%), one of them presenting associated risk factors (Table I).

LVEF. Prior to chemotherapy, the mean LVEF was 62.91±7.43% and 61.10±5.18% in the absence of previous anthracycline treatment. The mean LVEF decreased to 57.40±5.34% after completion of chemotherapy. Since this decrease was not significant ($p=0.067$, Student's paired *t*- test), the basal LVEF was included as a serial measure of LVEF in all subsequent analyses. The mean LVEF decreased to 60.96±9.30%, 56.90±11.32%, 58.42±7.61 and 55.14±4.60% after anthracycline cumulative doses within the intervals of 101-200 mg/m^2, 201-300 mg/m^2, 301-400 mg/m^2 and 401-500 mg/m^2, respectively. The relationship between mean LVEF and the centres of cumulative doses intervals was significantly linear. The correlation between LVEF decrease and cumulative dose increase was highly significant ($r=0.926$, $p=0.0028$, Pearson's correlation, Figure 1).

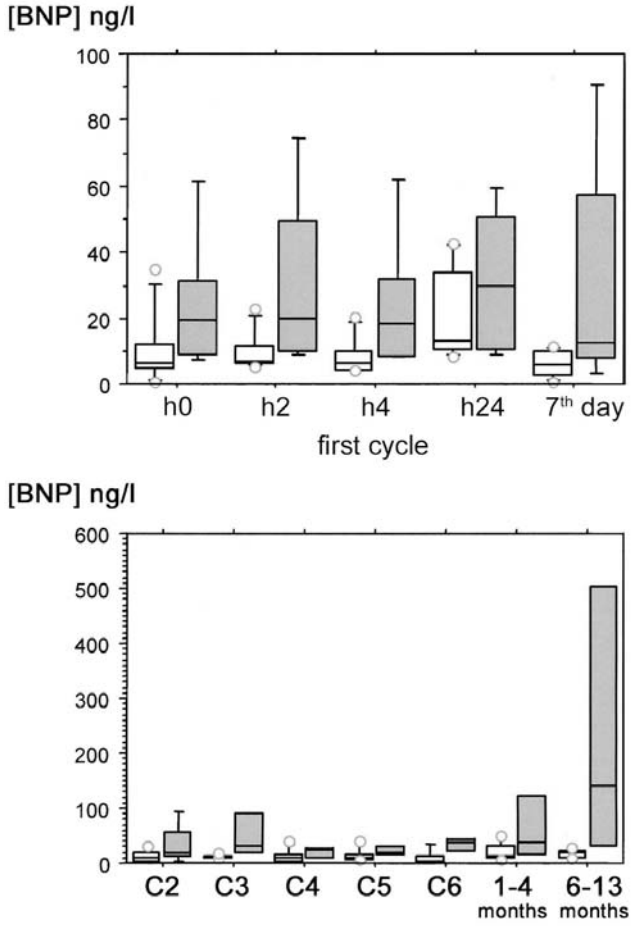

Figure 2. *Box plots of plasma BNP concentration during and after chemotherapy in the pilot group. C=chemotherapy cycle, unfilled boxes=neoadjuvant patients, filled boxes=pretreated patients.*

Cardiac output. Mean and SD cardiac output was 2978±1042 ml/min before chemotherapy. It increased to 3229±1073 ml/min on chemotherapy completion (non significant difference). Mean cardiac output was 3043±783 ml/min for the non previously-treated patients. During chemotherapy, the cardiac output remained stable up to an anthracycline cumulative dose of 500 mg/m^2, beyond which it increased to 4504±2500 ml/min. There was no significant correlation between cardiac output and the centres of anthracycline cumulative doses intervals. Likewise, the variations of LVEF and BNP were not significantly associated with any concomitant variation in cardiac output.

Plasma BNP. The mean±SD number of samples per patient during and after chemotherapy in the pilot study was 15.6±2.7. Forty-eight out of a total of 199 samples (24.1%) had a BNP concentration >40 ng/l, including 6/199>100 ng/l.

