Human heart-type fatty acid-binding protein as an early diagnostic marker of doxorubicin cardiac toxicity

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Abstract

Progressive cardiotoxicity following treatment with doxorubicin-based chemotherapy in patients with non-Hodgkin's lymphoma (NHL) may lead to late onset cardiomyopathy. So, early prediction of toxicity can lead to prevention of heart failure in these patients. The aim of this work was to investigate the role of H-FABP as an early diagnostic marker of anthracycline-induced cardiac toxicity together with brain natriuretic peptide (BNP) as an indication of ventricular dysfunction in such patients. Our study was conducted on 40 NHL patients who received 6 cycles of a doxorubicin containing chemotherapy protocol (CHOP), not exceeding the total allowed dose of doxorubicin (500 mg/m^2). Ten healthy controls were included in our study. Human heart-type fatty acid-binding protein (H-FABP) was assessed 24 hours after the first cycle of CHOP. Plasma levels of BNP were estimated both before starting chemotherapy and after the last cycle of CHOP. Resting echocardiography was also performed before and at the end of chemotherapy cycles. The ejection fraction (EF) of 8 of our patients decreased below 50% at the end of the sixth cycle. Elevated levels of both H-FABP and BNP were found in all patients wth EF below 50% and both markers showed a positive correlation with each other. We concluded that H-FABP may serve as a reliable early marker for prediction of cardiomyopathy induced by doxorubicin. Thus, in patients with elevated H-FABP, alternative treatment modalities with no cardiac toxicity may be considered in order to prevent subsequent heart failure in these patients.

Introduction

Anthracyclines are highly efficacious antineoplastic agents but their utility is limited by progressive cardiotoxicity. They include doxorubicin, daunorubicin, epirubicin and idarubicin.[1] Anthracyclines cause a dose-dependent cardiomyopathy that often leads to congestive heart failure. Late onset cardiomyopathy can appear months to years after treatment is completed.[2] The mechanism underlying cardiotoxic effects of anthracyclines is generally accepted to be via formation of free radicals generated by iron-doxorubicin complexes that damage cardiac cellular membranes.[3] Cardiac damage caused by anthracyclines is cumulative. With total doses of doxorubicin less than 500 mg/m^2, heart failure is seen in less than 7% of cases.[4]

Human heart fatty acid-binding protein (H-FABP) is a small protein abundant in the cytosol, which is readily released into the circulation following myocardial damage. Recent studies in laboratories and the emergency department have shown that heart-type fatty acid-binding protein (H-FABP), a more recently developed cardiac biomarker, is able to detect myocardial damage as soon as one hour after onset of ischemia and, therefore, is regarded the earliest plasma marker available.[5,6] A bedside test for H-FABP, providing results within 15 min,[7] could potentially reduce diagnostic uncertainty for patients suspected of ACS in primary care. Recently, Setsuta and colleagues reported that elevated levels of H-FABP were associated with subsequent cardiac events in patients with chronic heart failure due to a variety of causes.[8] On the other hand, brain natriuretic peptide (BNP) is an amino acid peptide chiefly secreted by the ventricular myocardium in response to strain. Thus, it may be viewed as a marker of myocardial load and the plasma measurement of BNP is being used increasingly in the diagnosis, prognosis and monitoring of patients with congestive heart failure.[9,10] Since heart failure is a complex clinical syndrome, a single biochemical marker may not reflect all of its characteristics. Thus, the serial and combined measurements of markers of myocyte injury may open new perspectives in heart failure. [8] The aim of our work was to investigate the value of H-FABP as an early diagnostic marker of anthracycline-induced cardiotoxicity together with BNP as an indicator of ventricular dysfunction in non-Hodgkin's lymphoma patients receiving doxorubicin-based chemotherapy.

Subjects and Methods

Our study was conducted on 40 patients with non-Hodgkin's lymphoma who received 6 cycles of an anthracycline containing regimen (CHOP) with the following doses:[11]

Cyclophosphamide:	750 mg/m^2	IV	Day 1
Doxorubicin:	50 mg/m^2	IV	Day 1
Vincristine:	1.4 mg/m^2	IV	Day 1
Prednisone:	100 mg/d	PO	Days 1-5

This cycle was repeated every 21 days.

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Patients' age ranged from 14 to 56 years with a mean of 36.2±10.02 years. Twenty-two of them were males, while 18 were females. Ten age and sex-matched healthy controls with no history of cardiac problems were included in our study.

None of our patients had symptoms or signs of cardiac disease at presentation, nor had they undergone previous cardiac surgery. Patients with severe hepatic or renal disease or other medical conditions in which combination chemotherapy may be contraindicated were excluded from our study. Also, patients with early stage disease (stage I and non-bulky stage II) who are candidates for involved-field irradiation in addition to chemotherapy were excluded.

At presentation, full history and clinical examination, complete blood picture, bone marrow examination and lymph node biopsy were carried out in all patients. Routine laboratory investigations, ECG and echocardiography were also performed.

