GRIGORE T. POPA UNIVERSITY OF MEDICINE AND PHARMACY IASI

HABILITATION THESIS

PEDIATRIC CARDIAC ANOMALIES – THE INTERPLAY BETWEEN ENDOGENOUS AND EXOGENOUS FACTORS

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LIST OF ABBREVIATIONS

AAP - American Academy of Pediatrics
ACAOS- Anomalous origin of the coronary artery from the opposite sinus of Valsalva
AD - Autism disease
ATP - Adenosine triphosphate
ALCAPA- Anomalous left coronary artery from the pulmonary artery
ALT - Alanine aminotransferase
ANA - Antinuclear antibodies
ASD - Atrial septal defect
AST - Aspartate transaminase
AV - Atrioventricular (ECG)
BP - Blood pressure
BDNF - Brain-derived neurotrophic factor
BT - Blalock-Taussig shunt (surgical technique)
BMI - Body mass index
CBC - Complete blood count
CC - Cranial circumference
CGH - Comparative genomic hybridization
CEA - Carcinoembryonic antigen
CF - Continuous feeding
CHD - Congenital heart disease
CMA - Chromosomal microarray analysis
CMR - Cardiac magnetic resonance
CNS - Central nervous system
CNV - Copy number variation
CoA – Coarctation of the aorta
CPK – Creatine phosphokinase
CPR - Cardiopulmonary arrest
CT – Computed tomography
CTI - Cardio-thoracic index
DCM - Dilated cardiomyopathy
DNA - Deoxyribonucleic acid
DORV - Double outlet right ventricle
EC - Endothelial cells
ED - Emergency department
EEG - Electroencephalogram
EMA - Epithelial membrane antigen
EN - Enteral nutrition
ERT - Enzyme replacing therapy
ESBL - Extended-spectrum β-lactamases
ESPGHAN - European Society for Paediatric Gastroenterology Hepatology and Nutrition
FISH - Fluorescence in situ hybridization
GA - Gestational age
GERD – Gastroesophageal reflux

GLUT1 - Glucose transporter 1
GRV - Gastric residue volume
GSH - Glutathione
GSSG - Glutathione disulfide
HAZ - - Height for age
HF - Heart failure
HIF – Hypoxia induced factor
HLHS - Hypoplastic left heart syndrome
HR - Heart rate
HU - Hounsfield units
iBF - Intermittent bolus feeding
IE - Infectious endocarditis
IH - Infantile hemangiomas
IO - Intraosseous infusion
IR - Infrared spectroscopy
FTIR - Fourier transform infrared spectroscopy
IN - Intranasal
IP - Intraperitoneal
IPPV - Intermittent positive pressure ventilation
IUGR - Intrauterine growth restriction
IVC - Inferior vena cava
Kb - Kilobyte
LDH – Lactic dehydrogenase
LDH (nanomaterials) - Layered double hydroxide
LKM - Liver-kidney microsomal antibody
LVH - Left ventricular hypertrophy
LVNC - Left ventricular non-compaction
MDR - Multi-drug resistant
MEN - Minimal enteral feeding
MiRNA - Micro RNA
MLPA - Multiplex Ligation-dependent Probe Amplification
MRI - Magnetic resonance imaging
NAC - N-acetylcysteine
NEC - Necrotizing enterocolitis
NMDAR - N-methyl-D-aspartate receptor
NGS - Next generation sequencing
3NT - 3-nitrotyrosine
NV - Normal value (biological investigations)
PA - Pulmonary atresia
PC - Pharmacological chaperone
PCR - Polymerase chain reaction
PD - Pompe disease
PDA - Patent *ductus arteriosus*
PEF - Primary endocardial fibroelastosis
PH - Pulmonary hypertension
PSVT - Paroxysmal superventricular tachycardia

PVR - Pulmonary vascular resistance
RBBB - Right bundle branch block
REE - Resting energy expenditure
RMT - Rhabdoid malignant tumor
RR - Respiratory rate
RV - Right ventricle
SEM - Scanning electron microscope
SD - Standard deviation
SMA - Superior mesenteric artery
SpO2 - Peripheral oxygen saturation
SVC - Superior vena cava
SVT - Supraventricular tachycardia
TEE - Transesophageal echocardiography
TF - Trophic enteral nutrition
TTE - Transthoracic echocardiography
TA - Tricuspid atresia
TGA - Transposition of the great arteries
TOF - Tetralogy of Fallot
US - Ultrasonography
VCVS - Velocardiofacial syndrome
VPA – Valproic acid
VSD - Ventricular septal defect
VT - Ventricular tachycardia
WAZ - Weight for age
WHZ - Weight for height

SUMMARY OF THE THESIS

Motto: "I think education is about being passionate about something. When you see passion and enthusiasm you can convey the educational message" -Stephen Robert Irwin

The Habilitation Thesis entitled "Pediatric cardiac anomalies- the interplay between endogenous and exogenous factors" represents, on one hand, a monograph of my medical, didactic and research activity, and on the other hand, an *opus primum* of my development directions as a doctor and didactic and research coordinator.

The habilitation thesis is structured in three parts that respect the criteria recommended and approved by the National Council for Attestation of University Degrees, Diplomas and Certificates (CNATDCU).

Section A details my professional experience, given my overspecialization in pediatric cardiology and many competencies in related fields, highlighting the original results obtained and published.

During the 25 years of activity, I have thoroughly studied, treated and researched genetic cardiac diseases, both structural and functional. Congenital cardiac malformations can occur either isolated or as a part of a syndromic disease. **Chapter 1** entitled "Cardiovascular genetics and genomics" includes results of studies that I have conducted to identify a targeted treatment of cardiac malformations and cardiac metabolic disorders based on their genetic blueprint.

The **second chapter**, "Pediatric cardiac and vascular tumors" focuses on the impact of molecular modifications on the development and prognosis of the most common benign tumors – hemangiomas- on one hand and the rarest cardiac tumors encountered in children- rhabdoid tumors, on the other hand. I chose to investigate and publish the result of my research in these 2 pathologies because the diseases at the 2 extremes of the spectrum, in this case the most common and the rarest, are frequently underdiagnosed or treated according to standardized, non-individualized therapeutic regimens, a fact that leads to suboptimal results.

Chapter III, entitled "Cardiac rhythm disturbances", approaches a recurrent theme in the pediatric cardiology area- how to identify, treat and prevent malignant cardiac rhythms in the pediatric population. I have been most fortunate to encounter miscellaneous cases with an unpredictable evolution, which required the application of the notions of international guidelines originally.

Chapter IV, "Lifestyle, epigenetics and cardiac malfunction" is dedicated to the multidisciplinary approach of both changeable and non-changeable risk factors involved in cardiac pathologies in the pediatric population. From obesity, a disease recording exponentially increasing trend of prevalence in our country, to intellectual disability, a ubiquitous feature encountered in genetic syndromes, psychiatric afflictions, improper lifestyle choices - I have investigated a large portion of themes and presented here a summary of my main conclusions.

Chapter V, the last chapter in this section, entitled "Cardiac infectious diseases", addresses the topic of infectious endocarditis, a formidable enemy of the pediatric cardiologist, while detailing the latest results of my research in the field of targeted antimicrobial therapy.

Section B details the main areas of interest of my scientific research, outlining future directions of investigation. Pediatric cardiology is a specialty found in an ongoing self-discovery process, much like our little patients. The terrain is especially permissive for those who understand following their passion and curiosity.

My scientific ambitions are related to the discovery of novel biomarkers that can be used to identify patients at risk of developing cardiac complications, such as heart failure, or pulmonary hypertension, as well as molecules that can be used for risk stratification and prognosis evaluation in the post-surgical scenario in patients who undergo cardiac surgery for congenital heart malformations.

The pleiotropic effects of cardiologic medication and their potential to influence the cognitive and motor development of patients with genetic disorders and other pathologies with cardiac enrollment is another research area that warrants my attention.

The age of targeted medical therapy brings fresh new perspectives in the therapeutic arena, especially concerning genetic metabolic disorders. I have been diagnosing and treating such diseases for a significant period and have come across multiple patient compliance issues, treatment side effects and pitfalls. As such, establishing a genotype-phenotype correlation, as well as supervising and acquiring state-of-the-art treatment for my patients is one of my goals.

Section C includes a list of bibliographic references cited in the habilitation thesis.

REZUMATUL TEZEI

Motto: "I think education is about being passionate about something. When you see passion and enthusiasm you can convey the educational message" -Stephen Robert Irwin

Teza de abilitare intitulată „Anomalii cardiace pediatrice - interacțiunea dintre factorii endogeni și exogeni" reprezintă, pe de o parte, activitatea mea medicală, didactică și de cercetare, iar pe de altă parte, *opus primum al* direcțiilor mele de dezvoltare ca medic, coordonator didactic și de cercetare.

Teza de abilitare este structurată în trei părți care respectă criteriile recomandate și aprobate de Consiliul Național de Atestare a Titlurilor, Diplomelor și Certificatelor Universitare (CNATDCU).

Secțiunea A detaliază experiența mea profesională, având în vedere supraspecializarea în cardiologie pediatrică și competențele în domenii conexe, evidențiind astfel rezultatele obținute și publicate.

Pe parcursul celor 25 de ani de activitate, am studiat și cercetat temeinic bolile cardiace genetice, atât structurale, cât și funcționale. Malformațiile cardiace congenitale pot apărea fie izolat, fie ca parte a unei boli sindromice.

Capitolul I intitulat „Genetica și genomica cardiovasculară" include rezultatele studiilor pe care le-am efectuat pentru a identifica un tratament țintit al malformațiilor cardiace și al tulburărilor metabolice cardiace urmând defectul genetic.

Al doilea capitol, „Tumori cardiace și vasculare pediatrice" se concentrează pe impactul modificărilor moleculare asupra dezvoltării și prognosticului celor mai frecvente tumori benigne – hemangioamele – pe de o parte și ale celor mai rare tumori cardiace întâlnite la copii – tumorile rabdoide, pe de altă parte. Am ales să public rezultatele cercetării mele deoarece aceste 2 patologii sunt la extrema spectrului - în acest caz cele mai frecvente și cele mai rare - și sunt subdiagnosticate sau tratate după regimuri terapeutice standardizate, neindividualizate, cu rezultate suboptimale.

Capitolul III, intitulat „Tulburările de ritm cardiac", abordează o temă recurentă în sfera cardiologiei pediatrice, respectiv modul de identificare, tratare și prevenire a artimiilor cardiace în populația pediatrică. Cazurile diverse, cu o evoluție imprevizibilă au necesitat aplicarea ghidurilor internaționale pentru atitudinea terapeutică.