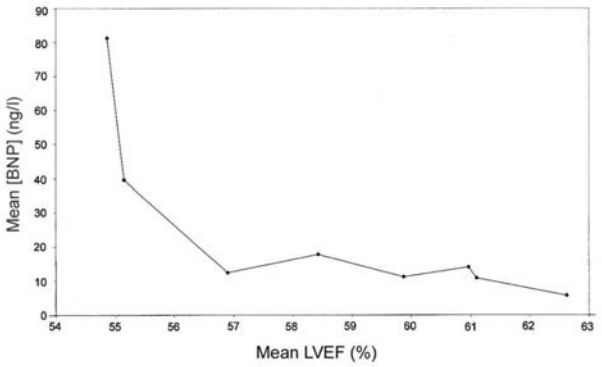

Figure 3. *Relationships between mean BNP concentrations and mean LVEF values (pilot group).*

Table II. *BNP, LVEF and anthracycline pharmacokinetics in the pilot group.*

		Mean	SD	Median	Range
BNP (ng/l)					
C1	baseline	16.21	17.05	10.51	61.23
	2h	18.85	20.29	10.74	69.23
	4h	14.66	16.12	8.42	57.73
	24h	24.99	17.94	16.40	51.20
	day 7	16.79	26.15	8.00	90.10
Doxorubicin (epirubicine) (µg/l)					
C1	baseline	0.00		0.00	0.00
	2h	44.04	13.48	49.30	39.30
	4h	35.93	13.91	39.55	44.00
	24h	12.85	4.09	14.00	12.60
	day 7	0.42	1.11	0.00	5.00
AUC (µg/l/h)		1069.00	286.00	1191.00	544-1387
Doxorubicinol (epirubicinol) (µg/l)					
C1	baseline	0.00		0.00	0.00
	2h	28.29	17.31	24.15	45.40
	4h	30.89	16.96	35.50	47.70
	24h	10.68	6.64	12.00	23.00
	day 7	0.47	1.64	7.09	5.70
LVEF (%)					
C1	baseline	63.28	7.68	59.30	25.70
	day 7	61.38	8.11	60.92	26.64

The mean BNP concentration before the first cycle was 16.21±17.05 ng/l. In previously-untreated patients, the mean BNP was 10.87±11.42 ng/l *versus* 23.69±22.01 ng/l for pre-treated patients (non significant difference). There was no significant difference in BNP concentrations after

Table III. *Results of the statistical models (pilot group).*

Model	Variable	Fixed-effect regression coefficient	Standard error	*p*-value	AIC*
A	intercept	1.7169	0.2622	< 0.0001	
	cumulative doses	0.0032	0.00047	< 0.0001	255.8
B	intercept	2.3302	0.279	< 0.0001	
	cumulative doses x time	0.00001	0.000001459	< 0.0001	267.3
C	intercept	2.9041	0.2802	< 0.0001	
	cardiac output	-0.2869	0.06886	< 0.0001	
	cumulative doses x cardiac output	0.00071	0.00012	< 0.0001	233.1
D	intercept	2.0338	0.2696	<0.0001	
	cumulative dose	0.005	0.0013	0.0027	
	time	1.7392	0.1677	<0.0001	
	cumulative dose x time	-0.0032	0.0004	<0.0001	
	[anthracycline]** x time	-0.0741	0.0125	< 0.0001	101
E	[anthracycline]** x time	0.0468	0.0156	0.0051	140
F	cumulative dose	0.0035	0.00046	< 0.0001	276.4
G	cumulative dose x time	0.00001	0.000001459	< 0.0001	290.7
H	cardiac output	-0.242	0.06853	0.0007	
	cumulative dose x cardiac output	0.00065	0.00012	< 0.0001	264.5

*AIC = Akaike information criterion.
** [anthracycline]= anthracycline concentration μg/l.
Models A, B and C: mixed models for the population average curve,
D and E: mixed model for BNP concentrations and results of anthracycline pharmacokinetics,
F, G and H: mixed models for the subject-specific curve.

radiotherapy according to the treated side. During the first cycle, the maximum of BNP concentration was observed at the 24th hour for all but two pre-treated patients (2nd hour). After the sixth chemotherapy cycle, the mean BNP levels increased to 20.41±18.12 ng/l (Figure 2). Since this increase was weakly significant ($p=0.044$), the baseline BNP value was included as a serial measure of BNP in all subsequent analyses. For six out of twelve patients, all serial BNP concentrations remained <40 ng/l in the samples taken during and after chemotherapy, four other patients showed a transient and mild (<100 ng/l) BNP elevation. The two patients who developed heart failure showed important BNP elevations at the end of chemotherapy or later, already exceeding 100 ng/l 52 and 178 days, respectively, after the first anthracycline course (Table I).

