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ORIGINAL ARTICLE

The role of biomarkers and echocardiography in the evaluation of cardiotoxicity risk in children treated for leukemia

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Summary

Purpose: *To describe the high-risk profile group, susceptible to develop anthracycline-induced cardiomyopathy in children with acute leukemia.*

Methods: *The study involved 35 pediatric patients diagnosed with acute lymphoblastic (ALL) or acute myeloblastic leukemia (AML), from March 2014 to December 2016. Serologic markers used for the analysis of cardiac dysfunction were troponin T, NT-proBNP and PCRhs. Also, the patients have had echocardiographic evaluation at the beginning of treatment to determine LVEF, SF and A, E, E' Doppler waves.*

Results: *Positive linear correlation was shown between NT-proBNP and leukocyte values, NT-proBNP and blast cells value, and NT-proBNP and LDH. Significant linear negative correlations between LVEF with leukocyte values, blast cells values, LDH, SF and leukocyte values, LVEF and NT-proBNP values and LVEF and troponin T values were also identified. A weak negative correlation between E/E' ratio and blast cells values has been observed. All of these correlations were statistically significant ($p<0.05$).*

Conclusions: *Leukocyte value, as well as the other serological markers assessed (NT-proBNP, Troponin T), are useful tools to evaluate the risk of anthracycline-induced cardiotoxicity. The variation of the biological markers at the beginning of the cytotoxic treatment confirms the presence of an early myocardial dysfunction, emphasizing the importance of systematic evaluation of this particular group of patients.*

Key words: *biomarker, cancer, cardiotoxicity, chemotherapy, leukemia, pediatric patients*

Introduction

Chemotherapy-induced cardiotoxicity is an important emerging health issue in cancer survivors. This is even more notable in pediatric cancer survivors, in whom the toxic effects of the antineoplastic treatment can affect early the myocardial tissue. Also, the longer life expectancy increases the impact of these long-term side effects on their overall quality of life and health status. Considering the increase in childhood cancer survivor rates (from 50% 5-year survival rate in the 1970s, to approximately 80% in 2016), cardiomyopathy is expected to be diagnosed more frequently in the future [1,2].

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Neoplastic pathology is generally rare in children, its annual incidence in the USA being 1.6 per 100,000 children [1,3]. However, its incidence continues to increase, cancer being the main cause of death caused by disease in children (12.8%) [1,2,4]. Of all cancers, hematopoietic cancers represent the most frequent type of cancer in this age group, up to 40% of all pediatric cancers. Leukemia, especially ALL, is the most frequent cancer diagnosis encountered in children [3].

Chemotherapy is the main treatment method used in pediatric leukemia, one of the main drug groups used being anthracyclines [5,6]. All chemotherapeutic agents are known for their toxicity on rapidly growing tissues (hair, gastro-intestinal mucosa), but anthracyclines are also well known for their toxicity on the myocardial tissue [7-9]. As shown by the Childhood Cancer Survivor Study, patients treated with anthracyclines in their youth, have an 8-fold higher risk of death caused by cardiac pathology as compared to the general population [10]. It has been estimated that in the present time there are over 363,000 survivors of pediatric cancers, with over 60% of them presumed to have been exposed to anthracyclines [9].

Anthracycline-induced cardiotoxicity can present during the cytotoxic treatment as an acute event (myocarditis, pericarditis or even acute heart failure), or after a short time from treatment cessation, or, most often as a late side effect of the cytotoxic drugs [6]. Chronic cardiotoxicity manifests as a progressive decrease in cardiac function, leading most often to chronic heart failure (CHF) with a very poor prognosis. Diagnosing this form of cardiomyopathy in its symptomatic form offers little possibilities regarding its treatment, affecting severely these patients' quality of life [6,11].

All these considered, it has proven to be extremely important to identify patients at risk of developing anthracycline-induced cardiomyopathy even before they start developing signs or symptoms of cardiac dysfunction [12]. This could allow the development of specific treatment protocols for these patients in order to slow down the progression to heart failure, or even prevent it.

