ASSESSMENT OF ANTHRACYLINE-INDUCED CARDIOTOXICITY WITH BIOCHEMICAL MARKERS

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Aim: Assessment of acute and chronic cardiotoxicity of anthracyclines in patients treated for acute leukemia (AL) with biochemical markers — “N-terminal pro brain natriuretic peptide” (NT-proBNP), cardiac troponin T (cTnT), creatine kinase MB (CK-MB mass), and echocardiography. Methods: Twenty-six adult AL patients (mean age 46.2 ± 12.4 years, 15 males) treated with 2—6 cycles of chemotherapy (CT) containing anthracyclines in the total cumulative dose of 464.3 ± 117.5 ng/m² were studied. Cardiac evaluation was performed at baseline, after first and last CT with anthracyclines and 6 months after CT. Results: Mean baseline NT-proBNP concentration was 117.7 ± 46.4 ng/L (slightly elevated in 3 patients). After first and last CT, NT-proBNP elevations to 299.7 ± 176.2 ng/L and 287.1 ± 147.4 ng/L were observed, respectively. Six months after CT, mean NT-proBNP concentration was 362.5 ± 304.9 ng/L (elevated in 16 patients). Changes in NT-proBNP were significant in comparison with the baseline values (p < 0.001). Six months after CT, two patients with marked NT-proBNP elevations during CT developed treatment-related cardiomyopathy with symptoms of heart failure. NT-proBNP correlated with systolic and diastolic LV dysfunction on echocardiography (r = 0.514; p < 0.01) and (r = 0.587; p < 0.01). CTnT concentrations were negative (bellow 0.01 µg/L) during CT in all patients. Six months after CT, delayed cTnT positivity occurred in 3 patients. CK-MB mass remained within the reference range in all patients. Conclusion: Our study shows that anthracycline treatment is associated with acute and chronic neurohumoral activation of cardiac dysfunction that is manifested by a significant increase in NT-proBNP. It seems that NT-proBNP could be useful in the early detection of anthracycline cardiotoxicity. CTnT negativity during anthracycline treatment suggests that anthracyclines, even in higher cumulative doses, do not cause detectable acute injury to cardiomyocyte structure. Further studies using more sensitive markers of cardiac damage will be needed in this context.

Key Words: cardiotoxicity, anthracyclines, biochemical markers, NT-proBNP, cTnT.
structural myocardial injury in patients treated for cancer. At present, biochemical markers have been evaluated inadequately in this context.

The aim of our study was to monitor acute and chronic cardiotoxicity of ANT in patients treated for acute leukemia (AL) with biochemical markers of cardiac injury (NT-proBNP, cTnT, CK-MB mass) and to compare the changes in biochemical markers with findings on ECHO.

METHODS

Twenty-six consecutive patients treated for newly diagnosed AL were studied. The study group consisted of 15 males and 11 females with the mean age of 46.2 ± 12.4 years (range: 22–61, median 49). Risk factors for cardiovascular disease at the baseline were as follows: arterial hypertension in 7 patients, type 2 diabetes mellitus (on diet) in 4 patients, hyperlipidemia in 5 patients, obesity in 7 patients and cigarette smoking in 8 patients. Familial susceptibility to cardiac disease was not found in any patient. All patients had normal renal and liver functions during the study. No patient was previously treated with chemotherapy (CT) or radiotherapy. The patients were given 2–6 cycles of conventional CT containing ANT agent in the total cumulative dose of 464.3 ± 117.5 mg/m² (range: 240–715, median 426); to calculate the total cumulative dose of anthracyclines, we applied conversion factors derived from the maximum recommended cumulative doses for individual agents used (idarubicin, daunorubicin, mitoxantrone). The ANT doses for a cycle of CT were: idarubicin (Zavedos) 3 x 10–12 mg/m², daunorubicin (Daunoblastina) 2–3 x 50 mg/m², mitoxantrone (Novantrone, Refador) 2–3 x 10 mg/m² in combination with cytarabine (Cytosar). Cardioprotection with dexrazoxane was not used. Afterwards, sixteen patients underwent preparative regimen and hematopoietic cell transplantation. The study was carried out with the ethics committee approval. All patients gave a written consent before they were included in the study.