Capitolul IV, „Stilul de viață, epigenetica și disfuncția cardiacă" este dedicat abordării multidisciplinare a factorilor de risc atât modificabili, cât și neschimbabili implicați în patologiile cardiace în populația pediatrică. Principalele concluzii prezentate în acest capitol cuprind teme plecând de la obezitate, o boală care înregistrează o tendință de creștere exponențială în țara noastră, până la dizabilitatea intelectuală - o trăsătură omniprezentă în sindroamele genetice, afecțiuni psihice și alegeri nepotrivite de stil de viață.

Capitolul V, ultimul capitol din această secțiune, intitulat „Boli infecțioase cardiace", abordează tema endocarditei infecțioase, un inamic redutabil al cardiologului pediatru, detaliind cele mai recente rezultate ale cercetării mele în domeniul terapiei antimicrobiene țintite.

Secțiunea B detaliază principalele domenii de interes ale cercetării mele științifice, conturând direcțiile viitoare de dezvoltare științifică. Cardiologia pediatrică este o specialitate într-un proces continuu de updatare. Terenul este provocator, mai ales pentru cei care înțeleg să-și urmeze pasiunea și curiozitatea.

Ambițiile mele științifice sunt legate de descoperirea de noi biomarkeri care pot fi utilizați pentru a identifica pacienții cu risc de a dezvolta complicații cardiace: insuficiența cardiacă sau hipertensiunea pulmonară, precum și modalităţi noi pentru stratificarea riscului și evaluarea prognosticului post-chirurgical la pacienții care suferă o intervenție chirurgicală cardiacă pentru malformații cardiace congenitale.

Efectele pleiotrope ale medicației cardiologice și potențialul lor de a influența dezvoltarea cognitivă și motrică a pacienților cu sindroame genetice sau cu alte patologii cu afectare cardiacă este un alt domeniu de cercetare care merită o atenție deosebită.

Epoca terapiei medicale țintite, individualizate aduce noi perspective în atitudinea terapeutică, în special în ceea ce privește tulburările metabolice genetice. De a lungul carierei mele am diagnosticat și tratat astfel de boli și m-am confruntat cu mai multe dificultăți în ceea ce privește complianța pacientului, efectele secundare ale tratamentului și diferinte capcane terapeutice sau diagnostice. Prin urmare, stabilirea unei corelații genotip-fenotip, precum și supravegherea și obținerea unui tratament de ultimă generație pentru pacienții mei este unul dintre obiectivele mele.

Secțiunea C include o listă de referințe bibliografice citate în cadrul tezei de abilitare.

SECTION A. BACKGROUND OF PROFESSIONAL, ACADEMIC AND SCIENTIFIC ACHIEVEMENTS

Professional achievements

I have the immense joy of working as a pediatric cardiologist since 1997, the year I graduated from Gr.T Popa University of Medicine and Pharmacy. The final examination consisted in a written examination, which sought the validation of basic and specialized medical knowledge, and the elaboration of a scientific thesis, which in my case bore the title "Interventricular septal defect in children". The main objectives of my diploma thesis was to study the anatomy, pathophysiology, embryology, and genetics of the aspects comprising the spectrum of interventricular defects, as well as clinical manifestations and paraclinical investigations, and possible complications that may occur in children with interventricular septal defect. The elaboration of the diploma project, structured in 17 chapters developed on 78 pages, was a meticulous and rigorous process and involved capitalizing on the knowledge gained during the six years of study.

I began my residency at Saint Mary Clinical and Emergency Hospital for Children. During the 5 years I worked as a medical resident, I completed internship rotations in pediatrics, pediatric surgery, infectious diseases, nephrology, and pediatric intensive care, gathering valuable theoretical knowledge and practical skills for developing my future medical career and acquiring experience in managing life-threatening scenarios involving chronically ill patients. At the end of the 5 years of residency, I passed the specialist doctor exam, and I graduated with 9.73 - the highest grade in my series. The theoretical knowledge as well as the practical skills acquired as a resident, as well as the one-year specialization course carried out at the Universite Catholique de Louvain, Belgium, proved to be of great value.

The next step for the consolidation of my career was the completion of the doctoral studies, organized by the Faculty of Medicine at the University of Medicine in Iasi, under the supervision of Prof. Ioan Tansanu, a corresponding member of the New York Academy of Sciences.

I defended my doctoral thesis entitled "Nephrotic Syndrome in children" in 2007. The paper was 253 pages long and was structured in two major distinct parts: the general part and the personal part, followed by a section of general conclusions of the paper. I approached the general section by detailing epidemiological clinical and paraclinical aspects, treatment options, complications and prognosis, achieving a successful systematization of the latest information in the field, in an interesting, and concise form.

The personal part presented my research objectives in this important syndrome in the pediatric pathology, characterized by an unpredictable evolution, sometimes towards renal failure. This doctoral thesis is one of the pillars of resistance for the monography "Nephrotic Syndrome in children", a book published in CNCSIS publishing house where I am the only author and publisher, a book to which two personalities of Iasi Pediatrics are scientific references: Prof. Ioan Tansanu, corresponding member of the New York Academy of Sciences and Associate Professor Eugen Cârdei.

Driven by the desire for continuous improvement, I took the course of "General ultrasound" at the Institute of Gastroenterology Iaşi between 15.04 and 15.07.2001, which I graduated with 9.80.

In 2003, after graduating from the training program and passing the attestation exam, I obtained the Certificate of Competence in General Ultrasound.

In 2006, I completed the 9-week General Echocardiography course, taught at the Institute of Cardiovascular Diseases and Transplantation in Târgu-Mureş.

In 2008 and 2009 I completed the Level 1 and Advanced Echocardiography courses organized by the "Carol Davila" University of Medicine and Pharmacy at the Institute of Cardiovascular Diseases in Bucharest, obtaining the Certificate of Competence in Echocardiography.

The same year, I received the physician title in Pediatrics- Order of the Ministry of Health no.1200 / 01.X.2009 and continued working in the Saint Mary Clinical and Emergency Hospital for Children as Head of Pediatric Cardiology . Being responsible for the management of patients with cardiovascular pathologies, I made it my purpose to be up to date with the latest advancements in diagnosing and treating cardiac diseases.

Therefore, in 2009, I attended the AEPC Arrhythmia Teaching Course in Zurich, which focused on general principles of diagnostics of arrhythmias, high risk arrhythmias, arrhythmias in congenital heart disease and cardiomyopathies device therapy .

In April 2010, I participated at and graduated the European Echocardiography Course "Clinical Applications of Echocardiography: Old and New. Teaching Course with Live Demonstrations", held in Bucharest with EBAC accreditation. In May 2010, I attended and graduated The Update Course at the 44th Annual Meeting of the Association for European Pediatric Cardiology, Innsbruck, Austria. In May 2011, I took part in the training " The Update Course at the 45th annual meeting of the Association for European Pediatric Cardiology" held in Granada, Spain, under the auspices of EBAC.

The acquisition of practical and theoretical skills was validated again by obtaining in 2012 the certificate of complementary studies in Pediatric Intensive Care and Pediatric Cardiology issued by the Romanian Ministry of Health.

In 2016, I passed the exam attesting my competence as a cardiology specialist and in 2019 I received the specialty diploma in pediatric cardiology.

At present, I hold the title of senior specialist in pediatrics, obtained in 2009, senior specialist in pediatric cardiology, obtained in 2019 and specialist in cardiology, since 2016. My professional career so far has also been marked by my attendance in multiple specialized courses targeting pediatric cardiovascular emergencies and related fields.

I also obtained the certificate of complementary studies in "Management of Health Services" issued by the Romanian Ministry of Health- General Directorate of Human Legal and Litigation Resources, (Certificate Series C. No. 038998) and the certificate of graduation "Management of Health Services" (Certificate Series AT No. 0233, issued by the National School of Public Health, Management and Improvement in the Sanitary Field). My expertise in health policies, healthcare and management allowed me to actively participate in the improvement plans of my department and the hospital I work in.

As such, I coordinate programs and I am a member in several committees, as listed below:

- Coordinator of the „National Treatment Program for Rare Diseases – Pulmonary Hypertension” ;
- Member of the Commission for the implementation of standards for hospital accreditation (Decision no. 660 / 12.08.2013 of the Saint Mary Emergency Clinical Hospital for Children, Iasi);
- Member of the Transfusion and Haemovigilance Commission (Decision no. 655 / 12.08.2013 of the Saint Mary Emergency Clinical Hospital for Children, Iasi);
- Member of the Commission for the implementation and analysis of the results of use protocols and practice guides (Decision no. 657 / 12.08.2013 of the Saint Mary Hospital Emergency Clinic for Children Iasi)
- Member of the Medicines Commission (Decision no. 663 / 12.08.2013 of the Saint Mary Hospital Emergency Clinic for Children Iasi)
- Member of the Medical Council (Decision no. 664 / 12.08.2013 of the Saint Mary Clinical Hospital of Emergency for Children Iasi)

In 2012, I designed and implement a modernization project of the Pediatric Cardiology Department, and thus obtained:

- 3 OXY-PED pulse oximeters,
- 5 pulse oximeter OXY400BABY,
- 1 B3 multiparameter patient monitor,
- 2 PC3000 multiparameter patient monitors,
- 2 GIMA ABPM Holter,
- 1 EKG Holter 35130, 1 high-performance electrocardiograph.

The equipment was purchased by means of the MG 79213 Project "Save a Child's Heart" II approved by the Rotary International Foundation and conducted by the Rotary Club Iasi 2000 - 2241District Romania and the Republic of Moldova, as a host partner and Rotary Club Honolulu Sunset, Rotary Club Mililani Sunrise, Rotary Club Waikiki - District 5000 United States, as International Partner.

Since 2018, I am the coordinator of the pediatric cardiology residency program in Iasi and member of the National Pediatric Cardiology Commission.

In 2018, I also organized the first congress of pediatric cardiology in Romania.

The medical profession has a deeply humanitarian component. Beyond the time and energy invested in personal training, I fought to bring my contribution to society by helping my little patients spiritually as well.

Thus, beginning with 2009, we started the Christmas program "Be with MI for children."

As a result, the children admitted in the Cardiology Department, as well as those from other Pediatrics Clinics (some of them abandoned and deprived of material possibilities), enjoyed the wonderful gifts received from Santa Claus. The prevention of cardiovascular disease has been the reason why every year I participated in multiple actions in schools, on radio and television. I also actively participated in the annual "Heart Day" program initiated by the "My Child, My Heart" Association.

