UNIVERSITY OF MEDICINE AND PHARMACY

CRAIOVA

EARLY DIAGNOSIS OF CANCER TREATMENT RELATED MYOCARDIAL DYSFUNCTION USING 2D, 3D AND SPECKLE TRACKING ECOCARDIOGRAPHY

‑ DOCTORAL THESIS ABSTRACT ‑

PhD Supervisor:

Prof. Univ. Dr. Doina Cârstea

PhD Student:

Diana-Alexandra Cherata

CRAIOVA

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SUMMARY

KEYWORDS: cardiotoxicity, ecocardiography, *speckle-tracking, tridimensional,* myocardial dysfunction*, strain*, myocardial deformation, cancer treatment, cytostatics, Hematology, Cardio-Oncology, EACVI

INTRODUCTION

Cancer treatment has made considerable progress over the last decade, thus increasing the rate of neoplastic pathology remission or complete healing [1].

The outcome of this progress is expressed by the formation of a cohort of millions of surviving cancer patients with particularities in short-term cardiovascular (CV) risk, but also with a sufficient life expectancy to develop antineoplastic therapy long term cardiovascular side effects [2], associated with the aging phenomenon and with the whole pathology related with it, which leads to an increased number of comorbidities, especially CV [2].

Myocardial dysfunction occurs by affecting myocardial cell metabolism, by direct cardiac toxicity or by accelerating the progression of existing cardiac conditions, being one of the most common complications of antineoplastic therapy, with particularly significant negative effects on short and long term prognosis [2]. The association of myocardial dysfunction with neoplastic pathology can influence specific oncology therapies compliance and healing, thus, myocardial dysfunction becomes a cause of increased morbidity and even premature mortality for these patients [2].

This unique, continuous, progressive phenomenon begins with myocyte lesion, followed by a gradual and asymptomatic decrease of left ventricular ejection fraction (LVEF), which, not detected and not treated on time, leads to heart failure (HF) with specific clinical manifestations and prognostic concerns [3]. Moreover, once HF symptoms appear, the lack of recovery of left ventricular (LV) function in patients with myocardial dysfunction was reported as up to 40-58% [4], specific cardiac treatment being even more effective as it is initiated early, before myocardial changes become irreversible [5].

Research in this area is ongoing, as proof is that the recommendations of European and American cardiology associations regarding cancer treatment cardiovascular side effects are still consensus documents [3, 6].

Thesis objectives are based on the advantages of echocardiography for the evaluation of cancer treatment related myocardial dysfunction. We aimed to determinate its incidence and the correlations between echocardiographic parameters: LVEF and myocardial deformation parameters changes and antineoplastic therapies risk factors, demographic variables and cardiovascular risk factors in a group of patients diagnosed with haematological cancers under specific treatment.

This study addresses a realistic theme, the choice of the topic being justified by its novelty and importance in the context of current evolution in the field of Cardio-Oncology, considering that identification and understanding of early echocardiographic changes can contribute to the development of monitoring strategies that would improve cancer patients CV prognosis, echocardiographic parameters early changes being easy to be detected and monitored in clinical practice.

1   CANCER TREATMENT RELATED MYOCARDIAL DYSFUNCTION

1.1 DEFINITION, CLASSIFICATION, EPIDEMIOLOGY

Cancer treatment related myocardial dysfunction definition is conventionally based on the calculation of LVEF using both two-dimensional (2D) or tri-dimensional (3D) echocardiography, radionuclide ventriculography or nuclear magnetic resonance imaging (CMR) [3].

The most recent document, written under the auspices of the European Society of Cardiology (ESC) [6], aims to evaluate all available evidence on cardiac toxicity associated with oncological treatments and defines cancer treatment related myocardial dysfunction as symptomatic or asymptomatic 10% decrease of LVEF compared with pretreatment value to less than 53%, using Simpson biplan method [3,6]. Furthermore, this document encourages the use of global longitudinal strain (GLS) as a diagnostic criteria for cancer treatment related myocardial dysfunction, a parameter that evaluates myocardial contractile function: a relative percentage reduction of more than 15% from pretreatment value suggests cardio-myo-toxicity and early detection of cancer treatment related myocardial dysfunction with the likelihood of subsequent specific clinical manifestation [6].

Based on the type and magnitude of the cellular abnormalities and the degree of reversibility of these changes, in 2005 Ewer and Lippman [7] were considering cancer treatment related myocardial dysfunction as type I, characterized by myocyte irreversible dose-related structural damage and type II, a reversible form along with causal treatment cessation and with no relationship between cytostatic dose and CV effects.

Almost intuitively, with the advances in the oncology field, further studies have raised some concerns related to the relationship between clinical reality and this classification [8,9], because responsible cytostatic drugs for type I and type II myocardial dysfunction:

anthracyclines and "targeted" therapies, are rarely given as monotherapy, usually associated, preceded or followed by drugs belonging to other classes of cytostatic drugs, and thus the resulting cardiotoxic effect emerge from a synergistic or combined action.