Cardiac evaluation was performed at the baseline (before CT), the day after first CT (mean cumulative ANT dose 136.3 ± 28.3 mg/m², median 150), the day after last CT (mean cumulative ANT dose 464.3 ± 117.5 mg/m², median 426) and circa 6 months after completion of CT (range: 5–8 months, median 6)

Venous blood samples for assessment of biochemical markers were obtained from an indwelling catheter after 30 min of rest in supine position. The blood samples were withdrawn into chilled tubes containing EDTA. The whole blood was centrifuged, plasma was decanted, immediately frozen and stored at −27 °C until assayed (within 6 months after sampling). Plasma concentrations of biochemical markers were measured by electrochemiluminescence immunoassay on Elecsys 1010 analyzer according to the manufacturer’s guidelines (Roche Diagnostics, Minneapolis, USA). Concentrations of biochemical markers diagnostic for cardiotoxicity of oncology treatment have not been established yet. In our study, values above the reference range based on a number of studies and recommended by the manufacturer were considered elevated and suggesting cardiac injury associated with the treatment. The cut-off values for individual markers were as follows: for CK-MB mass 4.94 µg/L, for cTnT 0.01 µg/L (detection limit of the third generation assay), for NT-proBNP 100 ng/L (male gender) and 150 ng/L (female gender). In the study of Hess et al., 1980 healthy blood donors were tested for NT-proBNP and the results confirmed that the upper limit of normal (100 ng/L for males, 150 ng/L for females) is proper and can be used in clinical studies [14]. NT-proBNP concentrations above 500 ng/L irrespective of gender were considered markedly elevated.

The ECHO evaluation was performed on Hewlett Packard Image Point ultrasound by an experienced echocardiographer. Parameters of systolic and diastolic left ventricular (LV) function and presence of pericardial effusion were assessed. Systolic LV dysfunction was defined as ejection fraction (LVEF) less than or equal to 50%. Diastolic LV dysfunction was defined as E/A inversion and E-wave deceleration time above 220 ms on the transmitral Doppler curve (impaired relaxation). Pericardial effusion was defined as separation of pericardial leaves at least 2 mm in systole.

Statistical analysis was performed with the “Statistica for Windows, Version 5.0” program. Multivariate analysis of variance and McNemar tests were used. Correlations were evaluated with normal and Spearman correlation tests. The values are expressed as mean ± SD. Probability values (p) < 0.001 and < 0.01 were considered statistically significant.

RESULTS

Mean baseline plasma NT-proBNP concentration was 117.7 ± 46.4 ng/L. After first CT, NT-proBNP elevation to 299.7 ± 176.2 ng/L was observed. After last CT, NT-proBNP concentration was 287.1 ± 147.4 ng/L. Six months after CT, the mean plasma NT-proBNP concentration was 362.5 ± 304.9 ng/L. Changes in NT-proBNP concentrations were significant in comparison with the baseline values (p < 0.001) (Figure).

Figure. Plasma NT-proBNP concentrations during treatment and follow-up of AL (*p < 0.001 versus before CT). Notes: before CT — before chemotherapy (baseline); after first CT — after first chemotherapy with anthracyclines (cumulative dose 136.3 ± 28.3 mg/m²); after last CT — after last chemotherapy with anthracyclines (cumulative dose 464.3 ± 117.5 mg/m²); 6 Mo after CT — after 6 months after completion of chemotherapy; [n = 26; risk factors for cardiovascular disease at the baseline: ischemic heart disease 0, arterial hypertension 7, diabetes mellitus (type 2, on diet) 4, hyperlipidemia 5, obesity 7, cigarette smoking 8, familial susceptibility to cardiac disease 0 patients].
Table 1 shows the number of patients with elevated NT-proBNP values.