Table 1. Demographic and clinical characteristics

Characteristics	*n (%)*
Gender	
Male	17 (49)
Female	18 (51)
Age at diagnosis (years)	
1-10	25 (71)
>10	10 (29)
Mean ± SD	7.4 ± 4.35
Diagnosis	
ALL	25 (71)
T cell subtype	1
AML	9 (25)
M0	3
M7	1
Mixed type	1 (3)
Risk group	
SRG	13 (37.14)
MRG	12 (34.29)
HRG	10 (28.57)

SD: standard deviation, ALL: acute lymphoblastic leukemia, AML: acute myeloblastic leukemia, SRG: standard risk group, MRG: medium risk group, HRG: high risk group

Methods

The present study involved 35 pediatric patients diagnosed with ALL or AML, from March 2014 to December 2016. All the patients belonged to the Hemato-Oncology department of the Children's Emergency Hospital from Cluj-Napoca. Patients' data are listed in Table 1.

Inclusion criteria

- Children between 1 and 18 years old, with histologically positive diagnosis of ALL or AML.
- Chemotherapy protocol, which involved administration of anthracyclines.
- Biological parameters within acceptable limits in order to permit chemotherapy (hematologic, hepatic, renal function).
- Informed consent signed by both parents, approved by the Ethics Commission from Cluj-Napoca University of Medicine and Pharmacy.

Exclusion criteria

- Mediastinal radiotherapy in the patient's history.
- Left ventricular ejection fraction (LVEF) <50% at the beginning of chemotherapy.
- Preexisting heart diseases or arterial hypertension.
- Collagenosis and other systemic diseases.

Echocardiographic evaluation

The echocardiographs were performed using a VIVID S5 echocardiograph (General Electric).

Heart function was assessed using two windows: parasternal long axis and apical four chambers. The systolic function of the left ventricle was evaluated by calculating the LVEF and SF (shortening fraction) using two methods: M mode and volumetric method. The diastolic function of the left and right ventricle was assessed using Pulsed Wave Doppler (PW) at the apex of the mitral and tricuspid valve, from the apical four chamber window, with the patient in supine position. The E/A ratio was measured. To enhance the accuracy, Tissue Doppler Imaging (TDI) was performed, with the cursor being placed on the lateral side of the mitral and tricuspid valve. The E/E' ratio was measured, thus enabling the evaluation of LV filling pattern.

Serologic markers

Serologic markers used for the analysis of cardiac dysfunction were: troponin T, NT-proBNP and PCRhs. They were determined using the TNT hSST Roche kit, the NT-proBNP Roche kit and the C-reactive Proteing latex High Sensitive Assay (Roche). The device used in the evaluation of these biomarkers was COBAS 6000(e601)/ Roche/Germany.

Statistics

Data obtained was analyzed using Office Excel 2013. Normal data distribution was reported as mean ± standarddeviation. Student-t- two-tailed tests were used in order to evaluate the statistical significance of the correlation coefficients. Also, chi-square test and regression analysis were used in order to determine relationships between qualitative variables. A p value <0.05 was considered to be statistically significant.

Table 2. Biological markers

Markers	*Mean ± SD*
Hemoglobin (g%)	6.58 ± 2.50
Leukocytes (cells/mm³)	18.468 ± 30.713.51
Blast cells (cells/mm³)	12.820.56 ± 29.855.57
Platelets (no./mm³)	67.857.14 ± 69.621.07
LDH (U/l)	662.89 ± 927.23
Troponin T (pg/ml)	4.35 ± 2.59
CK-MB (U/l)	20.44 ± 12.92
NT-proBNP (pg/ml)	507.09 ± 1,754.31
PCRhs (mg/l)	29.65 ± 55.99

LDH: lactic dehydrogenase, CK-MB: creatinkinase MB (muscle-brain), NT-proBNP: terminal prohormone of brain natriuretic peptide, PCR: reactive proteine C high-sensitivity

Table 3. Echocardiographic parameters

Parameters	*Mean ± SD*
LVEF (%)	66.67 ± 8.85
SF (%)	38.25 ± 6.40
E/A	1.76 ± 0.34
E/E'	7.20 ± 1.71

LVEF: left ventricular ejection fraction, SF: shortage fraction, E/A: ratio of early diastole (the E wave) to peak velocity flow in late diastole caused by atrial contraction (the A wave), E/E': ratio between early mitral inflow velocity and mitral annular early diastolic velocity

Results

Characteristics of the studied group are shown in Table 1.

