Multimarker approach to evaluation of cardiac toxicity during preparative regimen and hematopoietic cell transplantation

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Cardiac toxicity of preparative regimen (PR) containing high-dose Cyclophosphamide (120 mg/kg) followed by hematopoietic cell transplantation (HCT) was evaluated with 6 biomarkers of cardiac injury: N-terminal pro brain natriuretic peptide (NT-proBNP), creatine kinase MB (CK-MB mass), cardiac troponins (cTnT, cTnI), heart-type fatty acid binding protein (H-FABP), glycogen phosphorylase BB (GPBB). Twenty-three patients (mean age 44.5±10.6 years, 15 males) with acute leukemia were studied. All biomarkers were measured the day before PR, the day after PR, the day after HCT and 14 days after HCT. We found NT-proBNP elevations above 500 ng/L in 6 (26.1 %) patients after PR, in 9 (39.1 %) after HCT and in 7 (30.4 %) 14 days after HCT. GPBB became elevated (above 7.30 μg/L) in 5 (21.7 %) patients after PR, remained elevated in 5 (21.7 %) after HCT and in 2 (8.7 %) 14 days after HCT. A significant correlation between elevation in NT-proBNP and GPBB was found. Other markers remained within the reference range early after PR and HCT. Our findings show that administration of PR and HCT for acute leukemia is associated with acute neurohumoral activation of cardiac dysfunction (significant rise in NT-proBNP) and may lead to GPBB elevation. These changes could indicate acute cardiac toxicity due to treatment and require further follow-up. The predictive value for development of cardiomyopathy in the future is unclear. Further studies will be needed to define the potential role of new biomarkers in this context.

Key words: cardiac toxicity, biomarkers, preparative regimen, transplantation, hematologic oncology

Cardiotoxicity is a well-known and potentially serious complication of cancer chemotherapy. The greatest risk for development of cardiotoxicity represent anthracyclines [1, 2] and high-dose (HD) chemotherapy especially regimens containing HD Cyclophosphamide [3–6]. Various methods have been recommended for monitoring of cardiotoxicity [7, 8]. In our conditions, echocardiography and electrocardiography are routinely used. Recently, biochemical markers of cardiac injury, especially cardiac troponins and natriuretic peptides, have been investigated in the assessment of cancer therapy-induced cardiotoxicity [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 and management of cardiac dysfunction and heart failure [11, 12]. Normal plasma BNP/NT-proBNP concentrations practically exclude heart failure due to high negative predictive value of the test [13, 14].

Cardiac troponins – cardiac troponin T (cTnT), cardiac troponin I (cTnI) – and myocardial isoenzyme of creatine kinase (CK-MB) are cardiospecific markers that show structural injury of cardiomyocytes from various causes, including cardiotoxic effect of chemotherapy [15, 16].

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Heart-type fatty acid binding protein (H-FABP) and glycogen phosphorylase isoenzyme BB (GPBB) are new perspective markers of myocardial ischemia and necrosis, recently evaluated in the diagnostics and risk stratification of acute coronary syndromes [17–20]. H-FABP is a relatively small cytoplasmic protein for the oxidation of fatty acids that is quite specific for cardiac muscle. H-FABP is rapidly released from the myocardium into the bloodstream after ischemic injury. Plasma H-FABP increases above the reference limit within 2 – 3 hours of the onset of myocardial injury and returns to normal within 18 – 30 hours. GPBB is a glycogenolytic enzyme providing glucose for heart muscle tissue. After glycogenolysis in ischemic tissue, GPBB is released from the sarcoplasmic reticulum into the cytoplasm and then into the circulation through the damaged cell membrane. GPBB is released into the circulation 2 – 4 hours after myocardial injury, returning to normal values within 24 – 36 hours of damage occurring. In the acute coronary syndrome setting, both markers are regarded early markers of cardiac injury due to acute myocardial ischemia. The main mechanism of cardiac injury caused by cancer chemotherapy is mainly non-ischemic and prior cyclic exposition to anthracycline agents may play a role (chronic and late cardiotoxicity). Therefore, it is difficult to estimate the kinetics of release of these biomarkers from cardiomyocytes in this setting. According to the available literature, data on using these new cardiac biomarkers in the assessment of cardiac toxicity of cancer chemotherapy are very limited.

