**“BABEȘ-BOLYAI” UNIVERSITY CLUJ-NAPOCA**
**Faculty of Biology and Geology**
**Doctoral School of Integrative Biology**

**DOCTORAL THESIS SUMMARY**

# Investigating Doxorubicin-Induced Cardiotoxicity: Circulating Biomarkers, Molecular and Pathological Insights

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# Table of Contents

## List of abbreviations

CK-MB – creatine kinase – myocardial band

CRP – C-reactive protein

CTOX - cardiotoxicity

CTRCD – cancer treatment-related cardiovascular dysfunction

DNA – deoxyribonucleic acid

DOX – doxorubicin

GAL-3 – galectin-3

MB – myoglobin

miRNA – micro ribonucleic acid

MMP – metalloproteinase

MPO – myeloperoxidase

NT-proBNP – N-terminal prohormone of brain natriuretic peptide

PIGF – placental growth factor

ROS – reactive oxygen species

sST2 – soluble suppression of tumorigenicity 2

**Keywords: doxorubicin, cardiotoxicity, circulating biomarkers, early diagnosis**

# SUMMARY

Doxorubicin (DOX) is a highly potent chemotherapeutic agent used in the treatment of many cancer types, from solid tumors to hematological malignancies, in both adult and childhood cases. The most concerning side effect of DOX is cardiotoxicity (CTOX) or cancer treatment-related cardiac dysfunction (CTRCD), which often progresses to irreversible heart failure (HF).

Symptoms of DOX-induced CTOX are, in most cases, silent or non-specific in the acute stage, making diagnosis and treatment difficult. While some patients start developing symptoms during or shortly after treatment, others are diagnosed many years after treatment completion.

Currently, CTOX diagnosis is based on a combination of non-specific heart-related laboratory tests and imaging studies, the latter requiring highly trained medical practitioners and advanced medical equipment that is not easily accessible and are often invasive, which brings additional stress to oncologic patients.

The best strategy for identifying DOX-induced CTOX is the use of specific circulating biomarkers, that can be easily assessed in all medical laboratories faster compared to imaging methods, with the additional benefits of reduced costs and minimal invasiveness.

Thus, this thesis aims to identify a set of circulating biomarkers for the early detection of DOX-induced CTOX which could facilitate timely diagnosis and therapeutic intervention. In pursuit of this aim, the following objectives were set:

- Identification of a set of circulating plasma biomarkers for DOX-induced CTOX focusing on their potential to predict early changes associated with the disease.
- Investigation of the relationship between the selected biomarkers and modifications induced by DOX treatment in other targeted organs beyond the heart.

Chapter I presents general information regarding DOX uses, mechanism of action and adverse reactions associated to the treatment.

Chapter II reviews current knowledge regarding cardiac pharmacological toxicity of cancer treatments, including biomarkers studied for early diagnosis and classical methods

currently used for diagnostic and prognostic purposes (Figura 1). This chapter was published in Farmacia 72(5): 975-986 (2024) as Anca, E., Banciu, M., Roșioru, C.R., Dobre, C., 2024. Cardiac Surveillance in Oncology: A review of circulating biomarkets and diagnosis methods in chemotherapy-induced cardiotoxicity. https://doi.org/10.31925/farmacia.2024.5.1