A recent study reported a 9% incidence of LV dysfunction after anthracyclines treatment in a cohort of 2625 patients, which in 98% of these cases the diagnose was made within the first 12 months of completing chemotherapy [9]. The incidence of cancer therapy related myocardial dysfunction varies widely, depending on the use of specific oncology treatment, its duration, and individual associated comorbidities [10]. (Table 1.1)

Table 1.1 Cancer therapy related myocardial dysfunction incidence according to main classes of cytostatic

Agent	Incidence (%)	Agent	Incidence (%)
Anthracyclines:		**Monoclonal antibodies:**	
Doxorubicin (Adriamicin):		**Trastuzumab**	1,7-20,1[a]
400 mg/ m^2	3-5		
550 mg/ m^2	7-26	**Bevacizumab**	1,6-4[b]
700 mg/ m^2	18-48		
		Pertuzumab	0,7-1,2
Idarubicin >90 mg/ m^2	5-18		
		Tirozin kinasis inhibitors:	
Epirubicin >900 mg/ m^2	0,9-11,4		
		Sunitinib	2,7-19
Mitoxantron >120 mg/m^2	2,6		
		Pazopanib	7-11
Lipozomal Anthracyclines >900 mg/m^2	2		
		Sorafenib	4-8
Alkilant Agents:		**Dasatinib**	2-4
Ciclophosfamid	7-28	**Imatinib mesilat**	0,2-2,1
		Lapatinib	0,2-1,5
Iphosfamid		**Nilotinib**	1
<10 g/m^2	0,5		
12,5–16 g/m^2	17	**Proteazom inhibitors:**	
Taxans:		**Carfilzomib**	11-25
Docetaxel	2,3-13	**Bortezomib**	2-5
Paclitaxel	1		

[a] When used in combination with anthracyclines and cyclophosphamide.
[b] Patients treated concomitantly with anthracyclines.

2016 ESC Position Paper on cancer treatments and cardiovascular toxicity developed under the auspices of the ESC Committee for Practice Guidelines, Eur Heart Journal [6]

1.2 DIAGNOSTIC PRINCIPLES AND METHODS

Currently, the main strategies for cancer treatment related myocardial dysfunction screening and diagnosis include cardiac imaging techniques (echocardiography, radionuclide angiography, cardiac magnetic resonance) as well as specific biomarkers for myocardial necrosis (troponin) and LV volume overload (natriuretic peptides) (Table 1.2)

Choosing the right strategy for the cancer treatment related myocardial dysfunction diagnosis depends on the cardiologist's experience and accessibility to these investigation methods, as well as some basic principles [6]:

• The same imaging and/or biomarker analysis technique should be used for myocardial dysfunction screening along and after antineoplastic treatment.

• Preferably, due to differences in interpretation related to the human factor, techniques and tests with the best reproducibility should be used.

• Imaging techniques that provide additional relevant clinical information are recommended.

• It is preferable to use the imaging technique with the highest image quality, without exposing patients to radiation.

• The frequency of monitoring will depend on the type of chemotherapy treatment, the total cumulative dose, the administration protocol and its duration, but especially on the oncological patient's initial cardiovascular risk [6].

Table 1.2 European Society of Cardiology proposed methods for cancer therapy related myocardial dysfunction diagnose [6]

Method	Available diagnostic criteria	Advantages	Major Limitations
Echocardiography: - Echo3D: LVEF - Echo2D: LVEF Simpson biplan method	• LVEF: drop of ≥10 % to a value ≤ 53% = Cancer treatment related myocardial dysfunction	• Availability • Lack of radiation • Hemodinamics and other cardiac structurs evaluation	• Inter-observer variability • Image quality
-EchoST (GLS)	• GLS: a relative percentage		• *Inter-vendor* variability,

	reduction of ≥15% from pretreatment value = early cancer treatment related myocardial dysfunction, risk for subsequent specific HF clinical manifestation		technical requirements
Radionuclide Angiography (MUGA)	• LVEF: drop of ≥10 % to a value ≤ 50% = Cancer treatment related myocardial dysfunction	• Reproducibility	• Radiation exposure • Limited information regarding other cardiac structures
Cardiac Magnetic Resonance Imaging (CMR)	• Used when relevant information for diagnosis or confirmation of LVEF drop under lower limit of normal are not provided by other techniques	• Accuracy • Reproducibility • Diffuse myocardial fibrosis detection	• Limited availability • Patient cooperation is necessary (claustrophobia, apnea, long acquisition time)
Cardiac Biomarkers: - Troponin I - Troponin T - BNP şi NT-proBNP	• The routine BNP and NT-proBNP dosage for patients at high risk for cancer treatment related myocardial dysfunction needs additional investigations	• Accuracy • Reproducibility • Availability • Increased sensitivity	• Insufficient evidence to establish the importance of subtle increases for the detection of cancer treatment related myocardial dysfunction • Variability of tests used for dosing
* Endomyocardial biopsy (BEM)	•A particularly sensitive and specific method to identify cancer treatment related myocardial dysfunction	• High sensibility	• Invasive technique • Low availability

EchoST - speckle tracking echocardiography, BNP - type B natriuretic peptide; GLS - global longitudinal strain; HF-heart failure, LVEF- left ventricular ejection fraction; MUGA - multiple gated angiography scan; NT-proBNP - the N-terminal prohormone of the natriuretic peptide type B; BEM- Endomyocardial biopsy* Diagnostic method not recommended by current guidelines

All patients receiving cardiotoxic chemotherapy should undergo a cardiology evaluation, which will mandatory include echocardiography for the assessment of LV function, repeated on a case-by-case basis during and after treatment. [6]

1.3 PREVENTION AND TREATMENT STRATEGIES

Cardioprotection represents all the strategies in order to reduce cancer therapy cardiovascular toxicity [11-17]. (Table 1.3)

Table 1.3 Strategies for antineoplastic treatments cardiovascular side effects prevention [11-17]

For all cytostatics and radiotherapy	Cardioprotection strategies
	• Cardiovascular risk factors identification and control • Co-morbidities diagnostic and treatment: HF, CAD and PAD • Avoid medications that may prolong the QT interval • Promt hydroelectrolytic rebalancing • Reduce mediastinal radiation doses.