At present, biomarkers of cardiac injury have been evaluated inadequately in patients treated with HD chemotherapy followed by hematopoietic cell transplantation (HCT).

The aim of the study was to evaluate cardiac toxicity during myeloablative preparative regimen (PR) and HCT in acute leukemia (AL) patients with 6 biomarkers of cardiac injury: NT-proBNP, CK-MB mass, cTnT (Roche Diagnostics, Minneapolis, MN, USA), cTnI, H-FABP and GPBB (Randox Laboratories Ltd., Crumlin, UK).

Patients and methods

Twenty-three consecutive adult patients with AL were studied. The patients consisted of 15 males and 8 females with the mean age of 44.5 ± 10.6 years (range: 22 – 60, median 44).

Six patients were treated for arterial hypertension, the other patients had no pre-existing cardiovascular disease. Renal and liver functions were normal during the study in all patients. The patients were previously treated with 2 – 6 cycles of conventional chemotherapy containing anthracyclines in the total cumulative dose of 452.2 ± 87.9 mg/m² (range: 240 – 609, median 429). Before PR, all patients had normal systolic left ventricular (LV) function on echocardiography, 3 patients had echocardiographic signs of diastolic LV dysfunction (impaired relaxation on the transmitral Doppler curve). PR consisted of Cyclophosphamide in the total dose of 120 mg/kg (60 mg/kg/day in a 3-hour intravenous infusion on 2 consecutive days) in all patients, in 17 patients in combination with peroral Busulphan 16 mg/kg (Bu/Cy2) and in 6 patients in combination with fractionated total body irradiation 12 Gy (Cy/TBI). In all cases, cryopreserved peripheral blood stem cells were used as the source for HCT. Thirteen patients were given allogeneic grafts and 10 autologous grafts. The study was carried out with the ethics committee approval. All patients gave a written consent before they were included in the study.

Serial measurements of cardiac biomarkers were performed the day before PR (baseline), the day after administration of 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 minutes of rest in supine position. The blood samples were withdrawn into chilled tubes containing EDTA. The whole blood was centri-
fuged, plasma was decanted, immediately frozen and stored at –70 °C until assayed (within 12 months after sampling). We measured all biomarkers according to the manufacturer’s guidelines as follows: NT-proBNP, CK-MB mass, cTnT (Roche Diagnostics; Elecsys analyzer), cTnI, H-FABP, GPBB (Randox Laboratories Ltd.; Evidence analyzer).

Concentrations of cardiac biomarkers diagnostic for cardiotoxicity of oncology treatment have not been established yet. On that ground, values above the reference range recommended by the manufacturers and based on a number of studies were considered elevated in our study. The cut-off values for single biomarkers were as follows: 4.80 μg/L for CK-MB mass, 0.01 μg/L for cTnT, 0.40 μg/L for cTnI, 4.50 μg/L for H-FABP, 7.30 μg/L for GPBB, for NT-proBNP 100 ng/L for male, 150 ng/L for female. NT-proBNP concentrations above 500 ng/L were considered markedly elevated and suggesting functional cardiac injury associated with the treatment.

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

Results

**NT-proBNP.** The day before PR, mean plasma NT-proBNP concentration was 109.9 ± 54.1 ng/L. The mean NT-proBNP concentration increased to 433.4 ± 393.4 ng/L after completion of PR. After HCT, a further increase to 825.6 ± 740.7 ng/L was observed. Fourteen days after HCT, the mean NT-proBNP concentration was 365.5 ± 252.0 ng/L. The differences were statistically significant in comparison with the baseline values (p < 0.01). See Figure 1. The number of patients with elevated NT-proBNP concentrations is shown in Table 1.