The mean BNP levels were 14.14±10.58 ng/l, 12.54±9.50 ng/l, 17.87±16.38 ng/l and 39.58±27.46 ng/l for the intervals of anthracycline cumulative dose of 101-200 mg/m^2, 201-300 mg/m^2, 301-400 mg/m^2 and 401-500 mg/m^2, respectively. The relationship between mean BNP concentrations and centres of cumulative doses intervals was linear and the correlation between BNP concentration and increasing anthracycline cumulative dose was significant ($r=0.855$, $p=0.0141$, Pearson's correlation, Figure 1).

After chemotherapy completion, the mean BNP concentrations reached 37.64±50.74 ng/l at 1-4 months and 107.78±232.06 ng/l at 6-13 months.

BNP, LVEF and clinical symptoms of cardiac toxicity. A linear relationship was observed between mean BNP and LVEF values according to the anthracycline cumulative dose, with a significant correlation between BNP increase and LVEF decrease ($r=0.771$, $p=0.025$, Pearson's correlation, Figure 3). A >20% decrease of LVEF was recorded for the two patients with heart failure, concomitant to a continuous increase in BNP concentrations (Table I).

Anthracycline pharmacokinetics. Doxorubicin and doxorubicinol (or epirubicin and epirubicinol) concentrations were measured at time zero, 2, 4 and 24 hours of first cycle

Table IV. *Summary of the biological and clinical data in selected patients from the test group who displayed clinical symptoms or a BNP>100 pg/ml, classified according to anthracycline cumulative dose.*

Patient no.	Age (years)	Maximum of BNP (ng/l)	Corresponding FEVG	no. of samples ≥100 ng/l/total	Anthracycline cumulative dose (mg/m^2)	Clinical symptoms	Pre-existing cardiomyopathy	Risk factors	Origin of cardio-pulmonary disorders	End-point status
34	51	119.02	54%	3/7	144	dyspnea NYHA II (reversible)	none	none	unrelated to anthracycline	stable disease
30	32	213.83	42%	2/5	199	cardiomegaly right heart failure	none	none	unrelated to anthracycline	deceased from cancer
63	68	292.04	53%	1/1	209	tachycardia dyspnea NYHA II due to pulmonary embolism	none	HT overweight (BMI* 29.6)	unrelated to anthracycline	deceased from cancer
50	62	172.8	41%	1/2	237	none	cardiomyopathy due to HT*	HT	unrelated to anthracycline	deceased from cancer
18	57	602.64	24%	1/1	289	dyspnea NYHA III (non reversible) pleural effusion	none	none	due to anthracycline	deceased from cardio-pulmonary failure
20	78	226.29	58%	1/2	427	dyspnea NYHA IV pleural effusion	none	pulmonary hypertension	unrelated to anthracycline	deceased from cancer
24	30	686.83	23%	1/3	505	right heart failure pleural effusion jugular vein thrombosis	none	none	unrelated to anthracycline	deceased from heart failure and cancer
11	55	2532.9	40%	8/9	538	dyspnea NYHA II (reversible) heart failure	none	HT	unrelated to anthracycline	regression under chemotherapy
61	47	376.5	30%	3/4	672	dyspnea NYHA II	none	none	due to anthracycline	deceased from cancer
42	68	674.16	50%	6/7	681	dyspnea NYHA II acute pulmonary oedema pleural effusion	none	none	unrelated to anthracycline	stable disease
9	53	1042	23%	3/5	714	dyspnea NYHA III (non reversible) cardiomegaly	none	HT** liver cirrhosis	mixed***	deceased from heart failure and cancer

*HT= arterial hypertension, BMI= body mass index (kg/m^2).
**HT= arterial hypertension.
***Mixed = pre-existent cardio-pulmonary disorders worsened by anthracycline.

and the area under the plasma concentration curve (AUC) was calculated. Additional measures were performed seven days after the first injection and prior to each subsequent cycle. No detectable concentrations of anthracycline or metabolites were obtained at baseline and before all subsequent cycles for all patients. The maximum concentration of anthracycline was reached at the second hour, except for one patient (4th hour). The results of pharmacokinetics are summarised in Table II. A positive correlation between the concentrations of doxorubicin or epirubicin and doxorubicinol or epirubicinol was obtained only at the 24th hour ($\varrho=0.739$, $p=0.006$). We found the AUC values to be unrelated to patients' age or the existence or not of prior anthracycline treatment. No correlation was found between BNP and anthracyclines or metabolites concentrations at the different time-points, or between maximum BNP concentration and AUC values during the first cycle.