For type I myocardial dysfunction (Anthracyclines and analogs)	• Limited cumulative dose (mg/m^2):
	   Daunorubicin <800
	   Doxorubicin <500
	   Epirubicin <720
	   Mitoxantron <160
	   Idarubicin <150
	• Lipozomal Doxorubicin
	• Continuous perfusion administration
	• Dexrazoxan
	• ACEI or ARA
	• β-blockers
	• Statins
	• Phisical aerob activity

For type II myocardial dysfunction (Trastuzumab and analogs)	• ACEI • β-blockers

ARA- angiotensin receptor antagonists, PAD-peripheral artery disease, CAD-coronary artery disease, ACEI- angiotensin II converting enzyme inhibitors, HF-heart failure.

Current recommendations suggest that cardioprotective treatment with - angiotensin II converting enzyme inhibitors (ACEI) and/or beta blockers should be promptly initiated in case of asymptomatic decrease of LVEF below 53%, and that these patients should be monitored monthly using echocardiography, until treatment is completed and at 3, 6 and 12 months afterwards [3, 6].

Patients who develop symptomatic HF during or after antineoplastic treatment should be treated according to HF European Society of Cardiology current guidelines [4, 18].

No evidence is currently available to support specific cardioprotection when echocardiographic alterations of myocardial deformation parameters are detected [19].

2 STUDY OBJECTIVES

The main idea of the study based on the hypothesis according that the evaluation of myocardial deformation provides information on the risk for cancer treatment related myocardial dysfunction, a phenomenon that almost always precedes LVEF decrease in oncological patients [20].

The purpose of the study was to identify changes in myocardial deformation parameters and LVEF using 2D and 3D echocardiography during 6 months of study and thus their role in the early diagnosis of cancer treatment related myocardial dysfunction. Moreover, studying the relationship between these changes and antineoplastic treatment risk factors, haematological diagnosis, demographic factors and the presence of cardiovascular risk factors, contributes to a better risk stratification for cancer treatment related myocardial dysfunction.

The main objectives of the study are:

• Assessing the incidence of cancer treatment related myocardial dysfunction by LVEF calculation using 2D and 3D echocardiography over the 6 months of study;

• To determine myocardial deformation parameters changes evaluated by speckle-tracking echocardiography over the 6 months of study;

• To analyze the relationship between the studied echocardiographic parameters decrease and antineoplastic treatment risk factors (cytostatic class, dose, administered protocol, number of cures), haematological diagnosis, demographic factors and the presence of cardiovascular risk factors.

• To diagnose early, subclinical myocardial dysfunction using myocardial deformation parameters among the subgroups of patients with pathological changes of echocardiographic parameters.

• To document the crucial impact of modern echocardiographic techniques for risk stratification and early, subclinical diagnosis of cancer treatment related myocardial dysfunction.

Basically, we evaluated a group of patients diagnosed with haematological cancers under specific antineoplastic treatment throughout two moments: initially, pre-chemotherapy and 6 months after initiation of cytostatic treatment. On these two occasions, age, sex, cardiovascular risk factors (arterial hypertension, diabetes mellitus, dyslipidemia, positive family history for CV deseases, obesity and smoking), haematological diagnosis, treatment protocol, cytostatics classes and their association, together with the individual cumulative dose of anthracyclines, the number of therapy cycles performed and the association of radiotherapy.

All these variables were correlated with the echocardiographic parameters changes during the 6 months of study: LVEF, global longitudinal systolic strain - GLS, radial systolic strain - GRS and circumferential systolic strain – GCS, using conventional (2D echocardiography) and modern (3D echocardiography and speckle-traking echocardiography) echocardiographic methods.

For better characterization of longitudinal function, we also assessed: the simplified longitudinal strain (LSa4c), subendocardial longitudinal strain (LSsubendo) and subepicardial longitudinal strain (LSsubepi).

3 METHODS

Our study was organized in two stages. The first stage (October 2012 - May 2015) focused on the current knowledge and available data about cytostatic treatments action mode and especially on their cardiovascular effects, the risk factors associated with the occurrence of these adverse effects as well as the epidemiological data regarding the mortality and morbidity of patients with haematological, oncological and cardiovascular pathology. Most of this period was devoted to the knowledge of conventional and modern diagnosis methods and treatment in cardiology. Thus, this first part of completing the documentation and study structuring took place

in the Department of Cardiology of Filantropia Municipal Clinical Hospital, University of Medicine and Pharmacy Craiova, under the close supervision of MD. PhD Cârstea Doina.

The second stage (June 2015 - September 2016) materialized in a daily activity performed in the Echocardiography Laboratory, Department of Thoracic, Cardiac and Vascular Sciences, University of Padua, under the supervision of MD. PhD Luigi P. Badano and MD. PhD Denisa Muraru. During this period, I have mastered the ability to perform conventional echocardiographic examinations and special techniques (3D echocardiography and speckle tracking) especially applied to oncology and haematology patients, along with the process of collecting and interpret the study data underlying this research.