<table>
<thead>
<tr>
<th>Elevated NT-proBNP</th>
<th>Before CT</th>
<th>After first CT</th>
<th>After last CT</th>
<th>6 Mo after CT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Above 100/500 ng/L</td>
<td>3 (11.5%)</td>
<td>23 (88.5%)</td>
<td>23 (88.5%)</td>
<td>16 (61.5%)</td>
</tr>
<tr>
<td>Above 500 ng/L</td>
<td>0</td>
<td>5 (19.2%)</td>
<td>4 (15.4%)</td>
<td>4 (15.4%)</td>
</tr>
</tbody>
</table>

Note: see Figure.

Six months after CT, NT-proBNP concentrations correlated with systolic and diastolic LV dysfunction on ECHO — \( r = 0.514; p < 0.01 \) and \( r = 0.587; p < 0.01 \), respectively. Correlations between NT-proBNP concentrations and gender, age, previous history of arterial hypertension or other risk factors for cardiovascular disease, hemoglobin level, fever, the total cumulative dose of ANT were not statistically significant.

ANT had LVEF equal to 55% and were asymptomatic. In 1 patient, diastolic LV dysfunction with symptoms of heart failure was observed. Three patients with ANT positivity after treatment had impaired systolic or diastolic LV function on ECHO and NT-proBNP above 500 ng/L. CK-MB mass concentrations remained within the reference range (below 4.94 µg/L) in all patients. Changes in CK-MB mass concentrations were not significant in comparison with the baseline values.

Changes in evaluated ECHO parameters during the treatment and the follow-up are shown in Table 2. Six months after CT, 12 (46.2%) patients had ECHO signs of diastolic dysfunction and 2 (7.7%) patients developed systolic LV dysfunction with symptoms of heart failure (ANT-related cardiomyopathy). Other 3 patients had LVEF equal to 55% and were asymptomatic. In the whole cohort, LVEF decreased from 65.3±4.5% (before CT) to 60.2±5.7% (6 Mo after CT), which was statistically significant \( p < 0.01 \).

Table 2. Abnormal ECHO findings during treatment and follow-up of AL (n = 26)

<table>
<thead>
<tr>
<th>Abnormal ECHO findings</th>
<th>Before CT</th>
<th>After first CT</th>
<th>After last CT</th>
<th>6 Mo after CT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic LV dysfunction</td>
<td>0</td>
<td>1 (3.8%)</td>
<td>1 (3.8%)</td>
<td>2 (7.7%)</td>
</tr>
<tr>
<td>Diastolic LV dysfunction</td>
<td>1 (3.8%)</td>
<td>5 (19.2%)</td>
<td>6 (23.1%)</td>
<td>12 (46.2%)</td>
</tr>
<tr>
<td>Pericardial effusion</td>
<td>2 (7.7%)</td>
<td>9 (34.6%)</td>
<td>9 (34.6%)</td>
<td>5 (19.2%)</td>
</tr>
</tbody>
</table>

Notes: see Figure. Systolic LV dysfunction — EF less than or equal to 50%; diastolic LV dysfunction — E/A inversion, E-wave deceleration time above 220 ms (impaired relaxation); pericardial effusion — separation of pericardial leaves at least 2 mm in systole.

Correlations between development of abnormal ECHO findings (systolic and diastolic LV dysfunction, pericardial effusion) and the total cumulative dose of ANT, gender, age, risk factors for cardiovascular disease did not reach statistical significance.

**DISCUSSION**

ANT-based treatment is a well established therapeutic approach for several malignancies, but its clinical efficacy is often limited by associated cardiotoxicity leading to cardiomyopathy that may worsen the patient’s outcome. The most frequently adopted method for detection of cardiotoxicity is evaluation of LVEF by ECHO or radionuclide ventriculography [3, 4]. However, these techniques are only partially reliable and available and have low sensitivity for detection of early cardiac dysfunction that could be reversible with appropriate therapy [15]. Biochemical markers of cardiac injury, especially cardiac troponins and natriuretic peptides, have been recently studied in this context.

The applicability of natriuretic peptides (ANP, BNP, NT-proBNP) as markers for ANT-induced cardiotoxicity has been investigated in a few recent studies. The results suggest that natriuretic peptides could be of value in the detection of clinical and subclinical cardiotoxicity of ANT [16–21] as well as markers of cardiac toxicity after high-dose CT and hematopoietic stem cell transplantation [15, 22–24]. However, definitive clinical evaluation has been missing and natriuretic peptides have not been routinely used for monitoring of cardiotoxicity in clinical practice.