Median age of the included patients was 7.4 ± 4.35 years, with 2 peaks of incidences: 4-5 and 12-13 years.

The pathologies were represented by ALL (72% of the patients), 25% with AML, and 3% with mixed pathology. Of the patients, 37.14% were standard risk patients, 34.29% medium risk and 28.57% high risk.

Eight of the 35 patients died during the study, 4 due to progressive disease and 4 from sepsis secondary to aplasia during chemotherapy.

All biological markers analyzed had a normal distribution in the studied group. The mean value and the standard deviation (SD) are shown in Table 2. Also, the echocardiographic parameters had a normal distribution in the studied group, with their mean and SD are listed in Table 3.

Associations between specific cardiac biomarkers values at diagnosis and other biological parameters

There was a statistically significant, linear, positive correlation (r=0.68; p=0.00) between leukocyte values at diagnosis and NT-proBNP values (Figure 1). Also, NT-proBNP values had a positive correlation with LDH values (r=0.82; p=0.00) and blast cells value in peripheral blood (r=0.72; p=0.00). Thus, it can be estimated based on the determination coefficient the NT-proBNP variation was dependent on the leukocyte, blast cells and

Table 4. P values according to Pearson correlation coefficient

Variables	*Leukocytes*	*Hemoglobin*	*Thrombocytes*	*LDH*	*Blasts*	
					%	*p value*
Troponin T	0.65	0.25	0.23	0.98	0.3	0.81
CK-MB	0.81	0.85	0.27	0.81	0.31	0.73
NT-proBNP	0.00	0.052	0.36	0.00	0.01	0.00
PCRhs	0.61	0.8	0.64	0.5	0.26	0.65
LVEF	0.001	0.48	0.06	0.004	0.03	0.001
SF	0.16	0.67	0.67	0.16	0.1	0.15
E/A	0.71	0.51	0.32	0.88	0.6	0.57
E/E'	0.12	0.49	0.04	0.12	0.04	0.06

CK-MB: creatinkinase MB (muscle-brain), NT-proBNP: N-terminal prohormone of brain natriuretic peptide, PCRhs: reactive proteine C high-sensitivity, LVEF: left ventricular ejection fraction, SF: shortage fraction, E/A: ratio of early diastole (the E wave) to peak velocity flow in late diastole caused by atrial contraction (the A wave), E/E': ratio between early mitral inflow velocity and mitral annular early diastolic velocity

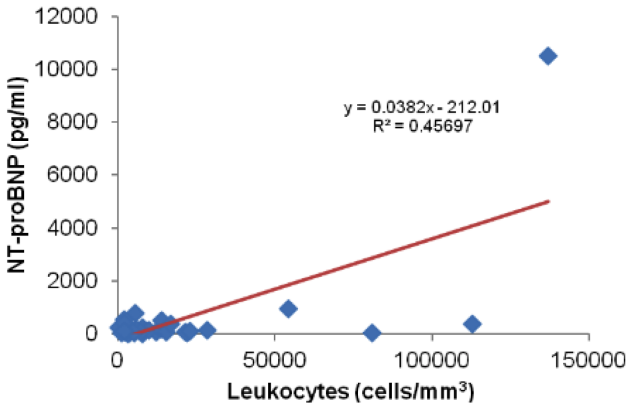

Figure 1. Dispersion diagram of NT-proBNP values according to leukocyte values.

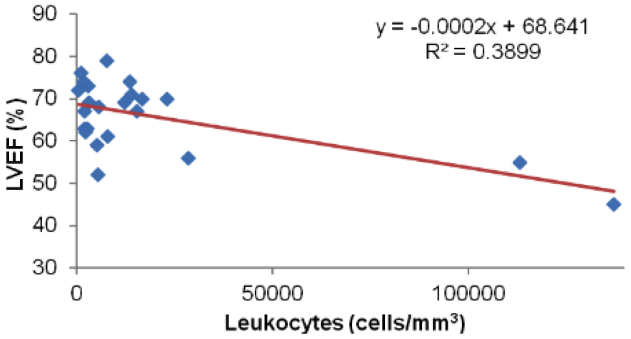

Figure 2. Dispersion diagram of LVEF values according to leukocyte values.

