BIOCHEMICAL MARKERS AND ASSESSMENT OF CARDIOTOXICITY DURING PREPARATIVE REGIMEN AND HEMATOPOIETIC CELL TRANSPLANTATION IN ACUTE LEUKEMIA

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INTRODUCTION: Cardiotoxicity is a relatively frequent and potentially serious complication of antitumor treatment. Anthracyclines and other high-dose chemotherapy represent the greatest risk. The aim of the study was to assess cardiotoxicity during preparative regimen (PR) and hematopoietic cell transplantation (HCT) in acute leukemia (AL) with biochemical markers — “N-terminal pro brain natriuretic peptide” (NT-proBNP), cardiac troponin T (cTnT) and creatine kinase MB (CK-MB mass). Methods: Nineteen adult AL patients previously treated with anthracyclines — idarubicine, daunorubicine, mitoxantrone with standard doses for a cycle as 3 х 12 mg/m², 3 х 50 mg/m², 3 х 10 mg/m² accordingly were studied. PR consisted of high-dose cyclophosphamide (HD-C) in combination with busulphan or total body irradiation (TBI). Plasma NT-proBNP, cTnT and CK-MB mass concentrations were measured the day before PR, the day after PR, the day after HCT and 14 days after HCT. Results: Before PR, mean plasma NT-proBNP value was 106.3 ± 55.7 ng/l. After PR, it increased to 426.1 ± 391.5 ng/l. After HCT, a further increase to 847.6 ± 780.6 ng/l was observed. Fourteen days after HCT, the mean NT-proBNP was 330.8±236.8 ng/l. The differences were statistically significant in comparison with the baseline values (p < 0.01). The NT-proBNP elevations were more pronounced in patients with cumulative doses (CD) of anthracyclines above 450 mg/m² (p < 0.05), in patients with PR containing HD-C and TBI (p < 0.05). In all patients, plasma cTnT and CK-MB mass concentrations remained unchangeable during PR and HCT. Conclusion: Our results suggest that administration of PR and HCT is in most AL patients associated with acute neurohumoral activation (significant rise in NT-proBNP). Persistent NT-proBNP elevations, in our study in 12 (63.2 %) patients, indicate subclinical cardiotoxicity (risk for development of heart failure) and require further follow-up. More pronounced NT-proBNP elevations in patients with higher CD of anthracyclines and in patients with PR containing combination of HD-C and TBI confirm that these therapeutic procedures seem to be more cardiotoxic and not very appropriate for patients with cumulation of risk factors for cardiotoxicity. Negative plasma cTnT and CK-MB mass concentrations show no detectable damage of cardiomyocyte structure during PR and HCT.

Key Words: cardiotoxicity, biochemical markers, transplantation, acute leukemia.

Cardiotoxicity is a well-known and potentially serious complication of antitumor treatment. The greatest risk for development of cardiotoxicity represent anthracyclines [1] and high-dose chemotherapy (HD-CT) especially regimens containing high-dose cyclophosphamide [2–5].

Myeloablative preparative regimen (PR) in acute leukemia (AL) contains high-dose cyclophosphamide in total dose of 120 mg/kg (HD-C), in some cases in combination with fractionated total body irradiation (TBI) 12 Gy. This is followed by hematopoietic cell transplantation (HCT). Moreover, these patients are pretreated with conventional chemotherapy (CT) containing anthracyclines in a relatively high cumulative dose (CD). All of these therapeutic procedures are potentially cardiotoxic and require thorough monitoring of cardiac functions during the treatment (acute cardiotoxicity) and after its completion (chronic and late cardiotoxicity).

Various methods have been recommended for monitoring of cardiotoxicity [6–8]. In our conditions, echocardiography and electrocardiography are routinely used. Recently, biochemical markers of cardiac damage, especially natriuretic peptides and cardiac troponins, are gaining ground in this field [9].

