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PhD THESIS

**TLR4 EXPRESSION IN CORRELATION WITH CARDIAC IRON
DEPOSITS BY T2 MRI * IN DOXORUBICIN TREATMENT -
TRANSLATIONAL CARDIOTOXICITY RISK ASSESSMENT**

A B S T R A C T

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INTRODUCTION

Brief history: Anthracyclines are among the most effective anti-cancer drugs ever developed. The first anthracyclines were isolated from pigment-producing *Streptomyces peucetius* in the early 1960s and were named doxorubicin (DOX) and daunorubicin (DNR).

Issue: Cardiotoxicity induced by certain anticancer pharmacological agents appears to be a potentially important problem among cancer survivors. For several decades, this subject has been associated almost exclusively with anthracyclines, for which dose-related cumulative heart damage has been the limiting step in their use. Although a number of efforts have been directed at predicting risk, to date there is no consensus on strategies for the prevention and monitoring of cardiotoxicity related to chemotherapy.

Recently, a new dimension of the problem has emerged when drugs targeting the activity of certain tyrosine kinases or tumor receptors have been recognized to have an undesirable effect on the cardiovascular system. Moreover, the higher-than-expected incidence of cardiac dysfunction that occurs in patients treated with a combination of old and new chemotherapeutics (eg anthracyclines and trastuzumab) has led physicians and researchers to find an effective approach to the problem.

Perspectives: From a pharmacological point of view, the presumed molecular mechanisms involved in chemotherapy-induced cardiotoxicity will be reviewed.

From a clinical point of view, current cardiotoxicity reduction strategies will be critically addressed. From this perspective, the precise identification of the antitarget (ie the unwanted target causing heart damage) and the development of guidelines for monitoring patients undergoing treatment with cardiotoxic agents appear to be the basis for the management of drug-induced cardiotoxicity.

Cardiovascular magnetic resonance imaging (MRI) is a non-invasive tomographic imaging technique. Its intrinsic advantages are the ability to acquire images in several planes without limiting the acoustic window and the habitus of the patients' body, the lack of radiation exposure, high contrast-noise ratios and real 3D volumetric coverage, without geometric assumptions. However, the unique opportunity that CMR offers is non-invasive characterization of myocardial tissue.

GENERAL PART

1. GENERAL INFORMATION ON ANTHRACYCLINE CARDIOTOXICITY

1.1. CLASSES OF CYTOSTATICS AND THE INCIDENCE OF CARDIOTOXICITY

Advances in oncology have led to the development of many antineoplastic agents for the treatment of cancer. The combination with other agents and modalities, together with dose escalation, has led to more toxicities, often requiring careful management and monitoring in healthcare.

Anthracyclines are among the most effective anti-cancer drugs ever developed. The first anthracyclines were isolated from pigment-producing *Streptomyces peucetius* in the early 1960s and were named doxorubicin (DOX) and daunorubicin (DNR).

Cardiovascular side effects resulting from oncological therapy may result in decreased quality of life and increased risk of mortality.

Anthracyclines and related compounds (doxorubicin, daunorubicin, idarubicin, epirubicin and anthroquinone mitoxantrone) are among the chemotherapeutic agents involved in cardiotoxicity. Anthracycline therapy is associated with an increased risk of heart failure with significantly increased morbidity and associated mortality (1).

The most distinct cardiotoxicity and with the most obvious clinical expression is by far a consequence of the use and administration of anthracyclines. Alkylating agents, such as cyclophosphamide, ifosfamide, cisplatin, carmustine, busulfan, have also been implicated in the phenomenon of chemotherapeutic-induced cardiotoxicity.

The anthracycline antibiotic family contains hundreds of analogues but only a few are in real clinical use. The best known and most widely used is doxorubicin. Anthracyclines are metabolized by reducing a ketone group to a hydroxyl group, which are less active than the parent compound.

Pharmacokinetic characteristics include a rapid (cellular / tissue) distribution step and a slow elimination phase, with the plasma half-life of doxorubicin and its metabolites in approximately 5 min, 1 hour and 30 hours, respectively. Because they are excreted mainly in the bile, special care should be taken in patients with hepatic impairment (2,3).

The latest anthracyclines, such as epirubicin, idarubicin or liposomal preparations, have higher lipophilicity and a supposedly higher safety. However, the risk of inducing cardiomyopathy is not diminished and there is always concern about tumor efficacy (4).

In the last 2 decades we have witnessed numerous attempts to identify new anthracyclines, which have been designed to prove superior to DOX or DNR in terms of cardiac activity and / or tolerability.