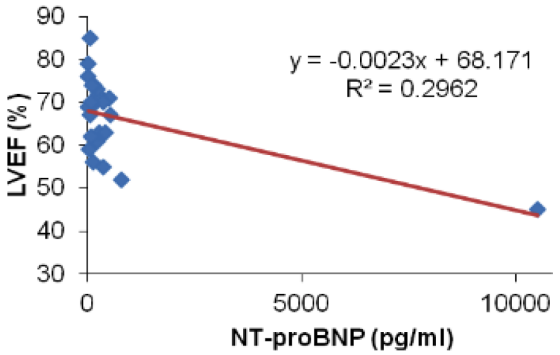

Figure 3. Dispersion diagram of LVEF values according to NT-proBNP values.

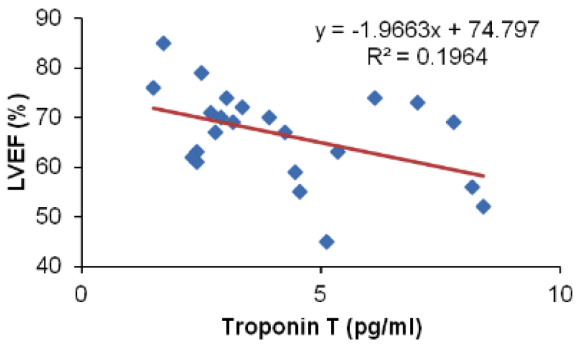

Figure 4. Dispersion diagram of LVEF values according to troponin T values.

LDH values variation. A mean value NT-proBNP for included patients was 507.09±1754.32 pg/ml with a high normal value considered to be 125 pg/ml.

Considering high normal level value of 14 pg/ml for troponin T, all included patients had values within normal range, the mean value for included group being 4.35±2.59 pg/ml.

On the contrary, for CK MB, for a high normal level of 24 UI/L, 35.39% of the included patients had values higher than the normal limits at diagnostis (upper level 24UI/L, 20.44±12.92 U/l).

CRPhs above 3 mg/l was seen in 53% of the included patients (mean value 29.65±55.99 mg/l).

Out of the total 35 patients included in our study, 14.71% had none of the cardiac function biomarkers above the normal limit, 41.17% had one marker above the normal range, 35.29% had two markers above the limits, and 8.82% had three markers above the normal values.

Other correlations between hematological parameters and Troponin T, CK-MB or PCRhs values were not statistically significant (p>0.05; Table 4).

Associations between echocardiographic and hematological parameters

There was a statistically significant, linear, negative correlation (r=-0.62) between leukocyte values at diagnosis and LVEF values (Figure 2). It could be estimated that 38.99% of the LVEF variation was dependent on the leukocyte values variation (r^2=0.3899; p=0.0016). Also, LVEF values had a significant negative correlation with LDH values (r=-0.56; p=0.004) and blast cells value in peripheral blood (r=-0.63; p=0.001). Thus, it could be concluded that LVEF value variation was dependent on the leukocyte, blast cells and LDH values variation.

Also, a weak negative correlation between E/E' ratio and blast cells values and thrombocyte values was observed (r=-0.42; p=0.046 for both correlations).

Other correlations between hematological parameters and LVEF, SF and E/A, E/E' values showed no statistical significance (p >0.05; Table 4).

Associations between echocardiographic and cardiac function biomarkers

There was a statistically significant, linear, negative correlation (r=-0.54; p=0.006) between LVEF values and NT-pro BNP values (Figure 3). It could be estimated that 29.62% of the LVEF variations were dependent on the NT-proBNP values variation (r^2=0.2962).