The crowning jewel of my professional activity is undoubtedly the network of partnerships that I have established with professionals from other states, in order to surgically solve the cases of congenital heart malformations diagnosed in my patients.

Academic achievements

I started my university career as a teaching assistant at the University of Medicine and Pharmacy "Gr.T.Popa" in the 4th Pediatric Clinic of the Saint Mary Clinical Hospital for Emergency for Children in Iasi, later becoming a lecturer in the 1st Pediatric Clinic at the same hospital.

In order to develop a teaching career, I took the DPPD courses, being awarded the grade 10. The disciplines I studied there, such as psychology of education, pedagogy, management of non-formal education, counseling and guidance, and sociology of education have been of real use to me in interacting with students in the educational process. A strong point was also the study of the discipline "intercultural education" in the psycho-pedagogical module, considering the fact that I currently teach foreign students. In order to improve the methods of medical research, I completed and graduated in 2002 the continuing education courses in the specialization "Clinical epidemiology. Medical research methodology. "

I currently hold the position of associate professor, awarded after a rigorous evaluation of my entire activity so far.The teaching activity has always been a point of interest for me, because by sharing with students and residents the experience I gained during my years of activity, I believe that I may contribute to the formation of future generations and thus reward past generations who in turn helped me. As John of Salisbury first said, "standing on the shoulders of giants" is the way our society progresses intellectually from one generation to another.

I am honored to teach my students and residents, to give courses and seminars, to organize and supervise the development of practical pediatric internships both for the students of the Faculty of General Medicine, the Romanian, English and French sections, as well as for the students of the Faculty of General Medical Care. I made a habit of using interactive means of transmitting information, starting from personal experience and specialized bibliography, in the elaboration of which I participated myself as an author. I am especially interested in the ever-evolving genetic field, that helps shed new light over the pathological mechanisms of various pediatric diseases in general, and cardiac anomalies in particular, and I always bring to my students and residents this fresh perspective.

The training of medical residents in the field of Pediatric Cardiology is my heraldry. I am one of the few senior specialists in pediatric cardiology in Romania and as the coordinator of the residency program in Iasi is my responsibility to train my residents for the challenges of a young specialty, in a continuous expansion, annexing extensive areas of multiple specialties, such as intensive care, neurology, nephrology, genetics and oncology. I organize weekly case presentations and debate sessions regarding the latest scientific articles and guidelines in our field of specialty. I have also coordinated Romanian and foreign students and their undergraduate thesis, with a total of over 120 undergraduate dissertations with a focus on pediatric cardiology and intensive care.

Scientific achievements

As a medical professional, I invested time and energy in scientific research, as part of my personal contribution to the development of pediatric cardiology. I started by developing important sources of information, both for colleagues in other specialties, as well as for resident doctors and students.

The field of pediatric cardiology must be seen as a universe in which multiple independent "galaxies" interact. The experience of 20 years of practice has shown me that beyond the obvious and clinically noisy manifestations of congenital heart defects, the pediatric heart suffers under the rule of systemic diseases.

My experience in applying international guidelines that address the problem of pediatric cardiology, allowed me to correlate the general directions with the reality in the territory, managing to establish good practice protocols based on thorough knowledge, all of which were summarized in books such as "**Clinical and therapeutic guidance in exogenous acute intoxications of children"** and "**Congenital malformations of the cardiovascular system in children"** published in 2002, followed by "**Pediatric toxic syndrome**" and "**Withdrawal syndrome in children**" which saw the light of day in 2009. In 2010, I took part in an ambitious project to document acute intoxications in children, which materialized in the scientific book entitled **"Acute poisoning in children**". The following year, I edited the "**Textbook of Pediatrics**" and in 2016 I collaborated with Prof. Ingrith Miron for editing "**Pediatrics**", an important reference book for medical residents and students. In 2016, the book I wrote "**Practical guide of diagnostic and treatment in pediatric pathologies**" won the **Gold medal** at the Grand Prize Euroinvent ceremony. In 2017, the book "Pediatric intensive care" which I wrote and edited, received the **Gold medal** at the Grand Prize Euroinvent ceremony, and in 2021, another project of mine, entitled "Textbook of acute poisoning in children" was awarded the **Grand Prize** at the Euroinvent Book Salon.

I participated in numerous projects and research grants such as:

- The International Grant : Réseau partenaire francophone pour la validation clinique des protocoles diagnostiques et thérapeutiques de la médecine fœtale dans la région de l'Europe Orientale (01.10.2012 -01.10.2014), financed by L'Agence Universitaire de la Francophonie;
- "100 hearts for 100 children" : POSDRU / 179 / 3.2 / S/ 151363 - March-Dec 2015 Training of specialists in the field of pediatric cardiology for a quality medical act in order to improve the quality of life - Project manager - Coordinating expert; The project was awarded the second place at the Structural Funds Gala;
- Jean Monnet Grant; Project manager for the research axis: Health and health policies in the EU, Grant Jean Monnet 2013- 2015/001 - 001. Contract 4934 / 17.12.2015;
- International project "H-CARE "Launching of Sector Skills Alliance for Training & Apprenticeship of Health Care and Food Supplements Sales persons (01.01.2014-30.06.2014)- Project Manager;

My scientific portfolio consists of 109 articles published *in extenso*, of which 39 in ISI-listed journals; 172 articles published in summary in ISI-listed journals, posters and other contributions; 4 specialized treatises as editor; 4 specialized textbooks as author; 165 book chapters. In total, 454 articles, books and book chapter bear witness to my scientific endeavors.

CHAPTER I. CARDIOVASCULAR GENETICS AND GENOMICS

Introduction

The formation of the human body is a plurivalent process that requires the perfect synchronization of biological, physical and chemical factors that act in morphogenesis. This high degree of complexity predisposes to the relatively frequent occurrence of errors, despite the prevention mechanisms available at the cellular level. In the perinatal and neonatal period, major structural abnormalities are an important cause of death and an overwhelming proportion of genetic factors determine them. The development of children and adolescents with structural defects with genetic and / or epigenetic foundation encounters an increased rate of morbidity provoked by systemic complications. Therefore, congenital anomalies are a major public health problem, requiring not only their early diagnosis, but also specific, correct and complete prophylaxis methods.

Congenital anomalies are structural changes caused by disorders of prenatal development. They are classified into malformations, disruptions, deformities and dysplasia. Malformations are primary structural defects of an organ that have occurred because of incomplete or abnormal differentiation. They take place early during embryonic development (Turnepenny et al, 2022). Congenital heart malformations are among the most common innate structural defects, with a cumulative incidence of about 10%, difficult to assess due to the high degree of intrauterine mortality associated with severe malformations and the percentage of spontaneous resolution of defects , such as septal defects or persistent arterial canal. Isolated congenital anomalies are the consequence of a strictly localized error at the level of an embryonic development unit (Covic et al., 2017).

Monotopic field defects are those that manifest either as a single error - for example isolated VSD - or as complex errors involving a single organ – TOF. Politopic field defects impact a region responsible for the development of several structures of the body, as is the case with VCFS.

Sequences are defined as effects of a cascade of events initiated by a single anomaly, they take on a malformative, deformative and disruptive aspect. Multiple congenital anomalies are represented by plurimalformative syndromes and malformative associations.

Syndromes are a constellation of major and / or minor abnormalities with a common cause, encountered in a predictable manner, in the same form, in patients from different backgrounds and families or even in related patients. The syndromes can be monogenic, chromosomal, teratogenic or only clinically recognizable entities, without a clearly determined etiopathogenesis. What gives a syndrome its identity is the combination of abnormalities, which, taken separately, can be found in many other clinical contexts (Turnepenny et al, 2022).

Malformative associations, unlike syndromes, do not have a common cause, but are specific configurations with a probability of manifestation of all components too high to be pure coincidence. The name of each association is the acronym of the ensemble that composes it.

The biological connection between generations is the result of biparental genetic material transmission at the time of gamete fusion. Errors in meiosis or mitosis lead to mutations and depending on the amount of genetic material affected mutations can be monogenic or chromosomal.

Every human cell normally has 22 pairs of autosomes and one pair of sex chromosomes, each composed of a single DNA molecule.

When referring to the chromosome morphology, the position of the centromere, the telomeres and the distribution of heterochromatin and euchromatin are the main aspects used for identification. Chromosomal abnormalities affect either the number or structure of chromosomes, and they can be constitutional or acquired, homogeneous or mosaic, autosomal or gonosomal.

Gene mutations affect the nucleotide sequence of a gene. Also, variations in the number of gene copies, resulting from insertions, deletions or duplications, can affect the activity of some genes, influencing the development and functioning of the body. Mutations characterized by unstable expansion of trinucleotide repeats cause abnormalities in gene expression or encoded protein function (Covic et al., 2017).

Sequencing of the human genome has shown that 10% of newborns' heart damage is due to *de novo* mutations. Genetic regions susceptible to mutations have also been identified, as so-called "hot spots" consisting of repetitive sequences or palindrome-type (Lewis, 2018).

The cardiovascular system is the first operational during intrauterine life, its activity beginning in the third week of embryonic development. Morphogenesis and the imprinting of cardiac function are processes that take place in parallel, requiring spatial and temporal coordination of cell populations in order to recruit, proliferate and differentiate them. Cardiac formation in vertebrates follows the same pattern, starting from the fusion of the endocardial tubes and the appearance of the excito-conductor system and the coronary circulation, with variations from one species to another, determined by cardiac septation and remodeling (Rickert-Sperling et al., 2016).

This similarity has allowed the study of the influence of various signaling pathways, various transcription factors, ligands and receptors, encoded by genes called essential, due to their irreplaceable activity. The genome of the mouse and zebrafish are the main sources of identification of the specific function associated with each gene, by gene manipulation, followed by documentation of phenotypic consequences. Animal models have also demonstrated the involvement of genes in multiple organ formation processes, thus explaining the association of constitutional and / or functional abnormalities.

After gastrulation, cells from the mesoderm migrate anteriorly and laterally to the embryonic axis, forming the primary cardiac field. Subsequently, they will differentiate *in situ,* simultaneously with the migration to the medial plane, and will form the primary cardiac tube.

The establishment of primary and secondary cardiogenic fields, responsible for the genesis of the left ventricle, and the right ventricle, atria and the middle tunic of the large vessels respectively, is dictated by an intricate network of signaling pathways and transcription factors (Fig.I.1*).*

Methods for identifying genetic mutations associated with congenital heart disease have progressed over time from light microscopy to genomic analysis. The chromosomal analysis allowed the detection of numerical and structural anomalies of the 23 pairs of chromosomes. Recent techniques, such as FISH, MLPA, and microarray-CGH can detect deletions or duplications at segments much too small to be visualized by standard karyotyping (Allen et al., 2021).