The search for a "better anthracycline" has led to around 2000 analogues, a figure that should not come as a surprise if one considers the number of chemical changes or substitutions and / or conjugations that can be introduced into the tetracyclic ring, the side chain, or amino sugar. However, only a few analogues have reached the stage of clinical development and approval; among them, epirubicin (EPI) and idarubicin (IDA) enjoy popularity as useful alternatives to DOX or DNR, respectively (Figure 1).

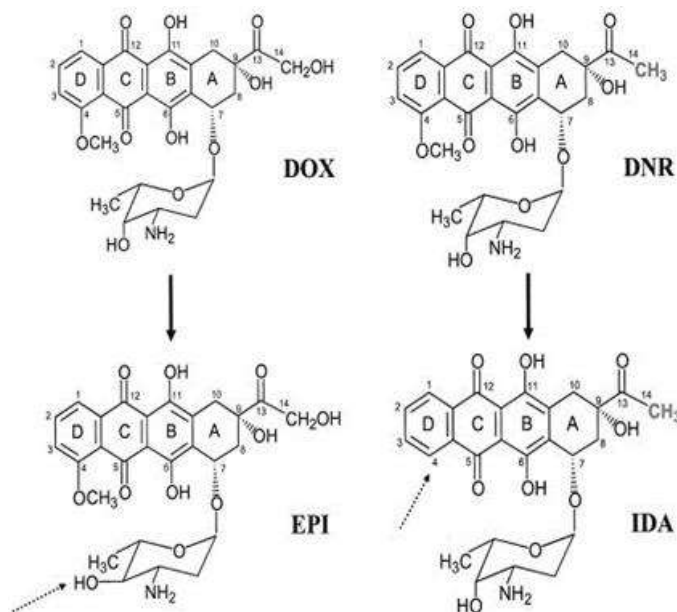


Figure 1 DOX, DNR, EPI and IDA structures. Gray-labeled residues indicate that the DNR or IDA side chain ends with a methyl instead of a primary alcohol compared to DOX or EPI. Dotted arrows indicate structural changes in PPE compared to DOX (axial-equatorial epimerization of the hydroxyl group to C-4 in daunosamine) or to IDA compared to DNR (lack of methoxy group to C-4 in ring D). (Adapted from Minotti et al. 2004).

EPI is a semisynthetic derivative of DOX obtained by axial-equatorial epimerization of the C-4 hydroxyl group in daunosamine (Figure 1). This change in position has a reduced effect on the mode of action and spectrum of activity of PPE compared to DOX but introduces pharmacokinetic and metabolic changes, such as increased volume of distribution (Vd), 4-O-glucuronidation, and therefore improved overall clearance (LC) or shorter terminal half-life.

Due to these kinetic and metabolic changes, EPI was used at cumulative doses almost double those of DOX, resulting in equal activity but without a significant increase in cardiotoxicity.

In practice, early studies in patients with advanced breast cancer showed that the mean doses for the development of laboratory rates of cardiotoxicity were 935 mg / m² EPI compared to 468 mg / m² DOX, and the cumulative median dose for heart failure (HF) symptomatic was 1134 mg / m² EPI compared to 492 mg / m² DOX.

These figures have been refined in subsequent studies, as a significant risk of HF has been documented in patients receiving cumulative doses greater than 950 mg / m²; thus the maximum recommended dose of PPE was carefully adjusted to 900 mg / m².

Thus, replacing DOX with PPE does not eliminate the risk of developing chronic cardiotoxicity. It should also be noted that the mechanisms underlying the reduced cardiotoxicity of PPE versus DOX may not be limited to glucuronidation and increased elimination. The actual mechanisms and dose dependence of improved PPE cardiac tolerance may therefore require further evaluation in both the preclinical and clinical contexts.

2. THE ROLE OF CARDIOVASCULAR MAGNETIC RESONANCE (CMR)

2.1. INTRODUCTION TO MAGNETIC RESONANCE

Cardiovascular magnetic resonance imaging (MRI) is a non-invasive tomographic imaging technique. Its intrinsic advantages are the ability to acquire images in several planes without limiting the acoustic window and the habitus of the patients' body, the lack of radiation exposure, high contrast-noise ratios and real 3D volumetric coverage, without geometric assumptions. However, the unique opportunity that CMR offers is non-invasive characterization of myocardial tissue.

CMR is contraindicated in patients with ferromagnetic implants, including non-MR conditioned cardiac devices. In particular, caution should be exercised in patients with breast cancer when evaluating breast tissue expanders that may contain some ferromagnetic components.

Additional limitations of CMR (Figure 6) include limited (but increasing) availability and expertise, cost, and potential patient claustrophobia. In patients with renal dysfunction and eGFR, administration of 30 ml / min / 1.73 m² of gadolinium-chelate contrast should be considered with caution due to the potential, albeit minimal, risk of nephrogenic systemic sclerosis (7).