Correlations between NT-proBNP concentrations and gender, age, history of arterial hypertension, body mass index, febrile episodes, CRP and hemoglobin levels were not significant. Correlation between baseline NT-proBNP or subsequent changes in NT-proBNP concentrations and the baseline parameters of LV function and LV diameters on echocardiography did not reach statistical significance.

In the peritransplant period, one patient (4.3 %) developed manifestation of cardiotoxicity – clinical signs of congestive heart failure, a significant decrease in systolic LV function on echocardiography (decrease in LVEF more than 15 % from the baseline value and LVEF decline to 50 %), NT-proBNP concentrations 659.0 ng/L (after PR) and 2228.0 ng/L (after HCT). The patient was treated with diuretics and ACE inhibitors with a good response. In this patient, baseline NT-proBNP was 319.9 ng/L, which was by far the highest value in the cohort.

**Markers of structural injury to cardiomyocytes.** Before PR, all biomarkers of cardiac injury were below the cut-off values in all patients. Serum GPBB concentrations increased above the cut-off in 5 patients the day after PR (values were 11.32 ± 4.13 μg/L, range 7.51 – 16.55), remained elevated in 5 patients the day after HCT (values were 9.87 ± 3.71 μg/L, range 7.45 – 15.09) and in 2 patients 14 days after HCT (values were 7.41 and 8.17 μg/L). The changes in GPBB were significant in comparison with the baseline value (p < 0.01). We found a significant correlation between elevation in GPBB and NT-proBNP above 500 ng/L (r = 0.477; p < 0.0001). In 1 patient with normal H-FABP concentrations early after PR and HCT, we found a delayed H-FABP elevation (4.97 μg/L) 14 days after HCT. Other biomarkers (CK-MB mass, cTnT, cTnI) remained below the cut-off in the peritransplant period in all patients. The number of patients with elevated markers of structural cardiac injury is shown in Table 2.

Discussion

Myeloablative therapy (PR) followed by HCT is a well established therapeutic approach for several malignancies, but its clinical efficacy may be limited by treatment-related

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<th>Table 1. Elevated NT-proBNP concentrations in the peritransplant period in AL patients (n=23)</th>
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<th>Table 2. Elevated biomarkers of cardiac injury in the peritransplant period in AL patients (n=23)</th>
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<td>H-FABP above 4.50 μg/L</td>
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cardiotoxicity, in particular when anthracyclines are used as a part of antecedent conventional chemotherapy [5, 21]. The most frequently adopted method for detection of cardiac toxicity is evaluation of LVEF by echocardiography or radionuclide ventriculography [7, 8]. 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 [22]. Biochemical markers of cardiac injury, especially cardiac troponins and natriuretic peptides, have been studied in this context.

Recently published studies reported significant BNP/NT-proBNP elevations after HD chemotherapy and HCT [22–25]. Persistent BNP/NT-proBNP elevations early after HD chemotherapy were observed in 33 – 47 % patients and were associated with development of cardiac dysfunction during the follow-up. The results suggested that monitoring of BNP/NT-proBNP could identify patients at risk for development of cardiac dysfunction after HD chemotherapy and HCT.

In our study, the patients were previously treated with conventional chemotherapy containing anthracyclines in a relatively high cumulative dose (median 429 mg/m²), which can explain the slightly elevated NT-proBNP concentrations in 4 (17.4 %) patients even before administration of PR. We found pronounced NT-proBNP elevations (above 500 ng/L) in 6 (26.1 %) patients early after PR and in 9 (39.1 %) patients early after HCT. Renal functions were normal in all patients. Overhydration was avoided by careful monitoring of fluid balance. In our previously published paper, we showed that solely intravenous hydration in AL patients did not cause a significant increase in NT-proBNP [26]. Since we did not find a correlation with other factors potentially influencing the NT-proBNP concentrations (gender, age, history of arterial hypertension, body mass index, febrile episodes, CRP and hemoglobin levels), we attribute these significant NT-proBNP elevations to acute functional myocardial injury caused by administration of PR and infusion of cryopreserved graft of hematopoietic stem cells. In our cohort, NT-proBNP concentrations remained markedly elevated in 7 (30.4 %) patients 14 days after HCT. These NT-proBNP elevations show persistent neurohumoral activation of cardiac dysfunction and indicate subclinical cardiotoxicity of the undergone treatment, which represents a risk for development of heart failure in the future and requires further follow-up.