Statistical models of BNP and LVEF variations during chemotherapy (pilot study)

a. Population average models. When modelling the average population curve (*i.e.*, $Z_i . \alpha_i = 0$), the BNP levels showed a significant association with the anthracycline cumulative dose ($p<0.0001$), the interaction between cumulative dose and time ($p<0.0001$), the cardiac output ($p<0.0001$) and the interaction between cumulative dose and cardiac output ($p<0.0001$, Fischer F test). The estimated parameters for the final models are summarised in Table III (models A, B and C). In the population average model including the pharmacokinetic data, the BNP levels showed a significant association with anthracycline cumulative dose ($p=0.0027$), time ($p<0.0001$), the interaction between cumulative dose and time ($p<0.0001$) and the interaction between anthracycline concentration and time ($p<0.0001$). The estimated parameters for the final model are summarised in Table III (model D). For all the models, the ratios of estimated fixed coefficients to their standard errors were all quite large, indicating a significant population model effect.

b. Subject-specific models. When focusing on individual responses, (*i.e.* BNP concentrations), an intercept (α_{0i}), associated with each patient (i) has been included as a random variable in the model to estimate the expected changes given a change in the covariates contained in X_i. The BNP concentrations showed a significant association with anthracycline cumulative dose ($p<0.0001$), the interaction between cumulative dose and time ($p<0.0001$), the cardiac output ($p=0.0007$) and the interaction between cumulative dose and cardiac output ($p<0.0001$, Fischer F test). In the final models retained, in all instances the random variables, (α_{0i}), were found to have highly significant effects. The estimated parameters for the final models are summarised in Table III (models F-H).

With the pharmacokinetic data, the BNP concentrations showed a significant relationship for the interaction between anthracycline concentration and time ($p<0.0051$, Table III, model E). In the latter model, the random variables, (α_{0i}), were found to have highly significant effects ($p\leq0.0004$) in all instances.

Test group. Sixty-seven patients were studied, within different time-frames during or after a potentially cardiotoxic treatment for a median follow-up time of 62 days (range 1-984 days), and a total of 237 blood samples were collected. Their mean age was 56 years (range 35-78 years). The mean and SD number of BNP assays per patient was 3.5±2.8 (range 1-11) and 1.9±1.3 (range 1-7) for parallel LVEF measures. Cardiac exploration was initiated by clinical symptoms for seven patients and was a part of systematic monitoring before starting a new treatment or during chemotherapy for the other patients. Fifty patients were explored during and/or after combined chemotherapy containing anthracycline, including four in the adjuvant setting and forty-six for recurrence. Seventeen patients received trastuzumab, associated with paclitaxel, docetaxel, or vinorelbine. At blood sampling, eighteen patients (12.7%) were in the range of cumulated anthracycline doses of 101-200 mg/m^2, 43 (30.3%) of 201-300 mg/m^2, 30 (21.1%) of 301-400 mg/m^2 , 28 (19.7%) 401-500 mg/m^2 and 23 cases (16.2%) were over 500 mg/m^2.

For 34 patients (50.7%), the plasma BNP never rose above 40 ng/l. A mild BNP elevation (<100 ng/l) was observed for seventeen (25.4%) patients and sixteen (23.9%) showed elevated (>100 ng/l) BNP concentrations. Left ventricular function was determined in one hundred and eighty cases in parallel with BNP assays, and twenty-one (31.3%) patients showed at least one LVEF result ≤48%.

BNP, LVEF and clinical symptoms in the test group. As with the pilot study, BNP elevations were found to be more frequent than LVEF decline and a linear increase of mean BNP concentrations as a function of anthracycline cumulated dose was found (regression coefficient r=0.315, $p=0.0032$). For LVEF, no linear regression according to anthracycline dose could be calculated ($p>0.05$). The inverse relationship between mean BNP concentrations and mean LVEF values shown in the pilot group was also evidenced in the test group (r=–0.395, $p=0.0004$).

Table IV summarises the clinical and biological results for eleven patients of the test group who showed BNP levels over 100 ng/l during monitoring, or clinical symptoms of cardiac dysfunction. The development of an anthracycline-induced cardiac toxicity was suspected for three patients (4.5%), who presented overt clinical symptoms. Two of them had no associated risk factors and were, thus, considered as true cases of anthracycline cardiac toxicity. The latter patient had other associated pathologies and was

classified as a mixed case. Two out of the three patients with serial BNP monitoring showed a continuous increase in BNP concentrations.