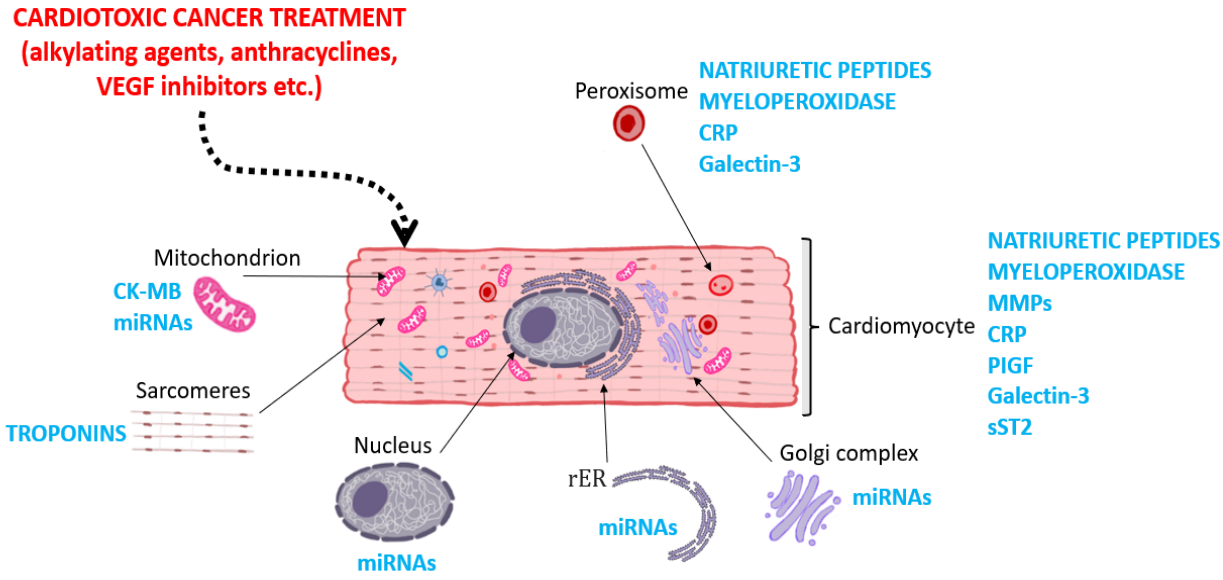


**Figure 1.** Overview of the targets for the biomarkers discussed in the review. Natriuretic peptides – volume/pressure overload; myeloperoxidase, metalloproteinases (MMPs), C-reactive protein (CRP), placental growth factor (PIGF), Galectin-3, soluble suppression of tumorigenicity 2 (sST2) – oxidative stress, inflammation, cell injury and necrosis, apoptosis; troponins – contraction mechanism; miRNAs – cell proliferation, differentiation and maturation, inflammation and metabolism reprogramming; creatine kinase – myocardial band (CK-MB) – energy homeostasis (created using Procreate®).

Chapter III aimed to assess a battery of circulating biomarkers in early and late DOX-induced CTOX along with histologic and ultrastructural analyses on the main tissues targeted by DOX toxicity. As the experiment was designed as a long-term study, investigating toxicity beyond the heart was considered of importance. To this aim, rats underwent a therapeutic protocol known to induce CTOX using two different DOX formulations and biomarkers were assessed from weekly plasma collections for the early manifestations of CTOX. For the investigation of late CTOX, a subset of the classical DOX-treated group was kept in the experiment without any additional treatment and plasma samples were collected weekly. Results indicated that plasma iron, calcium, Gal-3 and NT-proBNP levels could be correlated

to the extent of early cardiac damage produced by DOX during and shortly after treatment completion in both formulations. In late CTOX, biomarkers related to lipid metabolism such as total cholesterol and triglyceride levels could be correlated to cardiac risk as their plasma concentration continues to increase in the post-treatment period. In all organs analyzed, DOX led to progressive tissue dysfunction manifested as apoptotic-like cell appearance, myofibrillar disarray and vascular damage and immune cell infiltration. This chapter was partly published in Studia Universitatis Babeş-Bolyai Biologia 69(2): 7-22 (2024) as Anca, E., Sabău, F., Vădan, A., Marinescu, M., Licărete, E., Roșioru, C., Stoica, A.D., Dobre, C., Banciu, M., Assessment of circulating biomarkers in a rat model of doxorubicin-induced cardiotoxicity. https://doi.org/10.24193/subbbiol.2024.2.01

Chapter IV aimed to investigate the transcriptomic landscape of heart tissue in early CTOX to improve understanding of the pathways affected by DOX treatment and associate these alterations to the results described in chapter III, which corroborated, could facilitate early diagnosis of CTOX. A summary of the experiment is found in Figure 2.