Also, there was a statistically significant, linear, negative correlation (r=-0.41; p=0.044) between LVEF values and Troponin T values (Figure 4). It

Table 5. LVEF versus number of cardiac function markers outside the normal range

Number of markers outside of normal range	*0*	*1*	*2*	*3*
LVEF ≤ 65				
No of patients	1	2	5	1
LVEF > 65				
No of patients	4	5	3	2

LVEF: left ventricular ejection fraction

could be estimated that 16.8% of the LVEF variations were dependent on the Troponin T values variation (r^2=0.168). A statistically significant positive correlation was observed between E/A values and Troponin T (r=0.64; p=0.01) and CK-MB (r=0.45; p=0.029) values.

No other statistically significant correlations were observed.

We tried to establish whether there was any correlation between the total number of cardiac function markers above the normal range and LVEF values or not. For this, x^2 test was used and the observed frequencies are shown in Table 5. As no given guidelines for normal pediatric LVEF values exist, a cut-off value was determined in our study by using regression analysis. We thus established that a value of LVEF over 65% is considered normal, and values under this limit will be taken into consideration as an altered LVEF function. Given the resulted p value over 0.05 (p=0.396), we could state that the distribution observed was purely hazardous, there was no significant correlation in our studied group between LVEF value and the number of cardiac function markers situated outside the given normal values.

Discussion

Our results have shown that there is a certain degree of cardiac function alteration even from the start of chemotherapy. Thus, it is important not only to determine the initial value for these parameters, but also to re-evaluate them periodically, starting right from the first days of treatment. In this way, we can determine the group of patients who present a higher risk of developing anthracycline-induced cardiotoxicity during or after their treatment.

Cardiac function biomarkers

The scientific community has greatly debated the use of troponins in the evaluation of cardiac side effects in oncology. Dolci et al. wrote in 2008 a review on this subject, stating that troponin evaluation could predict up to 3 months ahead the development of a clinically significant LVEF dysfunction. Also, their study states that, for patients with high troponin levels receiving anthracyclines, the persistence of these high values for over a month after treatment is correlated with 85% probability of developing a major cardiac event in the first year following cytotoxic treatment [13]. Another article, which assessed the histological involvement of the anthracycline treatment showed that both cardiac Troponin I and T are effective in identifying doxorubicin-induced injury as indicated by vacuolization of cardiomyocytes of the atria or ventricles [14]. There are other studies, which state that Troponin I could be a more sensitive marker for the evaluation of the cardiotoxicity risk in this particular group of patients [15-17]. Sherief et al. have evaluated 50 patients, survivors of childhood leukemia, out of which none presented with high levels of Troponin T [18]. However, Cheung et al. have shown in their study that there is a correlation between high values of this parameter and the alteration of the myocardial contraction of the LVEF. In their study, Troponin T high values have also varied with the higher dose of anthracycline given and the leukemia relapse [19].

Our study has shown no patient with a troponin T value over the limit of 14 pg/ml (similar to the cut-off value used by Xue et al. in their study [20]), therefore emphasizing the low sensitivity of this marker in predicting cardiac function alterations, during and after cancer treatment. However, in our study Troponin T values have been correlated with LVEF and E/A ratio values, thus confirming its role as a cardiac function marker, but with a very low sensitivity for subclinical heart disease.

Another study evaluated the utility of troponines in 76 childhood cancer survivors with a median follow up of 9 years. Cardiac troponins T (cTnT) and I (cTnI) were evaluated but none of them were at measurable levels in order to provide additional information regarding the late cardiotoxicity of anthracyclines [21].

There are published data suggesting that high-sensitive cardiac troponin-I could predict cardiotoxicity induced by anthracyclines-containing regimens even in subclinical stages. A cut-off values of an absolute increase value of 30.7 ng/l seems to be statistically significant for predicting cardiac toxicity [22].

Nathan et al. in a published review underlined that a persistent high value of troponin after chemotherapy administration could identify a very high-risk population to develop cardiomyopathy (86% of them will develop a certain degree of disease during a period of 20 months) [23].

One of the most important inconvenience of the troponins are represented by the inconsistent release of the biomarker through the blood, which makes mandatory to serial samples from the patient in order to eventually discover pathologic values.