The echocardiographic protocol used in this study was designed in September 2015, being awarded by the European Association of Cardiovascular Imaging (EACVI) with a young researchers study-grant: Https://www.escardio.org/EACVI-past-grant-winners .

The study was prospective through an observational, descriptive-analytical research model, with time series (before and 6 months after the initiation of chemotherapy), carried out in a single research center: University of Padua, Department of Cardiology, University Hospital of Padua, Italy.

In our study, we defined myocardial dysfunction according to current recommendations (Expert Consensus for monitoring cardiac function during and after cancer therapy published under the auspice of European Society of Cardiology (ESC) [3, 12]), as a ≥ 10% symptomatic/asymptomatic reduction of LVEF to a value <53%.

Considering the data from the literature and the available recommendations, we considered the occurrence of early cancer treatment related myocardial dysfunction, patients at risk for possibly symptomatic decrease of LVEF, when myocardial deformation parameters registered a relative reduction of ≥15% from baseline, pre-chemotherapy value of [3,12,113].

3.1 STUDY POPULATION

Patients with haematological malignancies with cytostatic treatment indication from the Department of Hematology and Immunology of the University Hospital of Padua and referred a priori for a baseline echocardiography were eligible to be enrolled in our study.

Inclusion criteria were: age over 18 years; baseline LVEF ≥ 53%; no history of acute coronary event and/or revascularization procedure; no other cause of LVEF drop was found except for anticancer specific treatment during study enrollment (6 months); all patients were in sinus rhythm; informed consent has been signed.

Exclusion criteria were: patient refusal to participate in this study, known coronary diseases, HF symptoms, professional sports activity, pregnancy and LVEF <53% evaluated by 2D echocardiography.

3.2 ECHOCARDIOGRAPHIC PROTOCOL

We used Vivid E9 (General Electric Vingmed Ultrasound AS, Horten, Norway) ultrasound machine, equipped with dedicated probes for two-dimensional (M5S) and three-dimensional (4V) cord scans and a cut-out bed to facilitate maneuvering.

LVEF analysis using Simpson biplane method, 3D and speckle tracking datasets were performed offline, after the acquisition of the echocardiographic images, using a separate workstation using EchoPAC BT12 software (General Electric Vingmed Ultrasound).

4   RESULTS

This study evaluates the complete echocardiographic profile of 85 adult patients with haematological malignant neoplasms that underwent a baseline echocardiography in the cardiology ambulatory at University Hospital of Padua, Italy, between January and September 2016 and who were re-evaluated after 6 months of specific antineoplastic treatment.

1**. The studied population:**

• was represented by patients aged 21-86 years, 58.8% aged less than 65 years;

• included predominantly male patients: 63.53%;

• was composed of patients diagnosed with haematological neoplastic pathology, predominantly non-Hodgkin's lymphoma (NHL): 36.5%;

• included patients whose most common cardiovascular risk factor was hypertension: 33.8%.

In our study, we considered cancer treatment related myocardial dysfunction risk factors related to antineoplastic therapy: anthracycline treatment, cumulative Doxorubicin dose over 400

mg/m^2, "targeted" molecular therapies treatment, the use of combined cytostatic classes: anthracyclines , "targeted" molecular therapies and Cyclophosphamide, adjuvant treatment with mediastinal radiotherapy. We obtained the following results, aiming:

1. Left ventricular ejection fraction evaluated by 2D and 3D echocardiography modifications over the 6 months of study:

• The left ventricular ejection fraction evaluated both bi-dimensionally and three-dimensionally had a statistically significantly decrease over the 6 months of the study.

• There was no causality between the antineoplastic medication used in our study (anthracycline treatment, cumulative dose over 400 mg/ m^2 of Doxorubicin, "targeted" molecular therapies treatment, the use of combined cytostatic classes and adjuvant radiotherapy) and the 2D and 3D LVEF decrease over the 6 months of the study.

• The influence of cardiovascular risk factors (hypertension, diabetes, dyslipidemia, family history of CV diseases, smoking, obesity) for 2D and 3D LVEF decrease was not identified.

• 2D and 3D LVEF decrease was not determined by the gender or the age of the studied patients.

• Up to the 6th cytostatic cycle, 3D LVEF decrease was observed in more patients than 2D LVEF decrease (33% versus 22.4% of the studied subjects).

• After the 8th cytostatic cycle, 2D LVEF and 3D LVEF decreased for an approximately equal number of patients (45.6% and 48.3%, respectively).

2. Incidence of cancer treatment related myocardial dysfunction:

• The incidence of cancer treatment related myocardial dysfunction was 11.7% (10 cases) in our study diagnosed by 10% drop of LVEF from pre-chemotherapy value to a value of less than 53%, assessed by 2D echocardiography.

• Using 3D echocardiography evaluation, we observed an almost double incidence of cases that developed cancer treatment related myocardial dysfunction over the 6 months of the study (23.4%).

• First cases of LVEF decrease were observed after 4th cytostatic cycle, but the incidence of these cases increased with the number of cures, both in the two-dimensional and three-dimensional echocardiography evaluation.

• 3D echocardiography evaluation of LVEF diagnoses a larger number of patients who have developed cancer treatment related myocardial dysfunction and is therefore more sensitive for this purpose.

3. Global longitudinal strain modifications over the 6 months of study:

• GLS decrease assessed by speckle tracking echocardiography over the 6 months of the study was statistically significant.