2.2. T2 AND T2 MYOCARDIAL MAPING *

T2 myocardial mapping is a technique used to reconstruct a parametric image based on the T2 value measured in each voxel. Accumulation of water in the myocardium is associated with various types of pathology, such as acute myocardial infarction, myocarditis and graft rejection. Given a long time of transverse relaxation of water protons, T2 myocardial mapping appears to be promising for the detection of intramycardial water and even the quantification of myocardial edema. Verhaert et al. reported that T2 mapping may be useful for identifying at-risk myocardial regions and microvascular obstruction (21).

T2 * relaxation refers to the rapid decrease in transverse magnetization caused by the inhomogeneity of the local magnetic field. The magnetically recorded inhomogeneity comes from the inhomogeneity of the static magnetic field or from the differences in magnetic susceptibility between neighboring tissues, such as air-tissue interfaces, metal implants, paramagnetic contrast agents or iron deposition. Specifically, myocardial T2 * mapping is sensitive to tissue iron content and is therefore widely used to quantify the degree of myocardial iron deposition in patients with major thalassemia and repeated blood transfusion (22).

Myocardial T2 * MRI values are directly related to tissue iron levels. The minor effects of myocardial oxygenation and fibrosis are overwhelmed by the extremely dominant effect of iron in clinically relevant levels of myocardial iron overload. Myocardial T2 * values less than 20 ms indicate an iron overload, and this is considered severe when T2 * is less than 10 ms. Decreased T2 * myocardial activity is associated with systolic and diastolic ventricular dysfunction. For example, most cases of heart failure in thalassemia have occurred so far in patients with very low T2 * values (in the severe range).

Exceptions to this have occurred in patients with other causes of heart failure, such as concomitant congenital heart disease. In patients with heart failure suffering from aggressive chelation with continuous intravenous deferoxamine, longitudinal studies show that myocardial T2 * increases and this is accompanied by increases in ejection fraction and amelioration of heart failure. In cross-sectional studies, myocardial T2 * and ejection fraction of deferiprone patients were higher than those of deferoxamine.

The T2 * technique was initially developed in order to minimize imaging artifacts, for example, flow compensation was used and movement breathing was suppressed by holding the breath. For the measurement of the T2 * myocardium in vivo, a slice with a short short middle ventricular axis is obtained and a homogeneous region of interest is defined that includes both epicardial and endocardial regions, because iron is preferentially established in the epicardium compared to the endocardium. The analysis is limited to the septum to avoid susceptibility artifacts that occur from the veins of the anterior and posterior cardiac vessels and from the lungs (Figure 7). In addition, T2 * in septum has been shown to be a good indicator of global heart iron (22). In order to address heterogeneity in the distribution of iron in the myocardium, a multi-slice T2 * acquisition has also been proposed, but it is time consuming and the analysis is confused with the inclusion of susceptibility artifacts.

3. CARDIAC EFFECTS OF DOXORUBICIN - MAIN MOLECULAR - PHYSIOPATHOLOGICAL MECHANISMS

The cardiotoxicity of Doxorubicin most likely has a cause mediated by an induction of cellular apoptosis ("programmed" cell death), by producing and releasing multiple free radicals; according to recent studies, an overlap and a possible disorder for intracellular calcium homeostasis can be extrapolated (3). This effect is different from the antitumor target effect, which occurs by interfering with DNA replication, alkylation and cross-linking, RNA transcription and topoisomerase II inhibition (6).

3.1. OXIDATIVE STRESS AND CELL APOPTOSIS (SCHEDULED)

Oxidative stress can be characterized as a disorder in the economy and the balance between prooxidizing agents - antioxidants. All this multifactorial stress process has an obvious influence on the prooxidizing agents, thus being able to have a direction towards possible disorders and lesions of cellular nature and to the intracellular apparatuses and systems (24). This inequity in the distribution of these two types of agents may have as a starting point either an excess of reactive oxygen species - SRO - or a reduced or

inadequate amount of antioxidant agents. , such as superoxide dismutase (SOD), vitamins or a catalase.

From a pathophysiological perspective, oxidative stress is also blamed in the emergence and evolution of other different pathologies and ROS play a rather eloquent role in the occurrence of cardiotoxicity induced by anthracycline agents. Following the administration of Dox, free radicals appear, through the process of adding an electron to its quinone fraction, continuing this compound to have a return to its original status, by reducing an oxygen to a superoxide anion ($O_2^{\bullet -}$) (Figure 9).