Natriuretic peptides — atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP) and N-terminal pro brain natriuretic peptide (NT-proBNP) — are produced by myocardium in response to wall strain and pressure overload [10]. ANP is produced mainly in atria, BNP/ NT-proBNP predominantly in ventricles. In cardiology, natriuretic peptides are routinely used in diagnostics of heart failure [11]. Normal plasma BNP/NT-proBNP concentrations practically exclude heart failure due to high negative predictive value of the test [12].

Cardiac troponins — cardiac troponin T (cTnT), cardiac troponin I (cTnI) — and myocardial isoenzyme of...
creatinine kinase (CK-MB) are cardiосpecific markers that show structural damage of cardiomyocytes from various causes, including cardiotoxic effect of CT [13].

Assessment of cardiotoxicity of HD-CT with biochemical markers of structural or functional myocardial damage was the aim of only a few studies from recent time. In some studies, cardiac troponins [14–16] and natriuretic peptides [17–19] were suggested as predictors of late cardiac dysfunction after HD-CT and HCT. In these studies, different types of HD-CT were used in various, not only hematological malignancies. According to available literature, monitoring and comparison of acute cardiotoxicity of PR used in AL was not the subject of any study so far.

The aim of the presented study was to assess cardiotoxicity during PR and HCT in AL with biochemical markers – NT-proBNP, cTnT, CK–MB mass — and echocardiography (ECHO). We compared cardiotoxicity of two myeloablative PR used in AL. We assessed the impact of prior anthracycline treatment on plasma concentrations of biochemical markers of cardiac damage in the peritransplant period.

METHODS
Patients. Nineteen consecutive adult patients with AL were studied. The patients consisted of 13 males and 6 females with the mean age of 42.8 ± 10.0 years (range: 22–56). Four of the patients were treated for arterial hypertension, the other patients had no known pre-existing cardiovascular disease. Renal and liver functions were normal in all patients. The patients were treated with conventional CT containing anthracyclines in total CD of 446.4 ± 91.6 mg/m² (range: 240–609). To calculate the total CD of anthracyclines, we applied conversion factors derived from the maximum recommended cumulative doses for individual agents used (idarubicine, daunorubicine and mitoxantrone). The standard doses for a cycle of chemotherapy were: idarubicine 3 × 12 mg/m², daunorubicine 3 × 50 mg/m², mitoxantrone 3 × 10 mg/m². In all patients, PR consisted of intravenous Cyclophosphamide in total dose of 120 mg/kg (HD-C), in 13 patients in combination with peroral Busulphan 16 mg/kg (Bu/HD-C) and in 6 patients in combination with fractionated total body irradiation 12 Gy (TBI/HD-C). In all cases, cryopreserved peripheral blood stem cells were used as the source for HCT. Ten patients were given allogeneic grafts and 9 autologous grafts. The study was approved by the local ethical committee. All patients gave a written consent before they were included in the study.

Biochemistry. Serial measurements of plasma NT-proBNP, cTnT and CK-MB mass concentrations were performed the day before PR, the day after PR, the day after HCT and 14 days after HCT, i. e. at the time of bone marrow recovery.

Venous blood samples 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 immediately centrifuged, the plasma was decanted, frozen and stored at −27 °C until assayed. Plasma concentrations of biochemical markers were measured by electrochemiluminescence immunoassay on Elecsys 1010 analyzer (Roche Diagnostics).

Based on a number of studies, NT-proBNP values below 100 ng/l for male, 150 ng/l for female are considered normal and allow to rule out heart failure due to high negative predictive value of the test [12, 20]. We used these cut-off values for cardiac dysfunction. Values above cut-off respecting gender (100 ng/l for male, 150 ng/l for female) were considered elevated, NT-proBNP concentrations above 500 ng/l were considered markedly elevated. Elevated NT-proBNP concentrations in association with the given treatment show functional myocardial damage and are a sign of toxic effect of the treatment on myocardium. CtnT concentrations above 0.01 µg/l and CK-MB mass concentrations above 4.94 µg/l were taken as elevated and showing structural damage of cardiomyocytes caused by the treatment.