• GLS decrease was influenced by anthracycline treatment, by the cumulative dose of more than 400 mg/m^2 of Doxorubicin and by the cytostatic classes combination: anthracyclines, "targeted" molecular therapies and Cyclophosphamide, specific combination for R-CHOP and R-COMP treatment protocols.

• Early detection of cancer treatment related myocardial dysfunction using GLS decrease of more than 15% from pre-chemotherapy value was observed after the 4th cytostatic cycle, but in 50-75% of cases it occurred after the 6th one in the subgroups of pacients treated with anthracycline, with a cumulative dose of more than 400/m^2 of Doxorubicin and with the combination of anthracycline, "targeted" molecular therapies and Cyclofosfamide (AMC).

• GLS decrease over the 6 months of study was more important in patients diagnosed with NHL and Hodgkin's Lymphoma (HL) than in patients diagnosed with multiple myeloma (MM), chronic myeloid leukemia (CML), acute myeloid leukemia (AML) or chronic lymphoblastic leukemia (CLL).

• GLS alterations in the 6 months of study were not influenced by "targeted" molecular therapies (Dasatinib, Rituximab or protease inhibitors such as Bortezomib and Calfilzomib) and mediastinal radiotherapy.

• The decrease of GLS between the two moments, pre-chemotherapy and at 6 months of study was statistically influenced by the presence of hypertension and dyslipidemia.

• Early detection of cancer treatment related myocardial dysfunction using GLS decrease of more than 15% from pre-chemotherapy value was observed in hypertensive and dyslipidemia patients as early as the 4th cytostatics cycle but in 50-75% this event was observed after the 6th cytostatics cure.

• For the subgroups of patients who do not associate hypertension and dyslipidemia, early detection of cancer treatment related myocardial dysfunction using GLS assessment was observed after the 8th cytostatic cycle.

• GLS changed over the 6 months of study independent of age, sex, presence of family history of CV diseases, smoking, obesity and diabetes mellitus.

• GLS decrease was observed in 37% of the studied patients after the 6th cytostatics cycle and in 65% after the 8th cycle (Figure 4.1).

• GLS is an echocardiographic parameter that can be used for the early diagnosis of cancer treatment related myocardial dysfunction starting with the 4th cure of chemotherapy.

4. **Simplified longitudinal strain modifications over 6 months of study:**

• The changes observed for LSa4c parameter evaluated by speckle tracking echocardiography over 6 months of study were statistically significant in our study.

• LSa4c decrease was influenced by anthracycline treatment, by the cumulative dose of more than $400/m^2$ of Doxorubicin and by the cytostatic classes combination: anthracyclines, "targeted" molecular therapies and Cyclophosphamide, specific combination for R-CHOP and R-COMP treatment protocols.

• Early detection of cancer treatment related myocardial dysfunction using LSa4c decrease of more than 15% from pre-chemotherapy value was observed after the 4th cytostatics cycle, but in 50-75% of cases it occurred after the 6th cure in the subgroups of patients receiving anthracycline treatment, with a cumulative dose of more than $400/m^2$ of Doxorubicin and with AMC combination.

• LSa4c decrease over 6 months of study was more important in patients diagnosed with NHL and HL than in patients diagnosed with MM, CML, AML or CLL.

• Changes in the LSa4c parameter observed during the study period were not influenced by treatment with "targeted" molecular therapies represented by: Dasatinib, Rituximab or proteasome inhibitors of Bortezomib and Calfilzomib, and mediastinal radiotherapy.

• The decrease of LSa4c between the two moments, pre-chemotherapy and at 6 months of study was statistically influenced by the presence of hypertension and dyslipidemia.

• Early detection of cancer treatment related myocardial dysfunction using LSa4c decrease of more than 15% from pre-chemotherapy value was observed in hypertensive and dyslipidemia

patients as early as the 4th cytostatics cycle but in 50-75% this event was observed after the 6th cytostatics cure.

• For the subgroups of patients who do not associate hypertension and dyslipidemia, early detection of cancer treatment related myocardial dysfunction using LSa4c assessment was observed after the 8th cytostatic cycle.

• LSa4c changes over 6 months of study were independent of age, sex, presence of family history of CV diseases, smoking, obesity and diabetes mellitus.

• After the 6th cytostatic cycle, similar to GLS, the decrease of LSa4c was observed in 33% of the patients, and after the 8th cure in 59% of the study group (Figure 4.1).

• LSa4c is an echocardiographic parameter that can be used for the early diagnosis of cancer treatment related myocardial dysfunction starting with the 4th cure of chemotherapy.

5. Subendocardial longitudinal strain modifications over the 6 months of study:

• The changes recorded by LSsubendo evaluated by speckle tracking echocardiography after 6 months of study were statistically significant.

• Subendocardial longitudinal strain decrease was influenced by anthracycline treatment, by the cumulative dose of more than $400/m^2$ of Doxorubicin and by the cytostatic classes combination: anthracyclines, "targeted" molecular therapies and Cyclophosphamide, specific combination for R-CHOP and R-COMP treatment protocols.

• Lsubendo's decrease during the study period was also influenced by "targeted" molecular therapies, especially by Rituximab treatment.

• For patients diagnosed with NHL, HL and CLL, there was a statistically significant decrease in LSsubendo parameter than in patients diagnosed with MM, AML and CML.

• Early detection of cancer treatment related myocardial dysfunction using LSsubendo's decrease of more than 15% from pre-chemothery value was able after the 4th cytostatic cycle, but in 50-75% of cases it occurred after the 6th therapy cure for the subgroups of patients treated with anthracyclines (Figure 4.2), with cumulative doses of$> 400/m^2$ of Doxorubicin, and with AMC.