The usefulness of troponins is variable for early diagnosis of cardiomyopathy. For example, there is a well recognized elevation of troponins in anthracyclines treatments - 30% of them are with elevation of troponin I, which is considered predictive for future cardiac events [24]. More than 50% of patients with anthracyclines in their chemotherapy regimen could develop cardiac function abnormality in 10-20 years and 5% could develop congestive heart failure [25].

The specificity of troponin T elevation does not vary significant over time (in acute injury of the myocardium) with values from 86 to 98% in serial testings [26]. In chronic injuries of the myocardium (like cardiomyopathy) the level of cTn is rarely above 1 ng/ml and serial values do not change significantly [27]. Possible elevations of cTn could be seen in decompensations of cardiomyopathies, the level reached and longer period of time with elevated cTn could be related with prognosis of the patients [27].

More important seems to be the persistence of cTn levels after anthracycline chemotherapy. Persistent cTnT elevation in children with hematological neoplasias throughout the first 90 days following the treatment could predict the development of cardiac toxicity in the 4-year interval of follow-up [28]. In adult patients the absence of elevation of cTn reduces the risk of cardiotoxicity at 1 % [28]. The persistence of cTnI >0.08 ng/ml 1 month after cessation of chemotherapy increases the risk up to 84% compared to 37% for transient elevation [28].

NT-proBNP has been widely accepted as an important marker in the diagnosis and evaluation of cardiac failure in adults as well as in children. In 2010, Rusconi et al. have shown that a value of over 1000 pg/ml is associated with highly symptomatic children (III-IV NYHA functional class) with 95% sensitivity and 80% specificity [29]. In 2013, Lin et al. specified that a NT proBNP level of ≥598 ng/l, combined with a modified Ross criteria score ≥4, is highly diagnostic of heart failure in children [30]. Unfortunately, its utility in diagnosing a subclinical cardiac dysfunction has been poorly documented, with no current agreement on a specific cut-off value for asymptomatic patients. However, its utility in diagnosing a subclinical cardiac dysfunction has been poorly documented. There are few studies showing an increased value for this parameter following cancer treatment in pediatric patients with subclinical LV dysfunction [16].

In the studied group, 50% of the patients presented a value of NT-proBNP over 125 mg/l. As this marker shows increased myocardial stress, these high values could point out alterations in the cardiac kinetics or hemodynamics. Moreover, many studies have linked this marker with the heart failure severity, demonstrating its important prognostic role [13,31]. Therefore, patients presenting high NT-proBNP levels in our study are considered to be at risk of developing anthracycline-induced cardiomyopathy later in life, its values correlating negatively with the LVEF values, so this marker could predict cardiac function alteration even before LVEF reduction.

The primary mechanism for NT-proBNP elevation is myocardic wall stress, that is why the potential use of this biomarker is more likely for chronic disease than for acute injury of the myocardium and could be related with cardiac architecture remodeling [24]. There are conflicting data regarding the exact role of monitoring the levels of NT-proBNP. Despite the fact that NT-proBNP correlates well with LVEF declining levels in already published data, it is unclear whether it precedes or it is only a secondary phenomenon to cardiac failure [24].

There are some small studies which identify a possible role for NT-proBNP in the early detection of cardiotoxicity and also its role in risk stratification or as a prognostic factor [32]. In the PREDICT trial, which included 582 adult patients, serial measurement of NT-proBNP could help identify a high-risk population for cardiac event/cardiac failure. The cut-off value in this trial for NT-proBNP was 100 pg/ml and this cardiac biomarker had a high specificity and negative predictive value (85 and 92% prospectively) [33]. Published clinical experience in childhood cancer survivors regarding early detection of cardiomyopathy is scarce. An important percent (13 to 30%) of survivors had abnormal level of NT-proBNP with unknown significance [34]. Another published data suggested an inverse correlation between the level of NT-proBNP and left ventricle mass in children treated with regimens containing anthracyclines [35]. A published study of 200 children treated with a chemotherapy regimen with anthracyclines showed, with the exception of NT pro-BNP, no correlation between B-type natriuretic peptide and Troponin-T with LVEF [36].