Echocardiography. ECHO was performed before PR and in the early period after HCT (within 3 days after HCT). The ECHO evaluation was done with 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. In the study, systolic LV dysfunction was defined as ejection fraction (EF) below 55%. Diastolic LV dysfunction was defined as E/A inversion and deceleration time (DT) 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. Analysis of variance (ANOVA), paired two tailed t-tests and McNemar tests were used. Correlations were evaluated with normal and Spearman correlation tests. The values are expressed as mean ± SD. Probability values < 0.01 and < 0.05 were considered statistically significant.

RESULTS AND DISCUSSION
Significance of NT-proBNP for evaluation of cardiotoxicity. The day before PR, mean plasma NT-proBNP concentration was 106.3 ± 55.7 ng/l (slightly elevated in 4 patients). The mean NT-proBNP concentration increased to 426.1 ± 391.5 ng/l (elevated in 12 patients, markedly elevated in 5 patients) after completion of PR. After HCT, a further increase to 847.6 ± 780.6 ng/l (elevated in 14 patients, markedly elevated in 9 patients) was observed. At the time of bone marrow recovery (14 days after HCT), the mean NT-proBNP concentration was 330.8 ± 236.8 ng/l. Values remained elevated in 12 (63.2%) patients, markedly elevated in 5 (26.3%) patients. The differences were statistically significant in comparison with the baseline NT-proBNP value (p < 0.01, Fig. 1). The NT-proBNP elevations were more pronounced in patients with CD of anthracyclines above 450 mg/m² (p < 0.05), in patients with PR containing TBI and HD-C (p < 0.05, Fig. 2 and 3). The changes in NT-proBNP concentrations were not significantly different after infusion of allogeneic or autologous grafts. Associations between changes in NT-proBNP concentra-
tions and gender, age or history of arterial hypertension were not significant.

Fig. 1. Plasma NT-proBNP concentrations in the peritransplant period in AL patients. *p < 0.01 vs before PR.

Fig. 2. Plasma NT-proBNP concentrations in the peritransplant period according to CD of anthracyclines. (p < 0.05)

Fig. 3. Plasma NT-proBNP concentrations in the peritransplant period according to PR. (p < 0.05)

Explanatory notes to Fig. 1–3: before PR — the day before initiation of preparative regimen, after PR — the day after completion of preparative regimen, after HCT — the day after hematopoietic cell transplantation (graft infusion), day + 14 — 14 days after hematopoietic cell transplantation (bone marrow recovery).

Slightly elevated NT-proBNP concentrations before PR were in 4 (21.1%) patients. These elevations were likely caused by prior anthracycline-based CT. More pronounced NT-proBNP elevations in patients with higher CD of anthracyclines and in patients with PR containing combination of HD-CT and radiotherapy suggest that these therapeutic procedures are associated with higher myocardial strain, and thus seem to be more cardiotoxic. In our previously published study, we proved that solely intravenous hydration in AL patients does not lead to significant increase in NT-proBNP concentrations [21]. In the study of Snowden et al., administration of PR containing HD-C was associated with higher NT-proBNP elevation than other PR [17]. So far, there are no other studies comparing acute cardiotoxicity of different PR by means of natriuretic peptides.

Three studies from recent time have dealt with the assessment of cardiotoxicity of HD-CT and HCT [17–19]. In these studies, most patients had significant elevations in BNP/NT-proBNP after HD-CT and HCT. Persistent NT-proBNP elevations were observed in 33–47% patients. During follow-up, the patients with persistent BNP/NT-proBNP elevations had more often cardiac dysfunction than the other patients. The results show that monitoring of BNP/NT-proBNP could identify patients at risk for development of cardiac dysfunction after HD-CT and HCT.