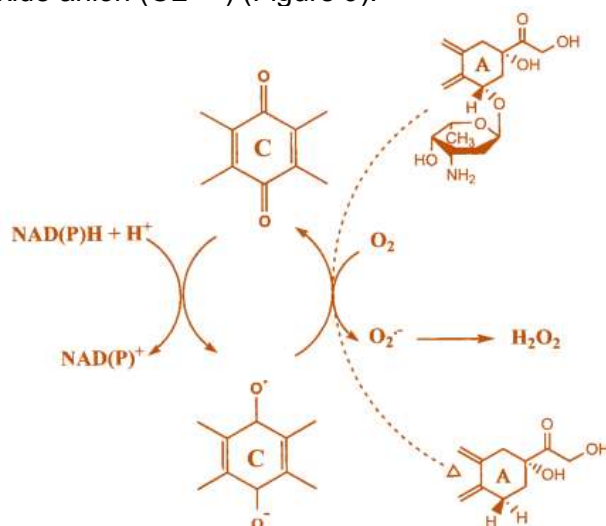


Figure 9 Production of oxygen free radicals (ROS) after the use of anthracycline [Y]; NAD (P) + - nicotinamide adenosine dinucleotide phosphate

Reactive oxygen species determine the induction of apoptosis, either following the extrinsic pathway [with Fas mediation (it is a type II transmembrane protein complex and belonging to the tumor necrosis factor family - TNF)] and following the intrinsic pathway (mitochondrial characteristic).

3.2. CELLULAR APOPTOSIS OR PROGRAMMED CELLULAR DEATH

Currently, the "RSO and iron" hypothesis is widely used to explain programmed cell death. Recently, new data have been circulating that Dox can interact directly with certain trigger factors for (programmed) cell death (25).

The main types of cell death are listed as follows:

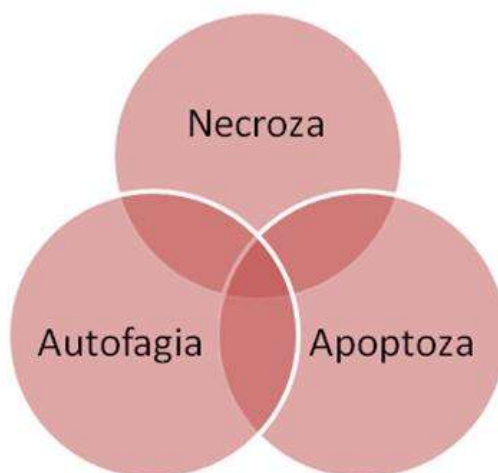


Figure 10 The main forms of programmed cell death

Necrosis is defined as an extended and uncontrolled cell death with an intense inflammatory cellular infiltrative process.

Apoptosis expresses a set of programmed cellular processes, but without a rupture of the plasma membrane, with multiple characteristic morphological changes, manifested by a decrease in cell volume, a condensation of chromatin and a fragmentation of the cell nucleus (nuclear).

Autophagy occurs at a local level, for example in a cell and shows an intense catabolic process, which includes a degradation of its own intracellular components by the initiation of a lysosomal mechanism.

Doxorubicin induces a process of mitochondrial DNA damage, mitochondrial membrane damage, multiple mitochondrial dysfunctions, and ATP depletion, all of which contribute to an intense and extensive process of necrosis (26–29).

There are currently multiple studies that support the idea that Dox induces a process of cellular apoptosis in the cardiomyocyte by activating the p53 component (30,31), the emergence of the so-called "down-regulation" process for GATA -4 (27,32) and for a degradation process of p300 (33,34). The transcription factor GATA-4 has a major role in the survival of postnatal cardiomyocytes, differentiated and also expresses an activator of the antiapoptosis gene Bcl-X (35). This negative regulatory process initiated by anthracycline compounds for GATA-4 is mediated by a transcriptional inhibition for the GATA-4 gene, this inhibition process being directly dependent on p53, which plays a role in blocking CBF / NFY binding to the CCAAT sequence, from the GATA-4 promoter (36). The transcriptional coactivation factor p300 has an implication for a multitude of intracellular reactions, including as a regulatory factor of cell cycles, cell differentiation and last but not least in the processes of tumor genesis and apoptosis (18,37). Certain kinases, for example p38 alpha and beta, activated by Dox, are corroborated with a phosphorylation of the factor p300 and are also responsible in its degradation process, all these phenomena being carried out in parallel with a cellular apoptosis that was observed in the level of primary neonatal cardiomyocytes, as well as a response to Dox administration (4). A pharmacological blockade for p38 is a preventive factor for p300 degradation. A recovery of GATA-4 (38) and p300 (39) inhibits doxorubicin-induced cardiomyocyte apoptosis.