Definition of a threshold of BNP for heart failure development in anthracycline-treated patients by ROC curve analysis. A ROC curve was calculated by adding patients from the pilot and test groups divided into two groups, with or without anthracycline-induced heart failure and known BNP values. Twelve samples from five patients coincided with clinical heart failure and two hundred and fifteen samples corresponded to patients without heart failure or before the diagnosis was ascertained. Patients with heart failure due to causes other than anthracycline cardiac toxicity were not included in the ROC curve calculation. The area under the ROC curve was 0.90 (95% CI: 0.854-0.936). At 51.3 ng/l of BNP, the sensitivity was 83.3% (95% CI: 51.6-97.4%) and the specificity was 90.2% (95% CI: 85.5-93.8%), (positive predictive value: 32.3%, negative predictive value: 99.0%). At a concentration of 97.2 ng/l, the sensitivity of BNP was 66.7% (95% CI: 34.9-89.9%) and the specificity was 99.5% (95% CI: 97.4-99.9%), with a positive predictive value of 88.9% and a negative predictive value of 98.2%. A hundred percent of positive predictive value was reached for BNP concentrations over 247.4 ng/l. From the ROC curve results, we propose to use a threshold of 100 ng/ml as a first alarm for the potential development of anthracycline cardiac toxicity.

Discussion

Cardiac markers might provide a useful means of monitoring drug-induced toxicity in cancer patients. In recent studies, the troponin isoform cTnI was found to be a good predictor of anthracycline-induced cardiotoxicity (19, 20). Plasma BNP might also be a good candidate for this aim once the short-term variations of BNP among patients treated by cardiotoxic chemotherapy have been described.

Overall, bivariate and multivariate analyses with the pilot and test groups showed that the increase of BNP was positively correlated to the anthracycline cumulative dose. In our experience, serial BNP assays showed frequent slight BNP elevations (<100 ng/l), beyond a cumulative anthracycline dose of 220 mg/m^2. Slight BNP elevations require careful interpretation, taking into account the variations linked to sex and age (16), and other elevations associated with many pathologies frequently encountered in breast cancer patients, such as renal insufficiency, pulmonary hypertension, arterial hypertension or cardiac damage secondary to liver cirrhosis.

The majority of BNP elevations in our series was reversible, particularly in patients not previously treated by anthracyclines. These transitory elevations can probably be ascribed to the acute or sub-acute reversible anthracycline cardiotoxicity. A BNP elevation after the first chemotherapy course in idarubicin-treated patients was reported by Nousiainen *et al.* (3) and Suzuki *et al.* (7). The latter work included patients previously treated by anthracyclines and reported 100% of BNP levels over cut-off before and after treatment. Surprisingly, we found that the importance of BNP elevation during the first chemotherapy cycle was statistically unrelated to the anthracycline circulating concentrations or to the AUC values. This suggests that anthracycline cardiotoxicity might be dependent on its tissue concentration rather than on circulating peak values. Short-term BNP elevations might parallel the frequently observed electrophysiological abnormalities or arrythmias, considered as an outward sign of anthracycline acute toxicity, however non-predictive of subsequent chronic cardiomyopathy.

LVEF values were found to be inversely correlated to anthracycline doses, resulting in an inverse correlation between plasma BNP and LVEF values in our patient groups. This inverse relationship was not demonstrated by previous studies, although Nousiainen *et al.* reported such a tendency over an anthracycline dose of 400 mg/m^2 (8). In all cases, BNP elevations preceded LVEF drop (the earlier one after the 4th cycle), which was generally observed after the end of chemotherapy, highlighting the functional toxicity due to anthracycline cumulative treatment.

The ROC curve analysis showed that a BNP concentration <100 ng/l has a very important negative predictive value for anthracycline-induced cardiac toxicity. For all the cases of our series, the anthracycline-induced cardiac toxicity was evidenced at the end of chemotherapy and was characterised by a continuous rise in BNP concentration, which was over 100 ng/l in all the serial samples preceding the LVEF drop.

Understandably, the present work has some limitations due to the small number of patients in the pilot study and the limited follow-up time. However, since changes in BNP levels were consistent and results from the test group in concordance with those from the pilot study, it is unlikely that a greater number of patients would have dramatically modified the results.

In view of future clinical applications, more experience with BNP measurements in patients treated by cardiotoxic drugs is necessary. For further studies, we propose to consider a pattern of BNP variations including the proposed threshold of 100 ng/l and a continuous BNP increase as predictive of heart failure.

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