In our patients, elevated NT-proBNP concentrations 14 days after HCT were found in a higher percentage of patients than in the study of Niwa et al — 63.2% versus 45.6% [18]. This difference could be explained by relatively high CD of anthracyclines in our patients — even before PR 21.1% patients had a slightly elevated NT-proBNP. The results can be influenced by the number of patients took that part in the study.

In the peritransplant period, one (5.3%) of the patients developed manifestation of cardiotoxicity — clinical signs of heart failure, mild systolic dysfunction (EF 52%), NT-proBNP concentrations 659.0 ng/l (after PR) and 2228.0 ng/l (after HCT). In this patient, baseline NT-proBNP was 319.9 ng/l, which was by far the highest value in the group. This patient had CD of anthracyclines above the maximum recommended CD (550 mg/m²), PR contained combination of TBI and HD-C and allogeneic HCT was performed.

Cardiospecific markers (cTnT and CK-MB mass). There were no differences in plasma level of cTnT and CK-MB mass during the PR and HCT, which earlier considered as showing no detectable damage of cardiomyocyte structure (Table 1). However, cTnT is probably not the most sensitive marker for cardiac damage caused by oncology treatment.

Table 1. Plasma concentrations of cardiospecific markers in the peritransplant period in AL patients

<table>
<thead>
<tr>
<th>Cardiospecific markers</th>
<th>Before PR</th>
<th>After PR</th>
<th>After HCT</th>
<th>Day +14</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>cTnT [µg/l]</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>NS</td>
</tr>
<tr>
<td>CK-MB mass [µg/l]</td>
<td>1.094 ± 1.218 ± 1.183 ± 1.117 ± 0.511 0.418 0.346 0.472</td>
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Measurement of cardiac troponins is a very sensitive method that is able to detect a minimal damage of cardiomyocytes after HD-CT [15, 22]. In the recently published study by Cardinale et al., cTnI positivity (≥ 0.08 µg/l) within 72 h after HD-CT was seen in nearly 30% of oncology patients. CtnI positivity was associated with a significantly higher decrease in LV EF and other cardiac events during 3-year follow-up [16].

Assessment of cTnT and cTnI is for clinical use equivalent. However, only assessment of cTnT is standardized at present [23]. According to the results of Cardinale et al., we would expect at least low positivity of cTnT in about 5 patients. Despite using a highly sensitive assay on cTnT with sensitivity of 0.01 µg/l, we did not find cTnT positivity in any of our patients after PR and HCT. This finding is in concordance with the study of Auner et al., in which administration of HD-C and TBI 12 Gy did not lead to elevation of cTnT in any of 30 hematonoology patients [24]. Either in the study of Benvento et al., administration of HD-C caused no elevation in cTnT [25]. At present, routine use of cardiac troponins for monitor-
ing of cardiotoxicity of antitumorous treatment cannot be established to clinical practice due to disunity of the available assays (lack of standardization) and inconsistency of results. The timing of sample collection and determination of cut-off value for treatment-related cardiotoxicity may play an important role.

Echocardiography. Changes in ECHO parameters during the peritransplant period are shown in Table 2. Correlations between plasma NT-proBNP concentrations and ECHO parameters did not reach statistical significance. In the early period after PR and HCT, we found a decrease in systolic LV function (LV EF 63.4 ± 1.9% vs 61.4 ± 3.5%), which was statistically significant (p < 0.05). In 1 (5.3%) of the patients, a decrease in LV EF function more than 10% with symptoms of heart failure developed. In the other patients, LV EF remained within normal range (above 55%). After PR and HCT, diastolic LV dysfunction newly appeared in 3 patients and small pericardial effusion in 2 patients. These changes in ECHO parameters are caused by combination of acute impact of PR and HCT and influence of the prior anthracycline treatment. Newly developed pericardial effusion after PR and HCT in 2 (10.5%) patients and presence of pericardial effusion altogether in 6 (31.6%) is lower than published in the literature. After PR containing HD-C, pericardial effusion has been reported in up to 90% of patients [26].