Autophagy, which as I specified is a lysosomal process for the degradation of its own cellular constituents, and plays an extremely important role in the renewal process of cardiomyocytes, in this case represented by long-lived postmitotic cells (31) . Thus, it can be said that autophagy recycles certain cellular constituents of degradation and that it is potentiated in certain pathological conditions, including heart hypertrophy and dilation, cardiomyopathies and heart failure. There are some data in the literature that indicate that the process of autophagy has a strong dual effect on the heart under stress: a strong benefit through the process of removing those protein aggregates and for destroyed intracellular organs, thus helping to maintain an energetic homeostasis and a negative result, of a harmful nature, through an excessive autophagic process, resulting in cell death (40).

5. EXPERIMENTAL STUDY

5.1. OBJECTIVES

The permanent cardiotoxic effects caused by conventional cytostatic therapy emphasize the need for early diagnostic methodologies with increased specificity and sensitivity to approve early detection of signs of cardiac dysfunction, a primary consequence for the cardiotoxic effects of cytostatic medication. The specificity and sensitivity of any test should allow for a separate examination of the risk / benefit ratio, which is a balance

between the likelihood of heart failure due to cumulative high doses of medication and the consequences of stopping that source therapy. antitumor.

In local specialized studies, with reference to this topic, there is very little information on the monitoring and management process for Dox cardiotoxicity. The analysis of this study may implicitly provide a broad set of solutions for establishing a method for reducing and preventing cardiotoxicity, even through the use of high doses of anthracyclines, with a higher rate of concomitant healing and an improvement in the quality of life of those patients.

The target of this thesis is an early identification and correlation for Dox cardiotoxicity by using a diverse range of paraclinical methods: imaging (nuclear magnetic resonance T2 *) and biological - biochemical markers specific to iron metabolism and economy [transferrin], and by a determination and analysis of a genetic risk score indicated by a genetic quantification for the presence of a TLR4 gene expressive couplet.

It is hoped that the main results of this study will be confirmed as useful in determining a new long-term screening tool for surveillance, prevention and further treatment, where necessary in patients who have survived neoplastic conditions. .

5.2. MATERIAL AND METHOD

5.2.1.GENERAL ASPECTS

This study was a prospective analytical, multicenter study that took place between 2018 and 2019.

Each subject included in the study underwent clinical anamnestic evaluations and complex paraclinical examinations. Patients who had symptoms of cardiac impairment prior to initiating chemotherapy were not included in this study.

Patients or their legal representatives have been informed (orally and in writing) in advance of the potential toxic effect of chemotherapy, including cardiotoxicity (by a attending physician). They were also presented with methods for assessing cardiac function and treatment options in the event of the development of cardiotoxicity.

The inclusion of patients in the study was performed only after obtaining individual informed consent in writing from them or their legal representatives.

The study was presented to the ethics commission within the Arad County Emergency Clinical Hospital and approved by this commission and complies with all national and European norms and regulations at the time of its development (Annex II).

Forms and a complete set for the legal annexes of this study are presented at the end of this thesis (Annex).

This study complied with applicable international regulations and was conducted in accordance with the ICH-GCP (International Conference on Harmonization - Good Clinical Practice) Recommendations and the Helsinki Declaration, adopted by the General Assembly of the World Medical Association since June 1964.

5.2.2. STUDIED POPULATION

Our cohort included 25 consecutive patients aged 18 to 65 years who received Dox treatment for haematological malignancies (leukemia, lymphoma or multiple myeloma) with a probability of survival > 6 months and a FEVS > 50% and who they gave written consent.

5.2.2.1. Inclusion / exclusion criteria

The main criteria applied in the selection of subjects in this study are presented below:

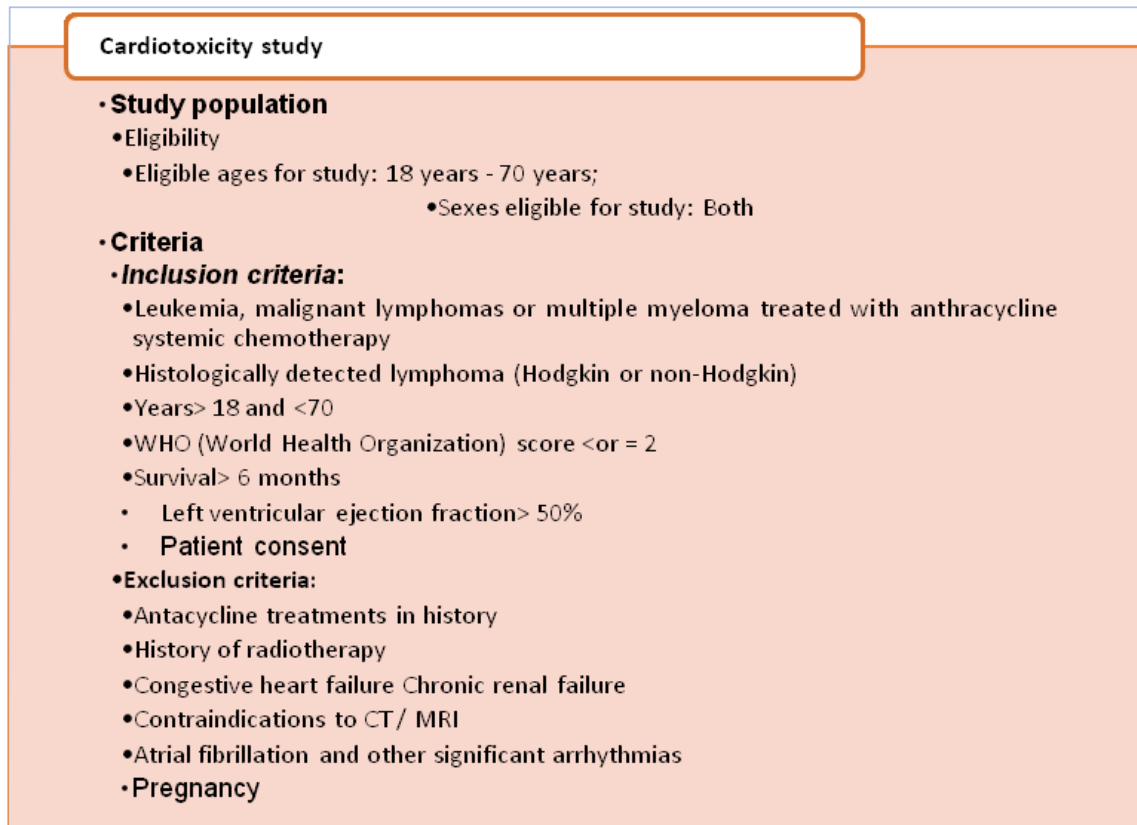


Figure 15 Overview of the study methodology - inclusion / exclusion criteria

5.2.3. STUDY METHODOLOGY

5.2.3.1. Evaluation methods

The main study methods:

- ♥ Biological products: Transferrin
 - from peripheral blood
- ♥ Cardiac magnetic resonance
 - T2 * - iron loading
- ♥ Genetic markers for TLR4
 - from peripheral blood
- ♥ Statistical analysis
 - Prospective Cohort Study: Using GraphPad Prism version 8.0.0 for Mac OS X.

5.2.3.2. General methodology of genetic and biological study investigation

A peripheral blood sample was taken from all patients on fasting for at least 12 hours to assess TLR4 gene expression and serum transferrin levels.

Gene expression was assessed and quantified by qRT-PCR using the following steps: blood collection (3 ml), RNA isolation, cDNA reverse transcription, qRT-PCR and quantification of relative expression. The blood sample was taken directly from a RNA preservation tube in the blood, Tempus (4342792 Applied Biosystems).

Serum transferrin levels were also quantified at follow-up (3 and 6 weeks).

A diagram of the whole process is illustrated in Figure 20.

5.2.3.3. Material and method of genetic study

5.2.3.3.1. Used materials

1. Tempus TM Blood RNA Tubes 50/Pkg
2. Tempus Spin RNA purification kit. 50/Pkg
3. 50 ml Conical Tubes (racked) 200/Pkg
4. RNase Inhibitor, 20 Units/ μ l, 100 reactions 2000 Units
5. Nuclease-free Water (1.75 ml/tube)
6. Eppendorf epTIPS LoRetention Reloads, PCR clean,
7. epTIPS LoRetention Reloads, PCR clean, 2-200 μ l, 10
8. epTIPS LoRetention Reloads, PCR clean, 50-1000 μ l,
9. twin.tec real-time PCR Plate 96, skirted (Wells white)
10. Eppendorf PCR tubes 0,5mL, colourless, 500 pcs
11. Eppendorf 0,2 ml PCR tubes, colourless, per 1,000 pcs.
12. Heat Sealing film, 100 pcs.
13. PCR Film (self-adhesive), 100 pcs.
14. ETHANOL PURISS P.A. ABSOLUTE puriss. p.a.,
15. High-Capacity cDNA Reverse Transcription Kit

Figure 17 Materials used - genetic study

5.3. RESULTS

The results of the investigations undertaken are further formulated and discussed in the light of data from the current medical literature and illustrated using graphs, tables and other forms of suggestive graphical representations.

5.3.1. CHARACTERIZATION OF THE STUDY BATCH

5.3.1.1. MAIN CHARACTERISTICS OF THE STUDY LOT

The total constituent group of the study considered consists of 25 subjects (patients) who present a various oncological pathology and who received a oncological treatment regimen with doxorubicine.