The Children's Oncology Group for long-term follow-up of childhood survivors recommendation version 4 does not sustain the necessity of including cardiac biomarkers in cardiomyopathy screening programs [37].

Regarding CK-MB values, 36% of the included patients presented with values over 24 U/l in our study. Many studies have shown that anthracycline treatment can cause not only late-onset CHF, but also acute cardiotoxicity presenting as coronary vasospasm or even acute myocardial infarction [38]. Although rare, these acute side effects are life-threatening, so it is vital to identify them as soon as possible, by obtaining an ECG prior and during anthracycline treatment and by evaluating CK-MB values periodically. On the other hand, an increased value for this marker could be the expression of cardiac ischemia in the context of a later-onset CHF, proving to be useful in monitoring these high-risk patients. In children exposed to anthracyclines in a small study which included 22 patients, CK-MB was in normal range before and during 72 hrs of treatment [39]. CK-MB remained within normal range even if the pediatric patients followed a high dose chemotherapy regimen needed in hematopoietic stem cell transplantation [40]. If the exposed total dose of anthracyclines is taken into account, CK-MB variations seem to be without statistical significance in a study that included 131 patients [41].

PCRhs values are closely correlated with the risk of acute cardiac events. In the studied group, more than half of the patients presented values above 3 mg/l of this marker, putting themselves in the high-risk group for these types of cardiac pathology [42]. Thus, PCRhs could be used as a marker for acute anthracycline cardiotoxicity. However, cancer patients are known to have an increased proinflammatory status, which could in turn explain the increase PCRhs values. This is why periodic evaluation of this marker could help distinguish between the two causes, indicating the high-risk patients which need more careful future monitoring. In a published randomized study of 205 pediatric patients with anthracyclines chemo regimen, PCRhs level was similar between groups through all the study time and were not statistically associated with any echocardiographic variables [43].

Hematologic parameters and cardiac function

In the studied group, leukocyte value at diagnosis has proven to have an important role in the later development of a CMP (cardiomyopathy). Leukemia patients are classified into three risk groups according to a number of criteria, one of which is the number of leukocytes at diagnosis. A leukocyte count at diagnosis of over 50000/mm^3 includes the patient in the high-risk group (HRG), meaning a more aggressive treatment, thus a higher total dose of anthracyclines. However, a high level of leukocytes at diagnosis could also imply myocardial infiltration by cancer cells, thus leading to impaired cardiac function. This is why the sensitivity and specificity of this marker in predicting future cardiac dysfunction is hard to assess. In our study, hematological parameters (leukocyte, LDH and blast cell values) have been correlated positively with NT-proBNP values and negatively with LVEF values. Also, E/E' values have been correlated negatively with blast cell values. This is of high importance, as it points out the degree in which the level of malignant cell presence in the body affects cardiac function even in the absence of an oncological treatment. This might signal infiltration of the myocardium with malignant cells, meaning that all these patients suffer a pre-treatment alteration of their cardiac function, which would then be amplified by the cytotoxic treatment. This is of vital importance since, by proving the existence of this already influenced cardiac status, would urge the initiation of a cardio-protective treatment right from the diagnosis. Also, considering that the higher the number of leukocytes, thus the patient being in the high risk group, the higher the given dose of anthracycline will be, it is noticeable that these are the patients that will require the most intensive cardiac monitoring, and also long-term cardio-protective treatment.

Echocardiographic parameters

With regard to the echocardiographic evaluation, it is widely accepted (class I recommendation) that children receiving cardiotoxic chemotherapeutic agents should have a baseline evaluation as well as frequent follow-ups, in order to determine subclinical cardiac ischemia [43]. The measurement of the echocardiographic LVSF and LVEF are both non-invasive and available in most pediatric oncology centers, being the most widely used diagnostic method for detecting cardiotoxicity in children. However, these measurements have their limitations, their value depending on the exact methods used to obtain the LVSF or the LVEF [44]. Moreover, no studies have evaluated the predictive value of the echocardiographic LVSF as a surrogate marker for the future development of clinical heart failure after anthracycline therapy [45], as was confirmed by an extensive literature search. Generally, the cut-off value for SF is 28-29% and for LVEF 60-65%. The same cut-off values have been considered in this study. A more accurate approach is to measure the difference between their values from one examination to another: a decrease of LVEF of over 10% being considered significant for an alteration of the heart's function. The present study has established its cut-off value of 65% for LVEF using regression analysis in the given patient group.