• Changes in the LSsubendo parameter over 6 months of study were not influenced by mediastinal radiotherapy.

• The decrease of LSsubendo between the two moments, prechemotherapy and at 6 months of study was statistically influenced by the presence of hypertension and dyslipidemia.

• There is also a correlation between LSsubendo variation and the presence of family history of CV diseases and obesity, but with a much lower statistical significance.

• Early detection of cancer treatment related myocardial dysfunction using LSsubendo decrease of more than 15% from pre-chemotherapy value was observed in hypertensive and dyslipidemia patients as early as the 4th cytostatics cycle but in 50-75% this event was observed after the 6th cytostatics cure.

• For the subgroups of patients who do not associate hypertension and dyslipidemia, early detection of cancer treatment related myocardial dysfunction using LSsubendo assessment was observed after the 8th cytostatic cycle.

• The subendocardial longitudinal strain pathologically changes in our study were observed in the largest number of patients, so after the 6th chemotherapy cycle approximately 50% of the studied patients had a decrease in LSsubendo parameter and at 8th cure up to 88% of the patients had these abnormalities. (Figure 4.1)

• LSsubendo is the most appropriate echocardiographic parameter to be used for early diagnosis of cancer treatment related myocardial dysfunction, starting with the 3rd cytostatic cure (Figure 4.3).

6. Modification of the subepicardial longitudinal strain in the 6 months of study:

• The changes observed for LSsubepi parameter evaluated by speckle tracking echocardiography during the 6 months of the study were statistically significant.

• Subepicardial longitudinal strain decrease was influenced by anthracycline treatment, by the cumulative dose of more than $400/m^2$ of Doxorubicin, by "targeted" molecular therapies alone and by the cytostatic classes combination: anthracyclines, "targeted" molecular therapies and Cyclophosphamide, specific combination for R-CHOP and R-COMP treatment protocols.

• Changes in the LSsubepi parameter over 6 months of study were not influenced by mediastinal radiotherapy.

• Early detection of cancer treatment related myocardial dysfunction using LSsubepi's decrease of more than 15% from prechemothery value was able after the 4th cytostatic cycle, but in 75% of cases it occurred after the 6th therapy cure for the subgroups of patients treated with anthracyclines, with cumulative doses of> 400 / m2 of Doxorubicin, with "targeted" molecular therapies alone and with AMC combination.

• LSsubepi decrease was statistically significant in patients diagnosed with NHL, HL and CLL, compared to patients diagnosed with MM, AML and CML.

• LSsubepi decrease between the two moments, prechemotherapy and at 6 months of study was statistically influenced by the presence of hypertension and dyslipidemia.

• Early detection of cancer treatment related myocardial dysfunction using LSsubepi decrease of more than 15% from pre-chemotherapy value was observed in hypertensive and dyslipidemic patients as early as the 4th cytostatics cycle but in 50-75% this event was observed after the 6th cytostatics cure.

• For the subgroups of patients who do not associate hypertension and dyslipidemia, early detection of cancer treatment related myocardial dysfunction using LSsubepi assessment was observed after the 8th cytostatic cycle.

• Up to the 6th cytostatic cycle, the subepicardial longitudinal strain reduction was observed in 39% of the study patients, a percentage close to that of patients with changes in GLS and LSa4c parameters (Figure 4.1).

• After the 8th cure of cytostatic therapy, the decrease in LSsubepi is observed in 83% of the study patients, a percentage close to that of patients with changes in the LSsubendo parameter.

• LSsubepi is a parameter that can be used for the early diagnosis of myocardial dysfunction associated with cancer therapy starting with the 4th cure of chemotherapy, following LSsubendo, GLS and LSa4c myocardial deformation echocardiographic parameters.

7. **Circumferential global strain and radial global strain modifications over the 6 months of study:**