Blood samples were obtained for estimation of BNP before starting the first cycle of chemotherapy and after completion of the sixth cycle. Blood was withdrawn for assessment of H-FABP within 24 hours of administration of doxorubicin in the first cycle of CHOP[12] H-FABP was measured utilizing human cardiac fatty acid-binding protein ELISA test kit, Oxis research catalog number: 11230, Oxis International Inc., Foster City, CA, USA.[13]Re-evaluation of ECHO findings regarding the left ventricular ejection fraction and parameters of diastolic function[14] was carried out after the end of the sixth cycle of chemo-therapy.

Results

Patients' data are summarized in Table 1. The mean age of our patients at presentation was 36.2±10.02 years. Fifty-five percent were males, while 45% were females. All patients had normal ECG findings with no clinical evi-

dence of heart failure prior to chemotherapy.

After completing the first cycle of CHOP, plasma levels of H-FABP were found to be elevated in 10 patients. After completion of the sixth cycle of chemotherapy, 8 of the 10 patients with elevated H-FABP showed a significant reduction in the left ventricular ejection fraction to levels below 50% and the reduction in EF showed a significant correlation with H-FABP levels (Figure 1). Six of them also showed evidence of diastolic dysfunction in the form of impaired relaxation and an E/A ratio below 1. Two patients of those with post-chemotherapy ejection fraction values less than 50% had clinical manifestations of heart failure. Fifteen of our patients (37.5%) showed evidence of diastolic dysfunction after 6 cycles of chemotherapy but 3 of them already had E/A ratios below 1 when evaluated before starting chemotherapy. We divided our patients into two groups according to echocardiographic evidence of left ventricular systolic dysfunction after the sixth cycle of chemotherapy (Table 2): Group I; patients who had left ventricular ejection fraction levels below 50%; Group II; patients who had no echocardiographic evidence of heart failure.

On comparing the mean plasma BNP values in both groups, we found that prior to chemotherapy, they did not show a significant difference (mean value group I 70.28±9.45, group II 75.62±11.84). After six cycles of chemotherapy, both groups differed significantly as regards plasma BNP, with the mean value in group I being 70.4±9.527 and in group II being 260±18.51 (p<0.001). There was also a significant elevation of BNP values in group II patients after chemotherapy and this elevation correlated significantly with the reduced ejection fraction in these patients (Figure 2). There were no patients in whom clinical cardiac failure occurred without an associated rise in the BNP level above the threshold value. From the 10 patients who exhibited elevated H-FABP levels after the first chemotherapy cycle, 8 also showed high BNP levels at the end of the sixth cycle and both biochemical markers correlated positively with each other (Figure 3).

Table 1. Biochemical markers and ECHO findings in our patients.

	N.	Minimum	Maximum	Mean
Age	40	14	56	36.2± 10.02
BNP before chemotherapy (pg/mL)	40	50	89	71.35±10.04
BNP after chemotherapy (pg/mL)	40	50	280	108.32±77.66
H-FABP (ng/mL)	40	8	30	17.25±6.44
EF before chemotherapy (%)	40	58	72	65.57±4.04
EF after chemotherapy (%)	40	40	70	61.4±9.79

Table 2. Comparison between group I and II patients as regards BNP and H-FABP.

	N.	Mean	T	p
Age GI	32	38.84±10.02	-1.79	0.081
GII	8	41.75±8.44		
BNP before chemotherapy (pg/mL)				
GI	32	70.28±9.45	-1.185	0.265
GII	8	75.62±11.8		
BNP after chemotherapy (pg/mL)				
GI	32	70.4±9.52	-28.048	0.000*
GII	8	260±18.5		
H-FABP (ng/mL)				
GI	32	13.84±2.68	-13.103	0.000*
GII	8	27.15±2.53		
EF before chemotherapy (%)				
GI	32	65.84±4.14	0.903	0.384
GII	8	64.5±3.66		
EF after chemotherapy (%)				
GI	32	65.65±5.02	15.47	0.000*
GII	8	44.37±2.97		

** p Significant . GI: Patients with post chemotherapy EF above 50% . GII: Patients with post- chemotherapy EF below 50%.*

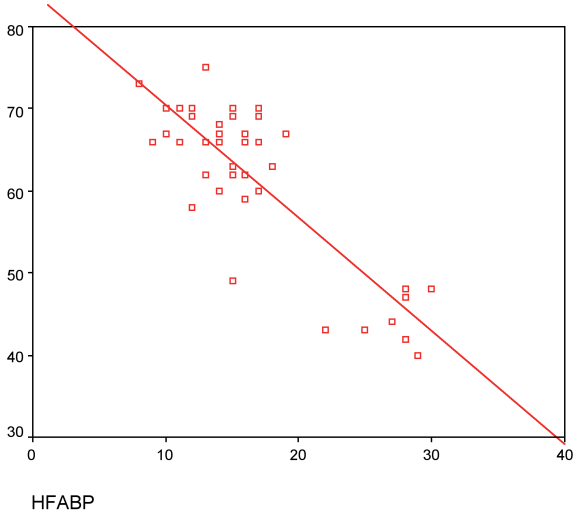

Figure 1. Correlation between ejection fraction after 6 cycles of CHOP and HFABP.