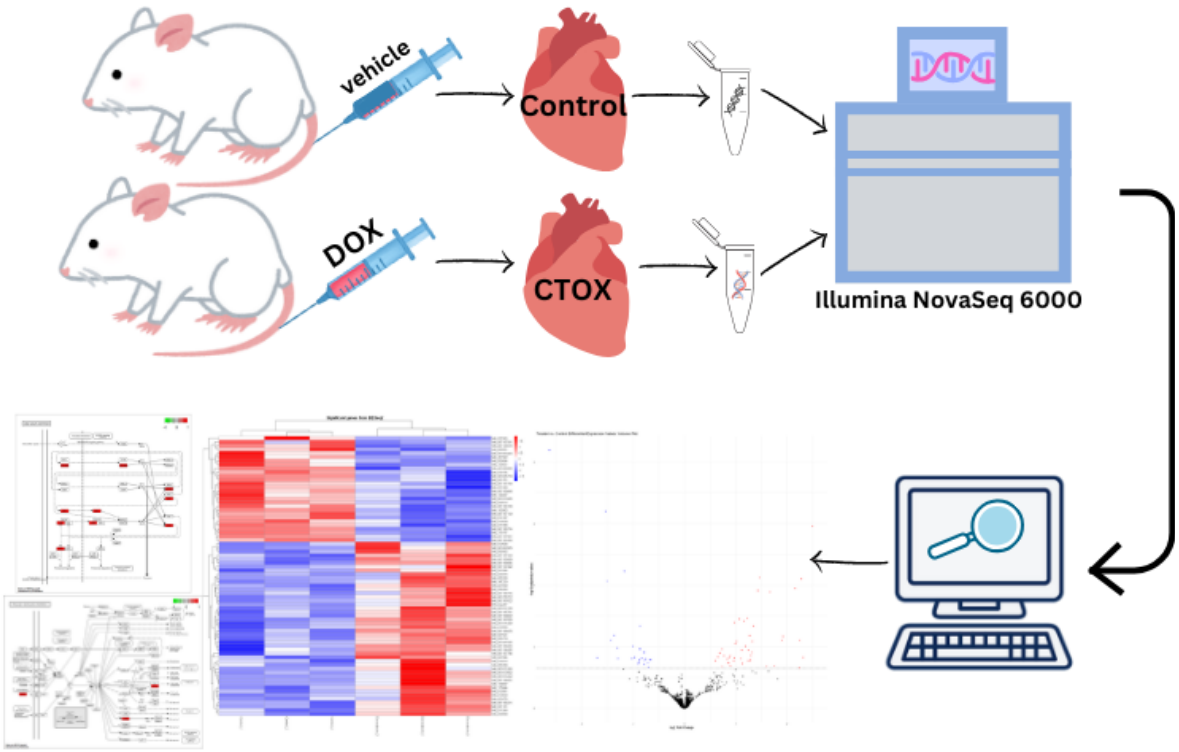


**Figure 2. Schematic summary of the experiment.** Adult male rats were treated i.v. with doxorubicin (DOX) or vehicle (Control) for 4 weeks. At the end of the treatment, rats were

sacrificed and RNA was extracted from heart tissue for transcriptomic analyses. Created using Canva (https://www.canva.com/).

Results presented in this chapter indicate a direct correlation between known DOX effects, circulating biomarkers and transcriptomic alterations. Briefly, findings indicated that this treatment induced significant alteration of pathways involved in inflammation, hormone signaling and circadian rhythm, which are all known to contribute to CTOX. The most important finding is related to the hypercholesterolemia observed in the biomarker study and the transcriptomic alteration of the circadian rhythm, as sleep impairment may contribute to the dysregulation of lipid metabolism and sustain the CTOX-promoting environment in the heart, thus preventing or delaying cardiac tissue recovery. This chapter was published as a preprint as Anca, E., Pavel, I.O., Licarete, E., Rosioru, C., Dobre, C., Banciu, M., Transcriptomic insights into early diagnosis of doxorubicin-induced cardiotoxicity in a rat model, 2025, *bioRxiv,* https://doi.org/10.1101/2025.04.01.646673

Chapter V presents the major findings and conclusion of this thesis, along with future perspectives as these results may be a starting point for the further investigation of circulating biomarkers that might facilitate early diagnosis and therapeutic interventions for DOX-induced CTOX.