Next generation sequencing, that allows sequencing of the entire genome, promises to pave the way for the discovery of genes involved in the pathogenesis of congenital heart disease.

Thanks to new methods of genetic investigation, the list of genes responsible for congenital heart diseases is constantly growing. They are transcriptional regulatory genes, genes involved in signal transduction, and genes encoding cardiac structural proteins.

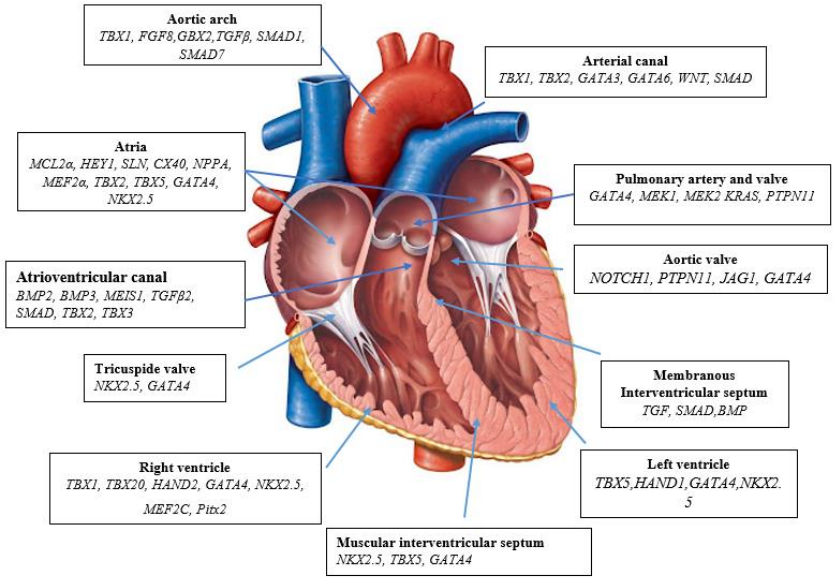


Figure I.1. Main genes involved in cardiac development and chamber specification

Isolated heart malformations caused by mutations in these genes are classified depending on the region of the heart that they affect, for better identification of their genetic substrate. In addition, for better characterization and management, isolated cardiac malformations can be analyzed according to their dependence on the persistence of the arterial canal.

I.1. Genetic, clinical and therapeutic aspects of isolated congenital heart diseases

I.1.1. Background

The fetal circulation follows a series pattern, where blood travels through the low resistance placental vessels to ensure adequate organ perfusion. The pulmonary circulation is bypassed because of the lungs vascular resistance. Instead, deoxygenated blood coming from the SVC passes through the *ductus arteriosus* and into the descending aorta, and oxygenated blood coming from the IVC goes through the *foramen ovale* and towards the ascending aorta. Gas exchange mainly occurs in the placental vessels.

Under normal circumstances, the transition from fetal to newborn circulation starts with a significant decrease in the pulmonary vascular resistance and the obliteration of the placental vessels following cord clamping. As a result, the pressure gradient between the right and left chambers of the heart changes in favor of the latter, prompting the closure of both *foramen ovale* and *ductus arteriosus.* In certain situations, the permeability of the *ductus arteriosus* is artificially prolonged, in order to prevent systemic or pulmonary complications associated with severe cardiac malformations.

CHDs are classified according to morphological and pathophysiological criteria into cardiac anomalies with increased pulmonary flow (such as septal defects with left to right shunt) and no pulmonary obstruction, cardiac defects with decreased pulmonary flow, congenital

anomalies that spare the septum, severe cardiac anomalies and congenital heart disease asymptomatic until adulthood (Joshi et al., 2020).

However, considering the latest scientific findings on the underlying genetic causes of congenital heart malformations, an embryological and functional classification, like the one proposed by Clark is indicated in order to facilitate the establishment of causal links, cascading effects and the establishment of screening indications for other anomalies / syndromes.

Clark's pathogenic classification enlists 6 categories, as explained bellow:

- Group I. Ectomesenchymal tissue migration abnormalities: defined as abnormalities in the migration of neural crest cells through the branchial arch vessels
- Group II. Abnormal intracardiac blood flow
- Group III. Cell death abnormalities: anomalies in the formation of the ventricular cavities and the interventricular septum caused by impaired cell death process
- Group IV. Extracellular matrix abnormalities: the matrix is involved in forming atrioventricular valves and insuring septal closure; anomalies falling under this category cause systemic and /or pulmonary circulation impairment
- Group V. Abnormal targeted growth: targeted growth is based on chemotactive tissue interaction and is the mechanism that ensures the inclusion of pulmonary vessels.
- Group VI. Abnormal situs and looping: cardiac looping ensures adequate arterio-ventricular coordination.

Detailed genetic mechanisms for each of these groups and exponents for each category are exemplified in Table I.1.

Table I.1. Classification of CHD according to Clark's criteria and genetic causes

CHD	**Monogenic etiology**	**Syndromes**	**Clark's pathogenic classification group**
ASD	*ACTC1, CITED2 GATA4, MYH6 NKX2.5, PITX2c TBX1,TBX20,TBX5*	Holt-Oram, Noonan, Kabuki, CFC, Down, Edwards, Patau, Turner Di George, 4p.16.3, 17p11.2, 17q21.31	Abnormal intracardiac blood flow- OS type ASD Extracellular matrix abnormalities OP type ASD
VSD	*GATA4, GATA6 HAS2, NKX2.5TBX5*	Holt-Oram, Noonan, Kabuki, CFC, Down, Edwards, Patau, Turner, 4p.16.3, 17p11.2, 17q21.31, 11q23 – microdeletions syndromes	Ectomesenchymal tissue migration abnormalities-type I VSD Abnormal intracardiac blood flow-type II VSD Extracellular matrix abnormalities-type III VSD
DORV	*ACVR2B,GDF1 NPHP4, TDGF1 TBX1, ZIC3*	Down, Edwards, Patau, Jacobs syndromes 5p15.2, 7q11.23, terminal 11q and 1p36 deletions	Ectomesenchymal tissue migration abnormalities
TGA	*AVCRB2, CENPU CFC1, EIF3J, GDF1, LEFTY2, MYH6, NAA15*	No genetic syndrome was found to be significantly associated	Ectomesenchymal tissue migration abnormalities

	NODAL, PRIMPOL, RAB10, SMAD2, SPG11, ZIC3	with TGA, except for heterotaxy	
TOF	*ALDH1A2, GJA5 GPC5, HAND1, HAS2, NKX2.5 NRP1, TBX1*	Down, Patau, Edwards 5p15.2, 7q11.23, terminal 11q and 1p36 deletions	Ectomesenchymal tissue migration abnormalities
Aortic coarctation	*ATP2A1,CCND3, CD247, PCDH11X, SCYL1, SMTN SSX6 TBX1 TRPM2 UCP1 ZNF630*	Turner, Noonan, Kabuki	Abnormal intracardiac blood flow
HLHS	*ERBB4, GJA1, HAND1, NKX2.5, NOTCH1*	Turner, Jacobsen, CHARGE, Wolf-Hirschhorn,Rubinstein-Taybi, Noonan Holt-Oram	Abnormal intracardiac blood flow
Tricuspid atresia	*GATA4, NKX2.5, ZFPM2*	22q11, 4q31, 8p23, 3p deletion syndromes, Edwards, Patau	Abnormal intracardiac blood flow
Ebstein's anomaly	*MYH7, NKX2.5*	Cri du chat, Williams Down	Cell death abnormalities
Pulmonary valve atresia	*CAMTA2, CUBN, DHFR NSD1*	DiGeorge, Sotos, Marfan	Abnormal intracardiac blood flow
Pulmonary valve stenosis	*BRA, HRAS, KRAS, MEK1, MEK2, PTPN11*	Noonan, Costello	Abnormal intracardiac blood flow

The personal contribution regarding the correlation between morphological, physiopathological, clinical and imagistic aspects in isolated cardiac malformations is exemplified by the articles mentioned below:

1. **Luca, A.C.;** Gavril, E.C.; Curpan, A.S.; Popescu, R.; Resmerita, I.; Panzaru, M.C.; Butnariu, L.I.; Gorduza, E.V.; Gramescu, M.; Rusu, C. Wolf-Hirschhorn Syndrome: clinical and genetic study of 7 new cases and mini review, *Children* 2021, pp.1-11, **IF 2,863**
2. **Luca**, **A.C.;** Tarca, E.; Rosu, S.T.; Cojocaru, E.; Trandafir, L.; Rusu, D.; Tarca, V. Socio-epidemiological factors with negative impact on infant mortality, and the occurance of birth defects, *Healthcare*, 2021**,** pp.1-12, **IF 2.645**
3. **Luca**, **A.C.;** Diaconescu, S.; Rusu, C.; Bozomitu, L.; Vlad, E.; Matei-Ciobanu, O.L.; Babici, R. Donea, L.; Strat, S. Pompe disease a late-onset misleading form of diagnosis in a patient with persistent hepatic cytolysis syndrome, *Ro J Pediatr.* 2020;69(2), pp 162-165.
4. **Luca**, **A.C.;** Lozneanu, L.; Miron, I.C.; Trandafir, L.M.; Cojocaru, E.; Paduret, I.A.; Mihaila, D.; Leon-Constantin, M.M ; Chiriac, S.; Iordache, A.C.;Tarca, E. Endocardial fibroelastosis and dilated cardiomyopathy – the past and future of the interface between histology and genetics, *Rom J Morphol Embryol* 2020, 61(4)**,** pp.999-1005, **IF 1.033**
5. **Luca**, **A.C.;** Braha, E. Cardiovascular involvement in Pompe Disease, *Ro J Pediatr.* 2017;66(1), pp.8-11
6. **Luca**, **A.C.;** Braha, E. Pre-existing maternal disease-risk factor for congenital abnormalities in the newborn, *Ro J Pediatr*. 2016;66(2), pp.141-144.
7. **Luca**, **A.C.;** Holoc, A.S.; Subotnicu, M.; Iordache, C. Clinic and therapeutic aspects in ductus-dependent congenital heart defects-Part I, *Romanian Journal of Pediatrics*, Vol. LXIV, No. 2, 2015, pp.122-125.