Recent studies have shown lack of sensitivity of the classical echocardiographic parameters (LVEF, SF) in the early diagnosis of cardiac dysfunction. The reduction of LVEF is considered a late phenomenon, which is a clinical result of the failure of myocardium systolic function recovering. McKillop et al. determined that an abnormal radionuclide LVEF at rest (≤45%) had a sensitivity of 53% and a specificity of 75% for detecting patients at moderate or high risk of developing CHF, with SF presenting an even lower sensitivity and specificity value [46]. Our study is consistent with these findings, proving an increased sensitivity of the biological markers as compared to the echocardiographic ones. However, this study has shown that there is not an exact biological profile that could predict a future decrease of cardiac function, as each patient presents with a particular set of altered biomarkers. One of the most important issues regarding echocardiography is represented by inter-operator variability.

The comparative analysis on risk groups of LVEF and SF showed a difference of about 3% and 4%, respectively, between the mean values for these parameters in the standard risk group (SRG) and high-risk group (HRG), but this difference was not statistically significant ($p>0.05$). However, it was noticed that in the case of ultrasound determinations, patients included in HRG are more likely to develop subsequent cardiotoxicity, with initially LVEF and SF slightly lower than those in the SRG.

With regard to the values of E and A waves, determined by PW, and E' respectively, determined by the TDI method, normal values were considered those proposed by Eidem et al. in 2004 [47]. For adults, four diastolic filling patterns are known depending on the E/A and E/E' ratio: normal, pseudonormal diastolic filling, impaired LV relaxation, and restrictive diastolic dysfunction. For children, there is currently no similar classification according to the echocardiographic parameters. However, due to the relative stabilization of diastolic velocities in children around 3 years of age, it is considered that the models used in adults can be applied to children over this age. In the studied group we identified three such patients, who had diastolic dysfunction according to the above-mentioned classification: two patients with impaired relaxation, presenting E/A and E/E' values below the normal limits, and one patient presenting with a restrictive dysfunction pattern with both parameters exceeding the maximum limit for their age group.

In recent studies, PW and TDI parameters are considered highly useful in the early diagnosis of diastolic dysfunction. Cengiz et al., declare in their study that the TDI method allows an early and accurate identification of diastolic dysfunction in patients treated with anthracyclines, the sensitivity of which increases with the time elapsed since cessation of treatment [48]. Also, Sherief et al. found a variation of TDI parameters in 26% of the 50-childhood leukemia surviving patients included in their study [18]. They point out that alteration of these parameters was also found in patients who had normal cardiac function in basal echocardiography. On the other hand, although Doppler evaluation improves early detection of cardiac dysfunction in these patients, there is limited knowledge regarding the normal values of these parameters, as well as the interpretation of values situated outside the reference limits in children [49,50]. This makes it difficult to diagnose cardiac dysfunction using PW and TDI, increasing the dependence on the examiner's expertise and interpretation.

Limitations and Conclusion

The present study was mainly limited in terms of the number of patients and their adherence to the study. Of the initial number of 35 patients, only 24 were present for echocardiography. For these reasons, the results are difficult to extrapolate. Our results offer a pre-chemotherapy image of pediatric oncological patients, in which even the leukemic cell load could determine a preexisting subclinical cardiomyopathy which could be over-expressed during specific treatment, as statistically demonstrated correlation between blastic load and NT-proBNP and E/E' values negatively correlated with blast cell values. This hypothesis could change the cardio-protective strategy by initiating the cardio-protective treatment right from the diagnosis. It is also necessary to monitor these patients at the end of treatment as well as 1 and 2 years after the end of treatment. In this way the predictive value of the biological markers and the echocardiographic parameters in anticipating cardiac dysfunction, could be determined properly.

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Conflict of interests

The authors declare no conflict of interests.

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