The thesis presents, as far as scientific literature indicates, the first study involving a multi-method approach to investigate DOX-induced CTOX, using a therapeutic protocol that mimics the clinical course of chemotherapy as well as the long-term implications of DOX treatment. This comprehensive investigation of DOX-induced CTOX integrates circulating biomarkers, tissue histological and ultrastructural analysis and transcriptomic profiling. The multimodal approach of this work brings insights on the dynamic progression of CTOX by examining the plasma biomarkers throughout treatment and in the post-treatment stage alongside tissue analysis bridged the gap between structural damage and molecular changes which allowed a detailed characterization of early and late effects of DOX.

In addition, the integrative approach of this work provides correlations between longitudinal biomarker profiling and structural changes across multiple tissues along with molecular changes for the heart. This is the first preclinical *in vivo* longitudinal study to connect a set of biomarkers to tissue- and molecular-level disruptions produced by DOX.

Future studies could be centered on the key mechanisms presented in this thesis. Firstly, DOX-induced mitochondrial dysfunction needs to be investigated in order to

understand if mitochondrial fusion which was observed at 4 weeks post-treatment is a compensatory mechanism or a maladaptive response to DOX injury that might suggest mitochondrial homeostasis as a potential area of investigation for developing efficient therapeutic interventions. Secondly, lipid dysregulation serves as an additional research direction, and further investigations targeted on this effect could potentially unveil effective protective strategies based on targeted lipid-lowering therapies.

Taken together, these findings advance the understanding of DOX-induced CTOX pathophysiology and highlight the need of long-term follow-up studies to determine the toxicity timeline of DOX.

## LIST OF PUBLICATIONS INCLUDED IN THE THESIS AS CHAPTERS

Chapter II

**Anca, E.**, Banciu, M., Roșioru, C.R., Dobre, C., 2024. Cardiac Surveillance in Oncology: A review of circulating biomarkets and diagnosis methods in chemotherapy-induced cardiotoxicity. Farmacia, 72(5): 975–986. https://doi.org/10.31925/farmacia.2024.5.1

Chapter III

**Anca, E.**, Sabău, F., Vădan, A., Marinescu, M., Licărete, E., Roșioru, C., Stoica, A. D., Dobre, C., Banciu, M., 2024. Assessment of circulating biomarkers in a rat model of doxorubicin-induced cardiotoxicity. Studia Universitatis Babeş-Bolyai Biologia, 69(2), pp. 7–22. https://doi.org/10.24193/subbbiol.2024.2.01

## LIST OF PUBLICATIONS NOT INCLUDED IN THE THESIS

Lang, C., **Anca, E.**, Vlad, C., Factori de risc comuni pentru cancer și bolile cardiovasculare, in: Vlad, C., Rădulescu, D., Drăgan, S., Burz, C. (Eds.), Manual de Cardio-Oncologie, Editura Medicală Universitară "Iuliu Hațieganu," Cluj-Napoca, 2020, ISBN 978-973-693-990-7: pp. 31–51

## ATTENDANCE AT INTERNATIONAL CONFERENCES

Poster presentation: **Anca, E.**, Vădan, A., Marinescu, M., Sabău, F., Roșioru, C., Dobre, C., Banciu, M., Circulating biomarkers in a rat model of doxorubicin-induced cardiotoxicity. Clinical Diagnostics & Research Virtual Event Series 2024, Nov. 13, 2024, https://www.labroots.com/file/view/PosterSubmission-Emilia%20Anca-54c6279e5ab6fc2c2ff5f6816119e0d6-2024-10-21%2010:54:48-preview.pdf