8. **Luca**, **A.C.**; Holoc, A.S.; Subotnicu, M.; Iordache, C. Clinic and therapeutic aspects in ductus-dependent congenital heart defects-Part II, *Romanian Journal of Pediatrics*, Vol. LXIV, No. 3, 2015, pp.246-251.
9. **Luca**, **A.C.**; Iordache, C.; Holoc A.S. Clinical and paraclinical aspects of Ebstein's Anomaly-severe form in newborns,*The Medical Surgical Journal*, vol 119, no.2, 2015, pp.390-394.
10. **Luca**, **A.C.**; Holoc, A.S.; Iordache, C. Clinical and therapeutic aspects in the severe aortic coarctation with duct dependent systemic circulation in the newborn, *Romanian Journal of Pediatrics, VOL. LXIV, NO. 4,* 2015, pp.406-409

Also, our vast experience in diagnosing and treating CHD was used as reference for elaborating protocols and management guidelines as well as BDI papers:

1. **Luca**, **A.C.**; Iordache C. Non ductal-dependant cardiac malformations, Book of Pediatric Intensive Care, Medical editure , Bucharest 2016 , ISBN 978-973-39-0805-0, pg.197-216.
2. **Luca**, **A.C.**; Iordache C. Ductal dependent cardiac malformations, Book of Pediatric Intensive Care, Medical editure , Bucharest 2016 , ISBN 978-973-39-0805-0, pg.217-228.
3. **Luca**, **A.C.**; Iordache C. Treatment of congenital cardiac malformations, Book of Pediatric Intensive Care, Medical editure , Bucharest 2016 , ISBN 978-973-39-0805-0, pg.229-234.
4. **Luca**, **A.C.**; Holoc A.S. Pulmonary hypertension, Book of Pediatric Intensive Care, Medical editure, Bucharest 2016, ISBN 978-973-39-0805-0, pg333-244.
5. **Luca**, **A.C.**; Iordache C. Congenital heart malformations, Pediatrics Book, GR.T.Popa, 2011, ISBN 978-606-544-046-3, pg.322-356.

The educational grant mentioned below offered the opportunity of training for professionals in the field of pediatric cardiology as well as for the establishment of international cooperation for the surgical resolution of cases with congenital heart malformations. The surgical interventions improved the survival chances of children and neonates, while providing the context for training specialists in the field of the latest interventional or classical surgery techniques used to solve heart defects.

Educational grant
„100 hearts for 100 children"- POSDRU/179/3.2/S/151363, GR.T.POPA University of Medicine and Pharmacy, Iasi, 2015-2020

I.1.2. Materials and Methods

Our research focused on the characterization of cardiac abnormalities starting from morphopathological, imaging and clinical aspects, focusing on the main axes of therapy depending on the specifics of the identified malformation.

Patient selection and data interpretation

Patients were selected from the archive of the Cardiology Department from the Saint Mary Clinical and Emergency Hospital for Children based on their CHD diagnostic. We compared our clinical data to that found in the scientific literature in order to elaborate on the best strategies for diagnosing and treating cardiac congenital malformations.

Cardiac diagnosis

Clinical examination, followed by electrocardiography and blood tests consisting of CBC, blood gas analysis, investigation of phosphocalcic metabolism, blood glucose, renal and hepatic function were used for the initial patient evaluations. 2D echocardiography was the preferred imagistic method to diagnose patients preoperatively.

I.1.3. Results

General morphopathology and physiopathology

In the first Clark category, **ventricular septal defects** have the most significant degree of prevalence of all congenital heart diseases, and can occur both as an isolated anomaly and in complex malformations, such as TOF or TGA, or associated with obstructive defects of the left ventricular ejection tract, such as subvalvular aortic stenosis and aortic coarctation (Munoz & Morel, 2020).

The consequence of such septal abnormalities is the occurrence of an interventricular shunt whose direction and magnitude depend on the size of the defect, pulmonary vascular resistance, systolic and diastolic function of the two ventricles and the presence of malformations. of right ventricular ejection tract (Park & Salamat, 2021).

Four types of septal abnormalities are described, depending on their location, namely**:**

• Type 1 VSD – infundibular: located below the level of the crescent valves, at the level of the conal septum or outlet, with a prevalence of 33% in Asian populations. Spontaneous closure of such a defect is rare;

• Type 2 VSD– perimembranous: located in the vicinity of the tricuspid cusp, being able to facilitate its adhesion to the defect and the creation of an „aneurysm" at the level of the septum with the limitation of the interventricular shunt;

• Type 3 VSD – inlet affects the interventricular wall below the level of the atrioventricular valve apparatus; such an abnormality is usually encountered in patients with Down syndrome;

• Type 4 VSD - muscular - is completely surrounded by muscle tissue; the incidence in children reaches 20%, but decreases to adulthood. Although the lesions are usually multiple, the tendency to spontaneous closure is increased (Sadeghpour et al., 2014).

Double outlet right ventricle is an abnormality of ventricular-arterial connections, presenting in multiple anatomical forms, depending on the location of the ventricular septal defect, the relationship between the large vessels and the ventricular cavities and the presence of pulmonary stenosis. The classification depends on morphological or functional criteria.

Transposition of the great vessels designates a congenital heart disease in which the aorta originates in the right ventricle and the pulmonary artery in the left ventricle. Complete transposition involves a ventriculoatrial concordance, which will allow a hypoxemic systemic blood circulation and a hyperoxemic pulmonary situation long-term, without surgery. Depending on the position of the aorta relative to the lung, we can encounter a complete dextro-transposition of great vessels or a levo-transposition (dTGA or lTGA). dTGA occurs in isolation or in the context of heterotaxy, a situation in which the risk of recurrence of the disease is high.

Congenitally corrected great vessel transposition also involves ventricular inversion, so that oxygenated blood that reaches the left atrium passes into the right ventricle and from there thorough the aorta into the systemic circulation. In this case, the patient's evolution may be complicated by other malformations, such as VSD, valve defects, or atrioventricular electrical conduction disturbances (Park & Salamat, 2021).

The Fallot tetralogy represents a classic model of complex congenital cardiovascular malformation, the morphological phenotype being characterized by:

• Obstruction of the right ventricular ejection tract, which most often occurs in the form of infundibular pulmonary stenosis (45%), less often valvular (10%). The remaining cases are represented by the coexistence of infundibular and valvular stenosis (30%) and pulmonary atresia (15%). In this last category of patients, multiple major aorto-pulmonary collaterals can be found, with or without patent arterial duct (Clowes, 2014). The degree of obstruction can be amplified in patients with TOF by hypoplasia of the pulmonary artery branches (Springer, 2019).

• Non-restrictive perimembranous VSD, which allows equalization of pressures in the two ventricular cavities

• The position of the aorta on the interventricular septum, described as overriding aorta caused by the dextroposition of the aortic root, resulting in its biventricular origin. When most communication is with the right ventricle, the right ventricle is considered to have a double outlet, in addition to TOF.

• Right ventricular hypertrophy

From the second Clark group**, aortic coarctation** is a defect found in 4-7% of CHD cases, with more than half of the patients associating aortic bicuspidy (Perloff, 2012).

Monogenic transmission of CoA is rare, accounting for only 1-2% of cases, most cases being sporadic, non-syndromic (Moosmann, 2015).

Interrupted aortic arch, the discontinuity between the ascending and the descending aorta, leads to critical obstruction in the systemic circulation. The blood flow in the descending aorta depends on the permeability of the ductus *arteriosus*. The decrease in pulmonary vascular resistance after the first week of life allows for increased pulmonary flow with the consequence of heart failure, installed when the *ductus arteriosus* starts to close, leading to tissue hypoxia, metabolic acidosis and renal failure.

Hypoplastic left heart syndrome is a category of pathologies that accumulates a maximum of 3.4% of congenital heart diseases, with an incidence of 0.016% of cases at birth, while being responsible for 23% of deaths in the first day of life and 15% of those in the first month of life (Allen et al., 2021). It is considered a set of diseases, consisting of left ventricular hypoplasia, atresia or severe stenosis of the aortic and mitral valves, hypoplasia of the ascending aorta and aortic arch. Associated abnormalities are ASD in 15% of cases, VSD in 10 %, CoA in a significant percentage of cases, about 75%; malformations of the central nervous system may also be present, the most frequently cited being microcephaly, agenesis of the corpus callosum, and holoprosencephaly (Springer, 2019). Despite significant progress in the clinical and surgical management of HLHS, including *in utero* interventions such as aortic and mitral valvuloplasty and atrial septoplasty, but with little impact on subsequent left ventricular development, the mortality and morbidity associated with this pathology remain an important concern. One of the main problems encountered in the control of this disease is the lack of sufficient information on its genetic basis. Currently, it is known that HLHS has a hereditary component, it has also been associated with chromosomal abnormalities, such as Turner and Jacobsen syndromes or, less frequently, CHARGE, Wolf-Hirschhorn, Rubinstein-Taybi, Noonan and Holt-Oram syndromes (Suuronen et al., 2015).

Ebstein's anomaly is the exponent of the third Clark category, and it is usually a non-syndromic heart malformation, accounting for approximately 1% of cases of congenital heart disease, with an estimated prevalence in 1 in 20000-50000 births, with an equal gender distribution (Giamberti, 2014). The disease mechanism of action consists in the adhesion of the posterior and

septal tricuspid cusps at the underlying myocardium, as well as the low insertion of the tricuspid valvular apparatus.

Valvular abnormalities, such as Pulmonary valve atresia or stenosis and tricuspid atresia fall under the second group in the Clark's classification.

Pulmonary valve atresia accounts for only 1% of cases of congenital heart defects, but it is a major cause of neonatal mortality, especially for newborns diagnosed with PA and intact interventricular septum. **Congenital pulmonary valvular stenosis** has been identified mainly in genetic syndromes, such as Noonan syndrome, caused by mutation in the *PTPN11* gene, Costello syndrome, in which the *HRAS* gene is involved, cardiofaciocutaneous syndrome, caused by mutations in *BRAF, MEK1, KRAS* genes, all of which have an impact on the MAPK signaling path (Allen, 2021).

Tricuspid atresia is found in about 3% of CHD cases, without manifesting a particular predilection for either sex, with a low degree of recurrence. Chromosomal deletions 22q11, 4q31, 8p23, 3p and trisomies 13 and 18 have been identified as causes of TA. Among the gene mutations associated with the pathogenesis of tricuspid atresia, the best candidates are the *ZFPM2* and *NKX2-5* genes. The *ZFPM2* gene modulates the activity of the *GATA4* gene, having a role in myocardial differentiation, and the analysis of null mice for Zfpm2 demonstrated the association of this genotype with the specific AT phenotype (Suuronen et al., 2015).