Administration of PR containing HD Cyclophosphamide (120 mg/kg) may be in the background of clinical manifestation of cardiac toxicity – in 1 (4.3 %) patient in our cohort. Development of acute heart failure in the patient with the highest baseline NT-proBNP concentration (319.9 ng/L) suggests that implementation of NT-proBNP assay to commonly performed pretransplant cardiac examinations could be useful in the identification of patients at high risk for development of acute heart failure and in the early diagnostics of cardiac dysfunction in the peritransplant period.

Assessment of cardiac toxicity of chemotherapy with classical biomarkers of cardiac injury (cardiospecific markers) – CK-MB mass, cTnT, cTnI – was the aim of a number of studies in the last decade. Measurement of CK-MB mass showed a low sensitivity for detection of chemotherapy-induced cardiotoxicity in the clinical setting [27, 28]. In some studies, cardiac troponins were suggested as predictors of late cardiac dysfunction after HD chemotherapy and HCT [29–31]. In the study of Cardinale et al, positivity of cardiac troponins early after HD chemotherapy for various malignancies was reported in nearly 30 % patients [31]. On the other hand, in several studies no elevations of cardiac troponins after HD chemotherapy were found [32, 33]. Our findings are in concordance with these studies – both cardiac troponins remained negative (cTnT below the sensitivity of the method, i.e. 0.01 μg/L, cTnI below 0.40 μg/L) after PR and HCT in all patients. According to our results, classical biomarkers of cardiac injury (CK-MB mass, cTnT, cTnI) do not seem to be of value in the detection of acute cardiotoxicity during PR and HCT in AL.

At present, routine use of cardiac troponins for monitoring of cardiotoxicity of cancer chemotherapy cannot be established to clinical practice due to disunity of the available assays and inconsistence of results. The timing of sample collection and determination of cut-points for treatment-related cardiotoxicity may play an important role.

H-FABP and GPBB have been recently investigated in the early diagnostics of cardiac injury. They have been suggested sensitive markers of myocardial ischemia and necrosis in patients with acute coronary syndromes [17–20, 34].

According to the available literature, our results are among the first data published on using these new biomarkers of cardiac injury in the assessment of cardiac toxicity of HD chemotherapy and oncology treatment in general. We found GPBB elevations above the reference range in 5 (21.7 %) patients early after administration of PR and HCT. All patients had normal renal and liver functions. Importantly, a significant correlation between elevation in GPBB and other marker of cardiac injury (NT-proBNP) was found. We therefore consider these changes as a sign of acute cardiac toxicity related to the administration of myeloablative PR followed by HCT. Positivity of GPBB in patients with negative cTnT and cTnI might suggest that GPBB could be a more sensitive marker for detection of acute cardiac injury following PR and HCT. Persistent GPBB elevations and delayed H-FABP 14 days after HCT could be a sign of subacute cardiac toxicity associated with the undergone chemotherapy (prior anthracycline treatment in combination with recent administration of PR) or might be due to cardiac injury related to other pathologies. Whether these changes will have predictive value for development of treatment-related cardiomyopathy in the future is not clear and must be evaluated during a prospective follow-up. Further studies in a larger number of patients will be needed to confirm our preliminary results and define the potential role of new biomarkers in the assessment of treatment-related cardiotoxicity in oncology.
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References