We are aware that our results are partially limited by the number of patients participating in the study and a relatively short period of the follow-up. These findings require a further prospective follow-up and confirming in further studies in a larger number of patients. Long-term cardiology follow-up is warranted in all oncology patients treated with anthracyclines and HD-CT followed by HCT.

Our results show that administration of PR and HCT is in most AL patients associated with acute neurohumoral activation (significant rise in NT-proBNP). In our study, NT-proBNP remained elevated in 12 (63.2%) patients at the time of bone marrow recovery. These persistent NT-proBNP elevations indicate subclinical cardiotoxicity (risk for development of heart failure) and require further follow-up. NT-proBNP elevations were significantly more pronounced in patients with higher CD of anthracyclines and in patients with PR containing combination of radiotherapy and HD-CT. Thus, these therapeutic procedures seem to be more cardiotoxic and not very appropriate for patients with cumulation of risk factors for cardiotoxicity. Predictive value of these changes is not clear and must be yet determined.

Administration of PR and HCT may lead to manifestations of cardiotoxicity — in our study in 1 (5.3%) of the patients. Development of acute heart failure in the patient with the highest baseline NT-proBNP concentration suggests that implementation of NT-proBNP to commonly performed pretransplant cardiac examinations could be useful in the identification of patients at high risk for development of cardiotoxicity in terms of heart failure and in the early diagnostics of cardiac dysfunction in the peritransplant period. Relatively high price of this assay and necessity of repeated measurements remains the limitation for implementation to routine clinical practice.

In our study, negative plasma cTnT and CK-MB mass concentration show no detectable damage of cardiomyocyte structure during PR and HCT. Thus, routine measurement of these cardioprotective markers in the peritransplant period in asymptomatic patients does not seem to be of value in the detection of cardiotoxicity. However, further studies using more sensitive markers of cardiac damage (such as ischemia modified albumin, fatty acid binding protein, glycerol phosphorylase BB) could bring a new view on this issue.

Cardiotoxicity of oncology treatment develops mainly in the combination of more potentially cardiotoxic procedures, as in case of AL treatment. Cardiotoxicity is a serious interdisciplinary problem that requires cooperation of an oncologist with a cardiologist.

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REFERENCES

Введение: кардиотоксичность — это относительно частые и потенциально опасные последствия противоопухолевой терапии. Наибольшую кардиотоксичность отмечают при применении высоких доз химиопрепаратов, в частности антибиотиков антрациклинового ряда. Целью данного исследования была оценка кардиотоксичности при лекарственной подготовке пациентов с острым лейкозом (ОЛ) и проведение им кардиотоксиологических стволовых клеток (ГСК), а также определение следующих биохимических параметров — Н-терминальный прон-бриннапин (NT-proBNP), сердечного тропонина (c TnT) и креатинкиназы MB (CK-MB).

Целью работы является оценка кардиотоксичности при проведении высоких доз химиопрепаратов у больных с острым лейкозом и проведенным гематопоэтическом стволовых клеток (ГСК) в сочетании с бусульфаном или радиолучевой терапией (РЛТ). Концентрацию NT-proBNP, c TnT и CK-MB определяли у пациентов до и после проведения предварительной терапии (ПЛ) и трансплантации ГСК. Результаты: уровень NT-proBNP перед проведением ПЛ составил 106,3 ± 55,7 нг/л, а после повышался до 426,1 ± 391,5 нг/л. Через 14 дней после трансплантации ГСК концентрация NT-proBNP достигла 330,8 ± 236,8 нг/л, при этом разница была статистически значимой (p < 0,05). Повышение уровня NT-proBNP в плазме крови за день до и через день после проведения ПЛ, а также за день до и через 14 дней после трансплантации ГСК. Следует отметить, что применение NT-proBNP как маркера кардиотоксичности является перспективным для дальнейших исследований и клинической практики. Биохимические маркеры, трансплантация, острый лейкоз.