Cardiac diagnostic

The patients diagnosed with duct-dependent cardiac malformations almost universally presented with tachypnea, cyanosis, severe respiratory distress, feeding difficulties, hypotonia, hypotrophy, and developmental delays. In children of all ages, signs of heart failure and systemic hypoperfusion of various degrees were identified. Clinical examination findings and paraclinical parameters are synthetized in Table I.2.

Table I.2. Clinical and biological parameters in patients with CHD

Type of CHD	Main symptoms	ECG findings	Blood tests (if specific)
Aortic stenosis	Pallor Tachypnea, Tachycardia Hypotension	Left atrial and ventricular hypertrophy T wave inversion ST segment depression	
Aortic coarctation	>10 mmHg difference between upper arm and leg blood pressure; Tachypnea Cyanosis Oxygen saturation differences between upper and lower extremities	Left ventricular hypertrophy Left ventricular strain or ischemia	Metabolic acidosis Prerenal failure
HLHS	Tachypnea Tachycardia Cyanosis Absent peripheric pulse Hypotension Pulmonary edema	Prolonged PR interval Wide QRS Low voltage or absent S wave in V1 Low voltage or absent R wave in V5 and V6	Metabolic acidosis Hypoglycemia

		Absent Q waves in precordial leads	
Interrupted aortic arch	Cyanosis	Biventricular hypertrophy Right atrial enlargement	Metabolic acidosis Hypocalcemia
Pulmonary artery stenosis	Tachypnea Cyanosis Feeding difficulties	Right axis deviation Right ventricular hypertrophy	
Pulmonary atresia	Anoxic crises Dyspnea Polypnea (during feeding or crying)	Right axis deviation; Right atrial hypertrophy;Left ventricular hypertrophy	Acidosis Hypoxia
Tricuspid atresia	Dyspnea Heart failure	Left axis deviation; Right atrial hypertrophy; Left ventricular hypertrophy	Polyglobulia
Ebstein's anomaly	Early cyanosis in severe forms	Right atrial hypertrophy WPW	Respiratory acidosis
TGA	Dyspnea Tachypnea Sweeting Rough cry Grunt	Right axis deviation Right ventricular hypertrophy	Metabolic acidosis

Cases 1 and 2 exemplify proper cardiac, medical and surgical management in complex CHD.

Case 1, a 10-days-old, term newborn, with birth weight of 2,700 g , the first child of an apparently healthy couple (mother-18 years old, father-22 years old), was referred for tachypnea, cyanosis, feeding dyspnea and systolic murmur.

Our clinical evaluation identified, normotensive anterior fontanelle, hypotrophy, pale and elastic tegument , generalized hypotonia, perioral and peripheral cyanosis, hepatomegaly, normal intestinal transit and physiological urinations.

The patient exhibited signs of severe respiratory distress: tachypnea, intercostal and subcostal retraction, RR of 35/minute, SpO2= 65-70%. Hemodynamic instability was severe : tachycardia, grade IV/6 systolic murmur in the 3rd right intercostal space and left parasternal border, absent femoral pulse , cold lower extremities, right upper limb blood pressure =70/50 mmHg, right lower limb blood pressure = 50/30 mmHg.

Biological investigations revealed microcytic hypochromic anemia, and thrombocytosis.

The thoracic radiography showed cardiomegaly, cardio-thoracic index of 0.61 and pulmonary congestion.

The electrocardiogram detected sinus rhythm, with a HR of 160 bpm, right QRS axis deviation (+120 grade), flattened T waves in the anterior leads, slightly elongated QT: 0.28 sec. The echocardiographic aspects revealed concentric left ventricle hypertrophy, bicuspid aortic valve. The color Doppler superposition at the coarctation level and in the trunk of the pulmonary artery showed a turbulent flow and the continuous Doppler superposition showed an envelope with

increased velocity in the systole. In suprasternal incidence (Fig.I.2.) we assessed the aortic arch, the coarctation area, and the post stenotic dilatation.

We instituted the treatment with Prostaglandin E1, in order to maintain the permeability of the arterial canal, and diuretics, to decrease cardiac preload and prevent heart failure.

After 20 days of hospitalization, the patient was transferred to a specialized center of pediatric cardio-vascular surgery, where the surgical correction was performed.

Aortic coarctation is suitable for surgical repair starting from the neonatal period, but also at older ages. There are a number of surgical techniques used to restore the normal diameter of the aorta, with or without resection of the stenotic segment. Extended excision of the affected segment and termino-terminal anastomosis is the method of choice for it preserves the subclavian artery and provides optimal therapy for patients who, in addition to aortic coarctation, have isthmic hypoplasia and possibly hypoplasia of the distal portion of the aortic arch.

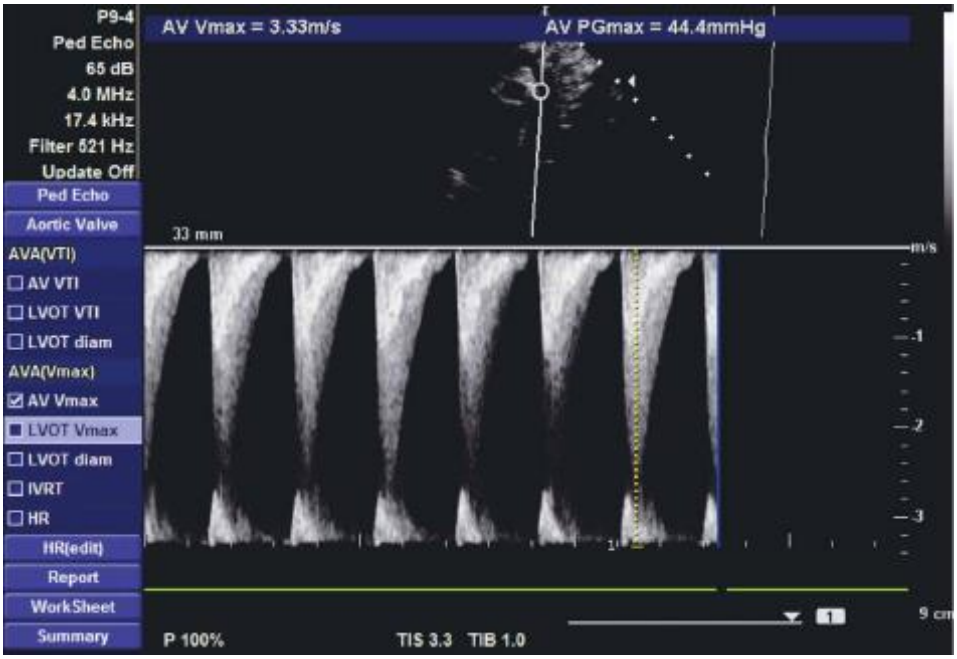


Figure I.2. 2D Transthoracic echocardiography, suprasternal section. Peak velocity is 3.33 m/sec and the pressure gradient is 44.4 mmHg which constitute as indications for severe impairment of blood flow

In our case, the resection of the segment that comprises the aortic coarctation, followed by termino-terminal anastomosis and the arterial channel ligature was done.

Other possible surgical techniques involve patch aortoplasty, lateral incision of the narrowed portion and the insertion of a polytetrafluoroethylene material in order to enlarge the affected arterial segment. The method is burdened by the risk of developing an aortic aneurysm at the site of patch insertion, and is reserved for elderly patients in whom a termino-terminal anastomosis cannot be obtained (Sadeghpour et al., 2014).

The post –surgical evolution of our patient was favorable, but with difficult hemodynamic adaptation, that required a prolonged inotropic support and vasodilatation treatment in increased doses for pressure values control. Also, erythrocyte mass transfusion was needed in order to treat anemia.

The echocardiography proved a normal flow in the descending aorta, without the existence of a gradient at anastomosis level. The pulmonary artery gradient was of 50 mm Hg (residual pulmonary high blood pressure), being considered as satisfactory.

The patient received treatment with captopril (3 mg/day), furosemide (1 mg/day), spironolactone (3.125 mg/day). The prophylaxis of infectious endocarditis was recommended, as well as periodical examinations in order to monitor blood pressure, echocardiographic aspects, particularly tracking an eventual stent narrowing.

Case 2, a 11-days-old male, term newborn, birth weight of 3400g, was hospitalized for generalized cyanosis, polypnea, tachycardia and a systolic murmur.

Our clinical examination revealed peripheral cyanosis, cold extremities, normotensive anterior fontanelle and hepatomegaly.

The patient presented signs of respiratory distress: RR = 45/min, SpO2= 70% without oxygen administration and SpO2= 98% after oxygen supplementation. There were also signs of hemodynamic instability: tachycardia, right upper limb BP= 67/47 mmHg; right lower limb BP=65/41 mmHg.

Cardio-thoracic radiography showed global cardiomegaly, CTI=0.7, normal pulmonary transparency.

The electrocardiogram showed sinus rhythm; HR= 140/min, right QRS axis deviation (+120 degrees), right atrial overload (high, sharp P waves in limb leads), major RBBB (QRS =180 msec in precordial leads) with secondary repolarization changes, type I AV block (PR=210 msec).

We performed TTE and found dilated right heart atrialized RV ,compression of the left ventricle and paradoxal movements of the interventricular septum, apical displacement of the tricuspid septal cusp insertion - 5.3/5.5mm/m2 body surface area *patent foramen ovale* with predominantly right to left shunt, and an ejection fraction of 38%.

All elements supported the Ebstein's anomaly diagnosis (Fig I.3.).

The infant received spironolactone during hospitalization and was transferred to the Institute of Cardiovascular Diseases in Targu Mures for possible univentricular corrections.

Our patient developed wide complex tachycardia before the surgical procedure. Cardiac arrhythmias in these cases are a negative prognostic factor and radiofrequency ablation is indicated. Heart failure was a major factor in the subsequent management of the case.

The degree of heart failure presented by the infant is supported by the small size of RV, 80% atrialized, tricuspid septal cusp hypoplasia, left ventricular dysfunction and pulmonary hypertension.