In our study, the baseline NT-proBNP values were slightly elevated in 3 (11.5%) patients. Only in 1 patient, diastolic LV dysfunction on ECHO was found. The other 2 patients had no clinical or ECHO signs of LV dysfunction, which shows that a relatively strict cut-off for NT-proBNP was used. However, these values have been suggested as the upper limit of the reference interval [10, 11, 14]. After first and last CT with ANT, we observed elevated NT-proBNP in 23 (88.5%) patients, i. e. in 20 (76.9%) patients NT-proBNP values increased above the cut-off. Administration of CT with ANT causes acute myocardial strain of LV and enhanced release of NT-proBNP irrespective of the reached cumulative dose of ANT. These NT-proBNP elevations could be considered a sign of acute subclinical cardiotoxicity of ANT. Six months after completion of CT, neurohumoral activation in sense of NT-proBNP elevation persisted in 16 (61.5%) patients, i. e. NT-proBNP values increased above the cut-off in 13 (50.0%) patients. NT-proBNP concentrations correlated with systolic and diastolic dysfunction on ECHO. Six months after treatment, 2 (7.7%) patients with NT-proBNP above 500 ng/L had ECHO signs of LV dysfunction and clinical symptoms of heart failure — these findings represent chronic clinical cardiotoxicity of ANT manifested as cardiomyopathy. The patients were treated with ACE inhibitors and diuretics with a good effect. These 2 patients with ANT-related cardiomyopathy had NT-proBNP values markedly elevated (above 500 ng/L) already after first and last CT with ANT. It seems that NT-proBNP could help in identification of patients at risk for development of ANT-related cardiomyopathy. In asymptomatic patients, persistent NT-proBNP elevations signify chronic subclinical cardiotoxicity, which requires a careful follow-up in the future.

Cardiospecific markers, especially cardiac troponins, have been studied in the detection of ANT-induced cardiotoxicity. Testing for cTnT and cTnI is equivalent for clinical use. However, only assessment of cTnT is standardized at present [25]. Experimental studies showed significant cTnT elevations that could serve as predictors for ANT-induced cardiomyopathy [26–28]. Clinical studies are limited and the results are ambiguous. In some studies, administration of ANT did not cause any elevation in cardiac troponins [29–31]. In other studies, cardiac tro-
ponins became positive after ANT treatment, correlated with the disease severity and might predict subsequent major cardiac events during the follow-up [32–34]. The results of clinical studies are inconsistent and cardiac troponins have not been established in clinical practice for monitoring of cardiotoxicity in oncology.

In our study, no patient had a detectable cTnT concentration early after ANT administration, even in higher cumulative doses. CT with ANT did not lead to detectable acute injury to cardiomyocyte structure. In this respect, it is unlikely that early assessment of cTnT during ANT treatment would be useful for screening for ANT-induced cardiotoxicity. On the other hand, cTnT positivity occurred 6 months after completion of treatment in 3 (11.5%) patients. These delayed elevations in cTnT were associated with cardiac dysfunction on ECHO (cardiomyopathy was diagnosed in 2 patients) and indicate chronic cardiotoxicity of ANT. According to our results, negative cTnT concentrations during ANT treatment do not identify patients with a low risk for development of cardiomyopathy in the future.

In our study, we did not find any elevation in CK-MB mass during ANT treatment and the follow-up, which was expected from previously published clinical studies [29, 35].

ECHO is the most frequently used non-invasive method for evaluation of cardiac function including toxic effect of oncology treatment. In our study, the incidence of systolic and diastolic LV dysfunction on ECHO advanced with increasing cumulative dose of ANT and with time after completion of CT. Impairment of diastolic LV function related to ANT treatment was diagnosed prior to impairment of systolic LV function. This finding is in agreement with the previously published studies [36–38]. Six months after completion of treatment, clinical manifestation of cardiotoxicity in terms of ANT-induced cardiomyopathy with heart failure developed in 2 (7.7%) patients. Asymptomatic changes in ECHO parameters are considered subclinical cardiotoxicity and require regular cardiology check-ups.