• GCS and GRS showed changes between the two moments assessed in our study but these were not consistent, not significant, and occurred in a small number of less than 10% of the studied patients. Therefore, these parameters were excluded from the subsequent statistical analysis.
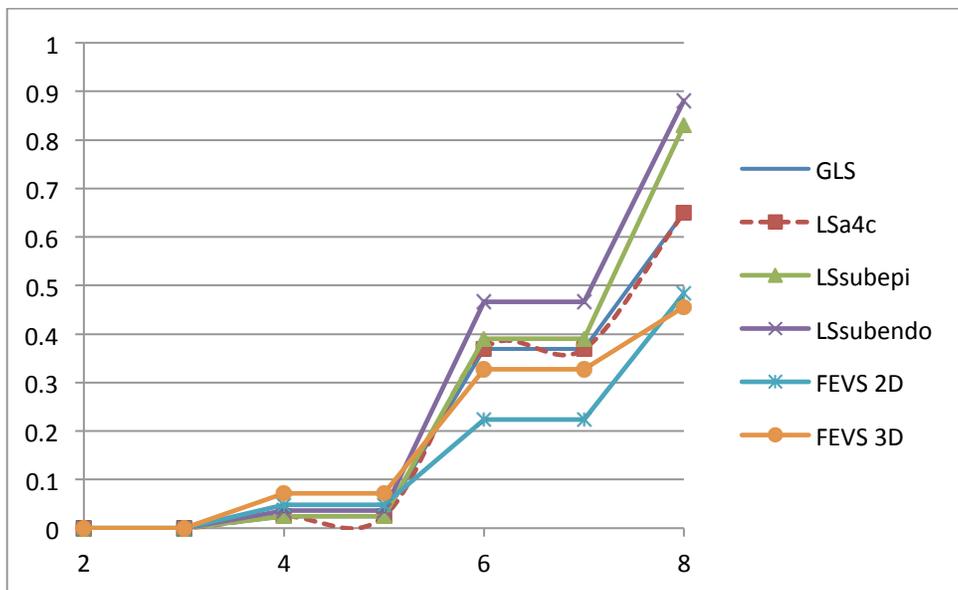
Figure 4.1 Kaplan-Meier curves for studied echocardiographic parameters variation depending on the number of patients in which we observed these variations and the number of cytostatic treatment cycles
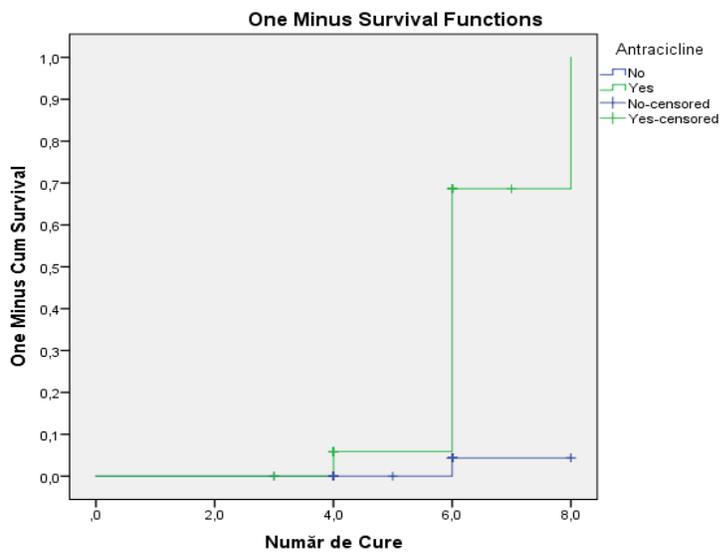

Figure 4.2 Kaplan-Meier curves for subendocardial longitudinal strain decrease with more than 15% from pre-chemotherapy value based on anthracycline treatment and the number of cytostatic cures
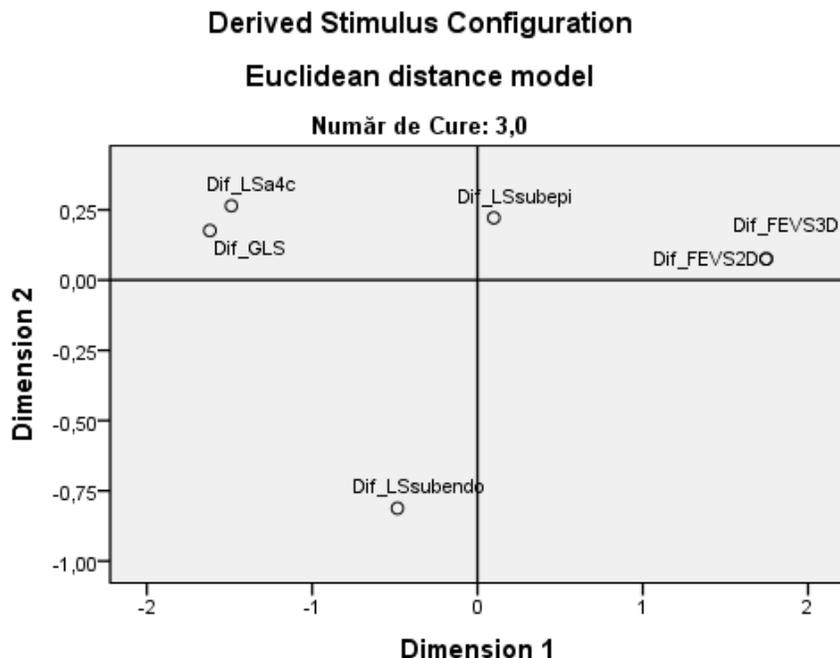
Figure 4.3 Euclidean distance pattern of the echocardiographic parameters after the 3rd chemotherapy cycle

5   DISCUSSIONS

This study analyzes in a detailed manner the echocardiographic profile of patients diagnosed with haematological neoplasms during specific cytostatic treatment. This goal was achieved through a complex study of myocardial deformation parameters (global longitudinal strain, simplified longitudinal strain, subendocardial longitudinal strain, subepicardial longitudinal strain, global circumferential strain and global radial strain), as well as the left ventricular ejection fraction. For this purpose, we used conventional 2D echocardiography and modern techniques: 3D echocardiography and speckle tracking echocardiography.

So we have endeavored to make a complex echocardiographic study. The results we obtained demonstrate that all analyzed echocardiographic parameters changed during the 6 months of study group follow up. We note that only for certain parameters of those listed, this changes were determined by specific cytostatic treatment and/or the presence of cardiovascular risk factors.

The existence of a physiological difference between the subendocardial and subepicardial fibers contraction strength has been known since Leonardo da Vinci described for the first time

the anatomy of the heart and the helical organization of the myocardial fibers. This fact was demonstrated by speckle tracking "multilayer" echocardiography for the first time in 2011, when a group of Italian researchers showed that there is a difference in subendocardial and subepicardial strain values in adult athletes [21].