Surgical therapy in **Ebstein's anomaly** has the following indications (Allen et al., 2021):

- During the neonatal period, when general status does not improve despite appropriate drug therapy;
- If the oxygen saturation of the blood falls below 80%, or the hemoglobin level exceeds 16g / dL, or heart failure sets in;
- NYHA or ROSS class III-IV;
- History of paradoxical emboli;
- Life-threatening arrhythmias in patients with concomitant preexcitation syndrome (Wolff Parkinson White)

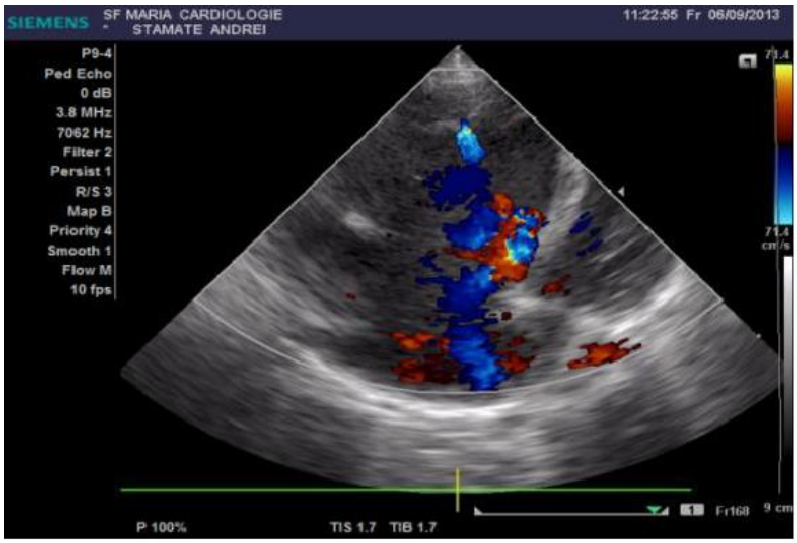


Figure I.3. *2D TTE - Doppler mode, apical 4 chambers view- Mitral regurgitation and dilation of the RV in a patient with Ebstein's anomaly*

Surgical procedures can be palliative and are intended for newborns with poor status. They involve in a first stage the BT-type shunt with ASD enlargement, provided that the left ventricle is functional, followed by a Fontan- type intervention. For cases with severe cyanosis, a bidirectional Glenn-type intervention may be attempted. Biventricular repair by the Danielson technique involves the plasty of the tricuspid valve resulting in a single-spike valve, the other two being often hypoplastic and unnecessary (Park & Salamat, 2021).

In general, tricuspid repair has much better long-term results than its replacement. Univentricular repair involves closing the tricuspid orifice through a fenestrated patch, widening the ASD, right atrial reduction, and placing a systemic-pulmonary shunt (Allen et al., 2021).

Pharmacological and surgical management

Pharmacological treatment of cardiac anomalies depends on the severity of the hemodynamic changes that they cause and is intended to prevent cardiovascular and respiratory failure.

The general principles that apply are:

- Oxygen supplementation in order to maintain a saturation of approximately 75-85% and no higher than that, in order to avoid premature closure of the *ductus arteriosus*
- Prostaglandin E1: 0.15-0.20 μg/kg/min and 0.20 μg/kg/min extra every 15 minutes, in order to maintain the patency of the *ductus arteriosus*. An option that is available only for neonates. Prostaglandin E treatment carries an increased risk of seizures, hypotension, bradypnea, tachycardia or ventricular block, which is why it is necessary to monitor the patient.
- Captopril 1 mg/kg for high blood pressure or Sildenafil l 0.5-2 mg/kg/dose for low blood pressure can be administered.
- Spironolactone 1 mg/kg in 1-3 divided doses or Furosemide 1 mg/kg/dose every 6 or 12 hours, provided that the diuresis is a minimum of 3 ml/kg/hour
- Sodium bicarbonate 4.2%: 2 mEq/kg/dose, intravenous, very slowly, ensuring adequate ventilation parameters is used in cases of metabolic acidosis
- Volume expanders, such as Ringer or sodium chloride 0.9%: 10 ml/kg, intravenous, if needed
- Inotropic agents : Dopamine 5-20 mcg/kg /min infusion

- Digoxin 0.04 mg/kg every 8 hours in the first day, followed by a maintenance dose, ¼ of the initial one divided in two. Digoxin poisoning manifests itself through vomiting, impaired general condition, or electrocardiographic modifications such as PR prolongation, ST segment depression, ventricular arrhythmias. In such cases, digoxin administration must be interrupted, Atropine 0.02-0.03 mg/kg should be given in bradyarrhytmias and Lidocaine of 0.5-1 mg/kg bolus followed by 0.02-.0.3 mg/kg/min in tachyarrhythmias.

Parenteral feeding is part of the treatment scheme for newborns, ensuring the appropriate caloric intake and the proportions between protein, lipids and carbohydrates. Enteral feeding through bolus feeding or continuous gavage can be initiated. A term ill newborn initially needs 40-60 kcal / kg / day, with a gradual increase to 90-120 kcal / kg / day. The prophylaxis of necrotizing enterocolitis is ensured through minimal enteral nutrition, approximately 10 to 20 ml/kg/day. The reason behind NEC is impaired intestinal perfusion during diastole combined with immature immune system and insufficiently developed mucosa (Cognata et al., 2019). MEN has been associated with a decreased incidence of NEC in this population (Martini et al., 2019).

Surgical management is the only corrective treatment.

Ventricular septal defects, especially those responsible for high interventricular shunt, need prompt surgical management if drug therapy is not enough to prevent heart failure and improve overall development. Adults with VSD either have a small defect, without hemodynamic impact, or have a major defect, which led to the installation of pulmonary hypertension, a situation that contraindicates surgery (Park & Salamat, 2021).

Also, if the systolic pressure in the pulmonary artery exceeds 50% of the systemic value, the defect must be closed surgically. It is considered that in the above situations, the intervention must be performed by the age of 1 year (Mann et al., 2015). In cases where surgery is an option, the direct closure of the defect can be performed, under the protection given by the hypothermic cardiopulmonary bypass, preferably without right ventriculotomy. Transatrial access is favored for perimembrane and inlet defects, by incision of the pulmonary artery in case of conal defects or by right apical ventriculotomy for apical defects. Atrioventricular septal defect can be closed using various surgical techniques, that use unique or double patches, and studies have shown similar results regardless of the surgical option, in terms of intra and perioperative mortality, residual shunt and left valvular regurgitation.

The surgical correction in **TOF** involves an artificially created systemic-pulmonary shunt, the Blalock-Taussing type (subclavian artery connected to the pulmonary artery on the same side) or modified Blalock-Taussing (interposition of a synthetic tube between the aforementioned vessels), followed by repair of intracardiac malformations by closing the septal defect and relieving the obstruction of the right ventricular ejection tract.

Adults with TOF are cadidates for surgery, considering that studies have shown that the results are satisfactory and the risk of surgery similar to pediatric cases (Sadeghpour et al., 2014).

Hypoplastic left heart syndrome can be repaired:

In a staged manner, starting with a Norwood procedure, which involves creating a connection between the right ventricle and the aorta by anastomosis between the pulmonary artery and the ascending aorta, adding a patch to enlarge the ascending aorta; the pulmonary flow is restored by a modified systemic-pulmonary shunt of Blalock-Taussing type or a central shunt , Sano type, that allows for a connection between the right ventricle and the pulmonary artery; the arterial canal is ligated and the interatrial septum is resected. The second stage consists of a bidirectional hemi-Fontan or Glenn type intervention in order to connect the superior vena cava to the pulmonary artery. The third stage involves Fontan type surgery (Da cruz et al., 2014).

By a hybrid procedure, followed by a Fontan operation that can be postponed. A band is applied to both branches of the pulmonary arteries, limiting pulmonary flow, and the arterial canal is permeabilized by a stent to maintain adequate systemic circulation. An atrial septotomy allows venous blood to return at the cardiac level. The second stage involves a two-way combination of Norwood and Glenn procedures, and the last stage consists of a Fontan-type intervention. The advantage of the second surgical approach to HLHS is that it allows the creation of a stable, balanced blood circulation, open heart procedures being postponed until the age of 3-6 months (Park & Salamat, 2021).

I.1.4. Discussions

Being caused by a wide range of gene mutations with overlapping phenotypic manifestations, isolated congenital heart diseases are suitable for genetic diagnosis by means of massive parallel sequencing. Whole exome sequencing ensures the evaluation of a gene panel traditionally associated with a cardiac malformation while whole genome sequencing allows for testing of the entire genome, in more complex cardiac anomalies. However, genetic investigations of isolated congenital heart abnormalities are economically justified only in cases of familial recurrence.

In cases where cardiac anomalies are accompanied by other organ malformations or malfunctions, targeted genetic testing by means of MLPA, karyotype, CGH or FISH is extremely useful, because, by uncovering the etiology, it helps establish the appropriate interdisciplinary management plan and even surgical options available.

A study conducted on a group of 95,253 patients under the age of 18, with various congenital heart defects who underwent surgery to correct the heart abnormality evaluated the risk for increased LOS and perioperative morbidities. Of these cases, 84% had no genetic identifiable cause, 9.9% had Trisomy 21, 0.2% Trisomy 13 and 18, 0.4% Turner syndrome, and 4.2% cases where monogenic abnormalities. In the group of patients with genetic abnormalities, increased LOS, nosocomial infections, cardiac arrest, renal failure and pulmonary hypertension were much more frequently encountered (Dillard et al., 2017).

Coordinating cardiac diagnostic with genetic testing, especially in syndromic cardiac malformations is therefore paramount for risk stratification, counselling and mapping a tailored management plan for patients.

Complex cardiac anomalies that require PDA in order to maintain a minimum pulmonary perfusion become apparent soon after birth. Changes in the neonatal circulation that encourage the closure of *ductus arteriosus* precipitate the cardiovascular and respiratory decompensation. Temporary salvaging measures already discussed relay mostly on supportive medication and artificial prolongation of the permeability of ductus arteriosus. However, the pressure in the pulmonary circulation eventually exceeds that of the systemic circulation, causing the reversal of the blood flow and leading to Eisenmerger syndrome a contraindication in most cases for surgical repair of the heart defect.

I.1.5. Conclusions

Comparing our results with the data available in the current literature, we noticed an overlap between the main characteristics of cardiac abnormalities, as they are found worldwide and those identified by us in our patients. Seeing as CHDs appear more frequently in patients diagnosed with genetic anomalies, this generated a future direction for our research, that is

establishing a genotype-phenotype correlation and discovering possible predictive factors for treatment response.

I.2. Genetic syndromes and congenital heart defects

I.2.1. Background

Genetic syndromes may be due to aneuploidy - abnormal chromosomal number-, to chromosomal structural alteration or could be the consequence of a mutation of a single gene. The association between genetic syndromes and heart malformations is based on the implication of genes involved in cardiac embryogenesis, as explained in Table I.3.