Since we did not find significant correlations between abnormal cardiac findings after ANT treatment and the total cumulative dose of ANT and other risk factors for development of cardiotoxicity, we conclude that thorough cardiology follow-up is warranted in all oncology patients treated with these agents. Our findings regarding assessment of ANT-induced cardiotoxicity with biochemical markers need a further prospective follow-up and evaluation in further studies on a larger number of patients.

Our study suggests that serial measurements of NT-proBNP could be a promising means for the early detection of clinical and subclinical cardiotoxicity of ANT during the treatment and the follow-up. NT-proBNP assessment is not a stand-alone test; it must be evaluated in conjunction with clinical findings and other imaging and laboratory methods. It seems that implementation of NT-proBNP into routinely performed diagnostic techniques would enable to detect subclinical myocardial damage earlier and to identify patients at risk for development of cardiac dysfunction in the future. According to our results, assessment of cTnT does not have predictive value for development of cardiomyopathy after ANT treatment and therefore does not seem to be of value in the early detection of cardiotoxicity. CK-MB mass assessment is a method with low sensitivity for detection of ANT-induced cardiotoxicity in a clinical setting. Further studies using more sensitive markers of cardiac damage will be necessary in the assessment of treatment-related cardiotoxicity in oncology.

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REFERENCES


ИСПОЛЬЗОВАНИЕ БИОХИМИЧЕСКИХ МАРКЕРОВ ДЛЯ ОЦЕНКИ КАРДИОТОКСИЧНОСТИ, ВЫЗВАННОЙ АНТРАЦИКЛИНАМИ

Цель: оценка острой и хронической кардиотоксичности, вызванной антраклиноми, у больных острым лейкозом (ОЛ), с использованием биохимических маркеров — "N-концевой натриуретический пропептид мозга" (NT-proBNP), сердечный тропонин T (CTnT), креатининкиназа MB (CK-MB) и нейроэндокринные гормоны. Методы: обследовано 26 больных с ОЛ (средний возраст 46,2 ± 12,4 года, 15 мужчин), получавших по 2–6 циклов химиотерапии (ХТ), включавшей антраклиноми в общей кумулятивной дозе 464,3 ± 117,5 мг/м2. Состояние сердца оценивали исходно, после первого и последнего цикла ХТ и через 6 мес после ХТ. Результаты: средняя исходная концентрация NT-proBNP составляла 117,7 ± 46,4 пг/л (была несколько повышена у 3 больных). После первого и последнего цикла ХТ отмечали повышение NT-proBNP до 299,7 ± 176,3 пг/л и 287,1 ± 147,4 пг/л соответственно. Через 6 мес после ХТ средняя концентрация NT-proBNP составляла 362,5 ± 304,9 пг/л (была повышена у 16 больных). Изменения концентрации NT-proBNP по сравнению с исходными значениями статистически значимы (р < 0,001). Через 6 мес после ХТ у всех больных с выраженным подъемом уровня NT-proBNP в процессе ХТ развилась кардиомиопатия с симптомами сердечной недостаточности. Уровни NT-proBNP коррелировали с наличием систолической и диастолической дисфункции при эхокардиографическом обследовании (r = 0,514; р < 0,01) и (r = 0,587; р < 0,01). Концентрация СТП была низкой (ниже 0,01 мг/л) у всех пациентов в процессе ХТ, но через 6 мес после ХТ у трех больных была повышенная в 1,5–2 раза. Выводы: данные показывают, что лечение антраклиноми ассоциировано с острой и хронической нейрогуморальной активацией дисфункции сердца, которая проявляется в значительном повышении концентрации NT-proBNP. Показатель NT-proBNP может быть использован для раннего выявления кардиотоксичности антраклиноми. Отрицательный показатель СТП показывает, что антраклиноми даже в высоких кумулятивных дозах не вызывают острого повреждения структуры кардиомиоцитов, а связан с чем требуется дальнейший поиск более чувствительных маркеров.

Ключевые слова: кардиотоксичность, антраклиноми, биохимические маркеры, NT-proBNP, СТП.