Consequently, subsequent studies on "multilayer" speckle tracking echocardiography have highlighted the importance of subendocardial and subepicardial deformation for early diagnosis of left ventricular dysfunction, before LVEF reaches pathological values. Although the number of these trials is low and includes small number of cases, the importance of "multilayer" myocardial deformation parameters for the early diagnosis of various pathologies is unanimously demonstrated [21-40].

To date, no studies have been published about the influence of specific antineoplastic treatments on "multilayer" myocardial deformation parameters. Following our results, we have been able to demonstrate that LV myocardial mechanics are affected early during cancer therapy, mainly by the subendocardial layer and LSsubendo parameter. The fact that subendocardial myocardial fibers are the most sensitive to the effects of chemotherapy, being the first affected during cancer treatment related myocardial dysfunction, has been observed since the 1990s through electron microscopy on experimental models by Llesuy et al. [41].

Certainly, "multilayer" longitudinal myocardial deformation parameters may be considered as GLS surrogates, much more used and known in clinical practice, with more than 150 studies regarding its importance for the early diagnosis of cancer treatment related myocardial dysfunction.

According to the literature data [42-49], our results regarding the decrease of the longitudinal myocardial deformation parameters along the 6 months of the study in patients with haematological neoplasms are consistent, the parameters of longitudinal myocardial deformation modifying early during the specific treatment, leading to a decrease in LVEF.

The hypothesis that LSa4c could be used as a surrogate marker for the longitudinal deformation evaluation launched by Fikrle et al. [50] has been confirmed in our study.

Early diagnosis of myocardial dysfunction is possible using longitudinal deformation parameters assessed by speckle tracking 2D echocardiography. The evaluation of longitudinal myocardial deformation parameters is fundamental for the early identification of mechanisms underlying cancer treatment related myocardial dysfunction.

6 CONCLUSIONS

1. The incidence of cancer treatment related myocardial dysfunction diagnosed by LVEF assessment using two-dimensional echocardiography was 11.7% in our study. We noticed that the incidence of myocardial dysfunction doubles (23.4%) when we used three-dimensional echocardiographic for LVEF assessment.

2. In our study, there was no causal relationship between LVEF decrease assessed using 2D or 3D echocardiography, and the presence of antineoplastic therapy risk factors, haematological diagnosis, the presence cardiovascular risk factors or demographic factors.

3. Pathological changes of LVEF incidence, assessed by 3D echocardiography was greater than LVEF changes assessed using 2D echocardiography incidence up to the 6th cytostatic treatment cure. The number of patients who experienced a decrease in LVEF, evaluated by 2D and 3D echocardiography, tends to equalize after the 8th cytostatic treatment course.

4. 3D echocardiography is the preferred imaging method for LVEF evaluation for the diagnosis of myocardial dysfunction in patients with haematological neoplastic disease up to the first 6 months of cytostatic treatment, knowing that certain myocardium lesions may become irreversible.

5. In our study, we noticed that the decrease of longitudinal myocardial deformation parameters values (GLS, LSa4c, LSsubendo, LSubepi) after 6 months of study showed a higher incidence compared to LVEF decrease evaluated by 2D or 3D echocardiography.

6. Pathological changes of longitudinal myocardial deformation parameters assessed by speckle-tracking echocardiography, represented by GLS, LSsubendo, LSsubepi, LSa4c, could be observed early during cytostatic treatment after the 3rd and 4th cytostatic treatment cure.

7. LSsubendo is the echocardiographic parameter that undergoes pathological changes from the 3rd cytostatic treatment cure and for most of the patients studied (88%), being the most sensitive longitudinal myocardial deformation parameter for the early diagnosis of cancer treatment related myocardial dysfunction.

8. We observed that LSa4c echocardiographic parameter modifies under the same conditions as GLS, and we consider that LSa4c could be a simpler, faster, and more repeatable option for assessing longitudinal myocardial deformation in patients with haematological cancers during the first 6 months of cytostatic treatment.

9. In our study, we diagnosed early cancer treatment related myocardial dysfunction associated with anthracyclines treatment, with an overdose of 400 mg/m^2 of Doxorubicin as well as in the subgroup of patients treated with regimens involving cytostatic classes as: anthracyclines, molecular targeted therapies and Cyclophosphamide.

10. Early cancer treatment related myocardial dysfunction diagnosed by longitudinal deformation parameters GLS, LSa4c, LSsubepi and LSsubendo was observed in the subgroups of patients associating hypertension and dyslipidemia.

11. Our study demonstrates that the evaluation of longitudinal myocardial deformation by speckle tracking echocardiography could be considered as a method of early diagnosis, evaluation and monitoring of patients treated for haematological cancers up to the first 6 months of cytostatic therapy.

12. The GCS and GRS changes did not meet significant statistically criteria, demonstrating that impairment of longitudinal myocardial function preceded the reduction of circumferential and radial function, the latter expressing transmural affection.

13. Our study emphasizes the importance of evaluating longitudinal myocardial deformation parameters for the early diagnosis of cancer therapy related myocardial dysfunction because it has been already demonstrated that the decrease in LVEF evaluated by 2D echocardiography, even by 3D echocardiography, is a relatively late stage of myocardial dysfunction, when functional reserve of myocardium has been exhausted.

14. Current recommendations on cancer treatment related myocardial dysfunction screening, diagnosis, prophylaxis and treatment are based on expert consensus statements; prospective cohort studies are required, willing to provide clear references and support for the publication of guidelines aimed for the early detection, prevention and treatment of this condition.

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