The group of subjects for control considered was composed of 25 subjects.

The essential characteristics and main elements used for the preparation of this lot are formulated on the basis of the following criteria:

- ♥ An average age with a value close to that of the study group
- ♥ and a uniform distribution for the distribution and distribution by sex.

Another element considered was the non-existence prior to the study for an oncological pathology (regardless of whether it was with or without treatment in the anthracycline class).

According to the data of the "age" reference category, the descriptive analysis process found that an increased number of patients were included in the age category <57 years, respectively 13 cases (66%), followed by the category > 57 years with 8 cases (22%), the lowest values were for 4 cases (12%) being distributed in the category > 60 years.

Out of a total of 25 patients taken into the study group and incorporated into the main cohort of analysis (100%), 8 patients (32%) were diagnosed with various haematological malignancies and 17 (68%) with solid tumors (Figure 30).

Of these cases, the following are distributed: ALL 3 (12%), 1 (4%) cases of AML, 4 (16%) cases with NHL.

16 patients (64%) did not receive treatment with other cardiotoxic antineoplastic agents, 3 patients (12%) received only cyclophosphamide, 3 (12%) received high-dose cyclophosphamide and ifosfamide, 2 (8%) cyclophosphamide and high-dose cytarabine, 1 patient (4%) was treated with actinomycin alone.

At the end of the study for enrolled subjects, the following cumulative doses of anthracyclines were quantified. The average cumulative dose is 450-500 mg / sqm, with a median of 432 mg / sqm, thus maintaining a cumulative anthracycline dose below a potential

risk value of 450 mg / sqm. The minimum doses administered to certain subjects in the study group were 240 mg / sqm, and the maximum 600 mg / sqm.

The distribution of data in the subjects included in the study is as follows: 3 subjects (12%) were classified in the category <200 mg / sqm, 6 patients (24%) in the category 200-450 mg / sqm, 8 patients (32%) were included in the category 450-550 mg / sqm, and 5 patients (20%) had cumulative doses of anthracycline agents that were above the limit doses of 550 mg / sqm: 2 patients (8%) in the group 550-700mg / sqm and 1 patient (4%)> 700mg / sqm.

The distribution according to the type of manifestation of cardiotoxicity is as follows: 16 patients (64%) did not show any cardiotoxic manifestations of clinical or paraclinical nature throughout the monitoring period, 6 patients (24%) exhibited elements of subclinical cardiotoxicity, and 3 patients (12%) expressed clinical manifestations of cardiotoxicity.

The distribution of cases according to this "temporal" classification was as follows (n = 9): 2 cases (22.22%) with cardiotoxic presentation with an early onset, 6 cases (66.66%) with chronic elements of cardiotoxicity and finally 1 case (11.11%) for late onset cardiotoxicity.

The mean amount of gene expression units was 0.121 for TLR4 (range 0.051-0.801). mRNA levels for the target genes were normalized with reference to a GAPDH gene; the result is presented as relative gene expression. The average amount of mRNA extracted was 113,571 µg / µl (Figure 50).

There is a strong (negative) descending linear relationship between the expression TLR4 and the CMR values T2 * ($r = -0.9106$, **** $P < 0.0001$), where r = the maximum simple correlation coefficient (Pearson). There is also a linear correlation (either positive or negative) with EF and Transferrin; no established relationship related to the genetic gender of patients. The main TLR4 correlations are presented in Table 8.

Serum transferrin levels (90 - 25.87 ng / mL) in the initial determination had a significant correlation ($r = 0.898$; $P < 0.0001$) with the 6-week determination (108 - 40.13 ng / mL). There were no statistical differences based on gender or correlation for this evaluated parameter (Figure 51).

5.4. DISCUSSIONS

Although traditional cytotoxic chemotherapy is still widely used, the clinical development and use of agents targeting signaling pathways has increased significantly. Disruption of these signaling pathways can lead to effects on cardiovascular tissue (CV) that can lead to adverse cardiac events, often referred to as cardiotoxicity. Cardiotoxicity may occur at any time during or after cancer therapy, depending on the pathophysiological effects of the agent, the patient's pre-existing cardiovascular risk factors, and the patient's physiological reserves. Radiation damage to exposed cardiovascular tissue occurs in a dose-dependent manner, leading to inflammatory response, cell death, and resulting fibrosis.

Understanding the mechanism of action of new (or investigative) anticancer agents on cardiovascular tissue can help identify specific effects to be evaluated in non-clinical studies and then prospective monitoring of these events in clinical trials. Cardiotoxicity attenuation and implementation of cardioprotective strategies may be considered before, during, or after therapy. The combination of multiple biomarkers and an integrated biomarker strategy and imaging measures are also areas of active investigation in cardio-oncology.