Discussion

Cardiotoxicity leading to congestive heart failure is a well-known complication of anthracyclines. Biochemical methods to assess and monitor cardiac function after anthracycline administration, if informative would be of utmost value.[15] We examined the diagnostic role of human heart-type fatty acid binding protein and brain natriuretic peptide to predict the impairment of left ventricular function in NHL patients treated by CHOP. We also studied the correlations between the plasma concentrations of these biomarkers and the functional alterations associated with doxorubicin-induced myocardial damage. A significant correlation between the left ventricular ejection fraction after 6 cycles of CHOP and plasma levels of both HFABP and BNP was found in our patients. Pichon and colleagues stated that an infra-clinical cardiotoxicity of anthracyclines as defined by BNP elevation is frequent but reversible and that patients who developed heart failure showed a continuous BNP increase and concentrations over 100 ng/mL.[16] On the other hand, Daugaard and colleagues concluded that in spite of correlations between peptide concentrations and reduced ejection fraction values, neither baseline levels nor serial measurements can safely substitute EF monitoring in patients undergoing anthracycline therapy.[10] Brain natriuretic peptide (BNP) was originally discovered in the porcine brain but was subsequently found to be predominantly a cardiac hormone.[17] Unlike atrial

natriuretic peptide (ANP), which is secreted by the atria in response to increased atrial pressure, BNP is derived chiefly from the cardiac ventricles in response to ventricular stresses.[18] The plasma levels of both peptides are inversely correlated with measures of cardiac function and recent studies have shown BNP to be a more sensitive marker of cardiac impairment than ANP.[19, 20] Raised plasma BNP levels have previously been shown to herald the clinical picture of cardiac failure by days to weeks.[21] Various guidelines based on changes in systolic and diastolic left ventricular function determined either by ECHO or by radionuclideventriculography (RVG) have been proposed for monitoring patients receiving anthracycline therapy. The advantages of ECHO over RVG are better availability, lower costs and lack of exposure to ionizing radiation.[16] Although H-FABP is viewed as a marker of myocyte injury and BNP is considered indicative of ventricular strain, the finding that both markers correlated with each other in our patients shows that some degree of myocyte injury is associated with increased ventricular load. It is well-known that myocardial structure is also altered in congestive heart failure (CHF). Non-contiguous areas of myocardial cell death and foci of replacement fibrosis are typical morphological changes in advanced CHF. Therefore, cytosolic proteins may be released into the circulation through leakage due to increased permeability of the membranes of injured myocytes.[22] Thus, the present study highlights the practical importance of measuring some biochemical markers such as plasma H-FABP and BNP to monitor left ventricular dysfunction in patients receiving anthracycline therapy as an early indication of subclinical cardiotoxicity.

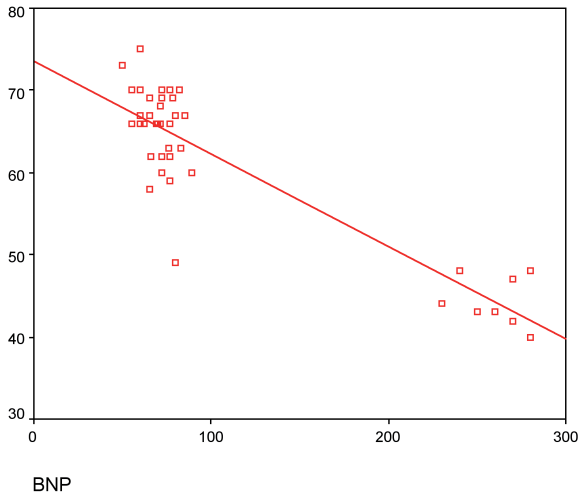

Figure 2. Correlation between ejection fraction after 6 cycles of CHOP and BNP levels.

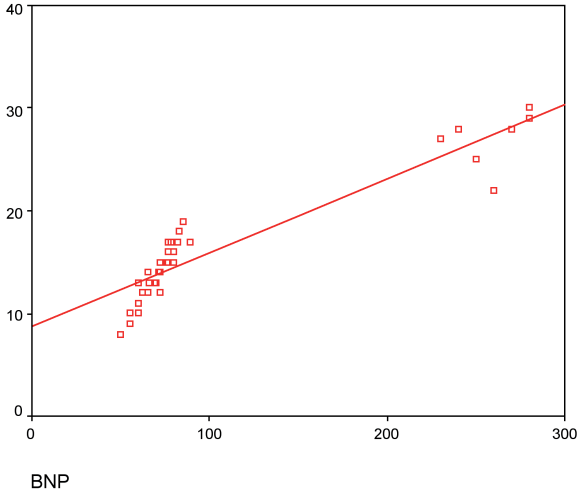

Figure 3. Correlation between HFABP& BNP in our patients.

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