In addition to the apoptotic forms of cell death, already studied and known, there are non-apoptotic forms of cell death, which can trigger a systematic process of cascade of inflammation by releasing molecular models associated with danger (DAMP), to be recognized by the born immune receptors (IIRs) (137). The mechanisms of pathophysiology that trigger a cardiomyocyte dysfunction secondary to an iron overload process are partially unknown (Figure 54).

We postulate that the entry of an endotoxin into the circulation triggers TLR4-mediated systemic inflammation (an early IIR response). Endotoxin is in this case the rationing scenario a key component for Gram-negative bacteria and also a TLR4 ligand (138,139). Under normal circumstances of homeostasis, a large amount of bacteria that

present endotoxin live in the intestine and are strictly limited by the intestinal mucosal barrier. But this barrier could be broken by doxorubicin, which is known to be able to disrupt the epithelium to induce oral ulcers, intestinal inflammation and hemorrhagic cystitis in cancer patients (140,141). If doxorubicin caused damage to the intestinal mucosa, endotoxin could enter the circulation and stimulate systemic inflammation.

Furthermore, doxorubicin has been found to upregulate TLR2 and 4 expression in cardiomyocytes (31). It is possible that this molecule directly raises TLR4 levels in macrophages, resulting in stronger inflammatory responses to endotoxin and more severe damage to various organs.

Usually, a significant left ventricular dysfunction (decrease by more than 10% for LVEF) has already occurred when cardiotoxicity is detected by imaging techniques (with clinical manifestations). Biomarkers, the most important cardiac natriuretic peptides and troponins, have been promising markers for identifying patients at potential risk of clinical symptoms of heart failure (142). However, so far, these biomarkers have not proved their practical utility as a screening and management tool for anthracycline-induced cardiotoxicity.

To be even more specific, the utility of troponin has been demonstrated mainly after the administration of antineoplastic and β -sympathomimetic drugs, although the routine use of these markers in monitoring patients receiving anthracycline therapy is far from being resolved (143).

Pre-existing comorbidities or unfavorable lifestyle (hypertension, diabetes, hyperlipidemia, reduced physical activity) have long been known to increase the risk of cardiotoxicity in patients receiving anthracyclines and were thus determined and analyzed in the batch study.

In our daily medical practice, identifying patients at high risk for cardiotoxicity is extremely important.

It should be noted that there is also a large individual variability in anthracycline sensitivity for each patient.

6. CONCLUSIONS AND PERSONAL CONTRIBUTIONS

- ♥ The present thesis aimed to study possible applications of cutting-edge imaging and biological-genetic methods, such as T2 * cardiac MRI and TLR4 analysis of peripheral blood, in the study of Doxorubicin-induced cardiotoxicity.
- ♥ At present, we do not yet have a consensual definition for cardiotoxicity.
- ♥ TLR signaling produces an activation and maturation of immune system cells and is indispensable for an effective immune response against pathogenic microorganisms as well as against malignant cells.
- ♥ Some experimental studies have shown that Doxorubicin may cause a direct increase in the level of intracellular iron in the pool of intracellular labile iron, which is normally the equivalent of about <5% of total intracellular iron.
- ♥ Physiologically, the vast majority of cells in the human body accept iron through the intervention of a plasma glycoprotein, transferrin.
- ♥ The pathophysiology of doxorubicin consists in the accumulation and overlap of its effects, having as main elements the formation of free radicals and the disturbance of iron metabolism.
- ♥ Although the ORS and iron theory is widely recognized as a primary mechanism in doxorubicin toxicity, the use of antioxidants in practice, during clinical trials and experimental animal reproduction models, has failed to cushion and reduces anthracycline-induced cardiotoxicity.
- ♥ Harnessing the beneficial effects of TLR4 stimulation remains a challenge for cancer research.
- ♥ Anthracycline cardiotoxicity is associated with cardiac inflammation, mediated by the nonspecific immune system.

- ♥ The working methodology designed for this study can be a standard for the elaboration and diversification of other experimental sketches in this field of interest (or not only, through the interdisciplinary or correlated approach of human study and experimental model, of this thesis).
- ♥ Some cardiac protective agents may be used, which are associated with a decrease in cardiotoxicity and facilitate the use of higher cumulative doses of anthracyclines. Cardiotoxicity to anthracyclines can also be minimized by using analogues that may be less cardiotoxic.
- ♥ Definitions and detailed scientific and technical requirements for drugs used in gene therapy and drugs used to prevent anthracycline-induced cardiotoxicity need to be updated. Furthermore, detailed scientific and technical requirements should be established for tissue engineered products as well as for medical devices containing advanced therapy medicinal products and combination gene therapy medicinal products.