Table I.3. CHD and other malformations in frequently diagnosed genetic syndromes

Syndrome	Incidence	Associated CHD	Comments
Aneuploidies			
Down	1/800	ASD, VSD ,TGA, PDA, PTC	Down syndrome favors a second-hit phenomenon, leading to other monogenic mutations that determine heart defects
Edwards	1/5000	ASD, VSD, TOF, PDA, valvular dysplasis	Malformations that occur in ES affect the majority of organs
Patau	1/15000	ASD, VSD, TGA, AoC,	Associates multisystem failure
Turner	1/5000	HLHS, AoC, VSD, ASD, PSVC,	Turner syndrome facilitates duplications in the 12p13.31 region,
Deletions			
DiGeorge	1/6000	TOF, PTA,VSD ASD, HLHS, pulmonary valve stenosis	Significant phenotypic variability
Williams	1/20000	Valvular, coronary arteries and systemic arteries stenosis	Increased risk for arterial hypertension, ischemic phenomenon and malignant arrhythmias
Jacobsen	1/100000	VSD, aortic and mitral valves anomalies	
Wolf-Hirschhron	1/50000	ASD, VSD, TOF, PDA	Associates neurological malformations and other miscellaneous findings
Smith-Magenis		DSA, DSV, BAV, PDA	
Koolen de Vries		DSA, DSV, DSAV	

The phenotypic variability of genetic syndromes in terms of congenital heart malformations was the subject of the article below:

Luca, A.C.; Gavril, E.C.; Curpan, A.S.; Popescu, R.; Resmerita, I. ; Panzaru, M.C.; Butnariu, L.I; Gorduza, E.V.; Gramescu, M.; Rusu, C. Wolf-Hirschhorn Syndrome: clinical and genetic study of 7 new cases and mini review, *Children* 2021, pp.2-11, **IF 2,863**

I.2.2. Material and methods

Patient Recruitment

We selected from our available medical files a cohort of seven unrelated patients with cytogenetic or molecular diagnosis of WHS confirmed by means of MLPA kits and confirmed by FISH where needed. This group was composed of four boys and three girls, with ages between 1 and 13 years. The cases were evaluated at the Cardiology Department and Regional Medical Genetics Center, of "St. Mary" Children Hospital, Iasi, for an average period of 4 years.

Research Methodology

Patients underwent a complete history and clinical examination. Imagistic techniques such as echocardiography and MRI were used to assess both cardiac and neurologic involvement.

All patients were subjected to karyotype investigations (using peripheral blood) and MLPA (P096, P245 MLPA Kit for microdeletion screening, follow-up with Kit P264, P358 for microdeletion confirmation)—MRC Holland®. Karyotype analysis was performed on peripheral blood lymphocyte cultures. At least 16 metaphases with 550 bands were evaluated under a microscope, and the captured images were analyzed using CytoVision. DNA extraction was performed from peripheral blood using the Genomic DNA Purification Wizard. The DNA was precipitated in 70% alcohol, suspended in hydration solution, and stored at 4 ◦C.

The standard MLPA analysis was performed according to the manufacturer's recommendations: MRC Holland, Amsterdam, The Netherlands.

Genomic DNA (100 ng) was denatured and hybridized with the probes in the kit, at 60 ◦C for 18–20 h. After a binding step at 54 ◦C for 15 min, PCR amplification was performed with 1 µL universal primers labeled with Cy5 in a SensoQuest thermocycler (The KemEnTec Nordic, Denmark). The amplification was done after the following program: 35 cycles of denaturation (95 ◦C/30 s), hybridization (60 ◦C/30 s) and elongation (72 ◦C/60 s), completed with final elongation 72 ◦C/20 min. Fluorescent amplification products were subsequently separated by capillary electrophoresis in a CEQ sequencer 8000 GeXP Genetic Analysis System (Beckman Coulter).

The number of DNA copies was estimated using the Coffalyser.net program, which calculates the ratio of fluorescence intensity from patients to that of control cases for each target sequence. The results were expressed as the ratio of the number of copies of alleles to control patients (normal): the ratio obtained is 1 if both alleles are present,~0.5–0.7 if an allele is absent and ~1.3–1.5 respectively if an allele is duplicated. A normal range was set at a threshold of 0.8 for deletion and 1.2 for duplication.

I.2.3. Results

People with Wolf-Hirschorn syndrome have a deletion in the 4p16.3 region, whose phenotypic consequences are intellectual disability, multiple congenital anomalies and the appearance of the "Greek fighter helmet skull" (prominent eyebrow arches, hypertelorism, prominent nasal root, micrognathism, mouth with drooping corners). Other associated clinical manifestations include skeletal anomalies, congenital heart defects, eye abnormalities, hearing loss, genitourinary tract defects, and immunological disorders (Battaglia et al.2015).

Cardiac involvement in Wolf-Hirschhorn syndrome

The distribution of cardiac defects and the correlation with other anomalies and the size of the deletion in our group is summarized in Table I.4.

The distribution of cardiac defects in the studied group emphasizes an interesting aspect: the main system affected by the dimensions of the deletion located at the level of the 4p arm is the cardiac one. If the other abnormalities have a quasi-equal distribution, respecting the typical

aspects of this syndrome, the cardiac malformations are even more serious the greater the deletion, hinting at the presence of important genes in the p arm of chromosome 4.

Table I.4. Size of deletion and cardiac phenotype

CHD	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	
ASD	+	+	+	+	+	+	+
VSD		+				+	
Tricuspid regurgitation		+				+	
Mitral regurgitation						+	
PDA							
Pulmonary valve stenosis			+				+
Pulmonary valve regurgitation						+	
Size of deletion	2 MB	2 MB	2,85MB	2 MB	2 MB	8MB	22MB
Brain anomalies	+		+	+			
Jacksonian seizures		+					
Renal anomalies				+	+		
Hearing loss						+	+

Traditionally, chromosome 4 anomalies have been associated with neurological, skeletal and malignant diseases mainly. Multiple genotype-phenotype studies established the 2 critical regions responsible for the WHS phenotype, namely WHSCR1, , limited to a 165 kb interval at about 2 Mb from the telomere and WHSCR2, within an interval of 300–600 kb between the loci D4S3327 (at 1.9 Mb from the telomere) and D4S98-D4S168 (at 1.6–1.3 Mb from the telomere) **(**Zollino et al., 2000).

Based on the extend of the deletion, different categories of the WHS phenotype can be defined: the ‘‘mild’’ form, caused by deletions not exceeding 3.5 Mb, the ‘‘classical’’ form, associated with an average deletion of 5–18 Mb that leads severe psychomotor delay, delayed or absent speech, late walking and, very commonly, major malformations. Large deletions, exceeding 22–25 Mb, determine a phenotype difficult to describe as WHS (Nevado et al., 2020).

The critical interval for heart malformations starts at 5 Mb from the telomere. Several authors indicated that NSD2 gene, found in that interval , could be responsible, as it interacts with NKX2.5 in order to regulate cardiac transcriptional network (Mekkawy et al., 2021). FGFRL1 gene was also suggested as a possible cause for CHD in WHS based on experiments performed on null mice for homologous Fgfrl1 gene showing skeletal and septal and valvular heart

abnormalities. However, the location of the gene is at the tip of the chromosome 4, and deletion of the first 1.4 Mb region of chromosome 4p has been observed in non-affected individuals (Tautz et al., 2010).

Genotype-phenotype associations

In our study, severe CHD were diagnosed in patients 6 and 7.

Case 6 is a 5-year-old girl, the first child of an unrelated young couple. Her karyotype is 46,XX,del(4)(p16.1-pter) and 8Mb deletion detected by FISH. Besides cardiac malformations, she suffers from severe intellectual disability, hearing loss, hypotonia, anodontia and facial dysmoprhism.

Case 7 is the third child of an otherwise normal family. Her karyotype was 46,XX,del(4)(p15.2-pter) and MLPA confirmed a ~22 Mb deletion in WHS region. She had developmental delay, and hearing loss (bilateral stenosis of auditory canal).

Considering the large dimensions of the deleted interval, further investigations for phenotype mapping will be required, in order to determine the incriminated gene/s that caused the CHD.

In *cases 1, 2 and 4,* the syndrome was caused by microdeletions (~2 Mb).

Case 1 is a 13-year-old boy, first child of a young, unrelated, apparently healthy couple, with no similar cases in the family. Postnatal development was severely delayed and the patient had recurrent respiratory infections. MRI showed agenesis of corpus callosum and mild dilatation of ventricular system. Karyotype was 46,XY and MLPA confirmed a ~2 Mb deletion in WHS region, implicating the critical genes mentioned before.

The second case, a 4-year-old girl, is the only child of non-related parents. Karyotype was 46,XX and MLPA confirmed a ~2 Mb deletion in WHS region.

Case 3 is a 2-year-old boy, second child of an unrelated, apparently healthy couple. Karyotype was 46,XY,r(4)(p15.1q35)/46,XY,−4,+mar/47,XY,r(4)(p15.1q35),+mar/46,XY,der(4),+mar and MLPA confirmed a ~2.85 Mb deletion in WHS region.

In *case 4,* only the 2 Mb deletion in region 4p15.33→ter was important for the phenotype. However, the genetic investigations revealed that the father was carrier of a balanced translocation: 46,XY,1qh+,t(4;17)(p15.33;p13.3), thus indicating the parental couple had a 10–12% risk for an abnormal embryo that associates either 4p15.33→ter monosomy and a small 17p13.3→ter trisomy or a small 17p13.3→ter monosomy and a 4p15.33→ter trisomy.

Case 5, a 1-year-old boy, in addition to CHD also had seizures, and frequent respiratory infections. Karyotype was 46,XY,del(4)(p16.3),del(22)(q11.23) and MLPA testing uncovered ~2 Mb 4p deletion with the additional deletion in 22q11.2 in RTDR1 gene. The gene is located in a region deleted in pediatric rhabdoid tumors of the brain, kidney and soft tissues, but mutations in RTDR1 gene have not been associated with the disease.

I.2.4. Discussions

Initially, the pathogenesis of WHS was thought to be owed to a single gene. Nowadays, it is known that the clinical variability is doubled by genetic variability. Different genes on 4p16.3 have been suggested to cause distinctive clinical manifestations. Intensity of seizures is attributed to the synergistic effect of several genes, such as *PIGG, CPLX1* and *LETM1*.

Epilepsy maps on the terminal 1.9 Mb region and facial dysmorphism on a 300 kb interval comprised between 1.9 and 1.6 Mb from the telomere. The region for growth delay is limited to

300 kb between 1.9 and 1.6 Mb from the telomere. Microcephaly had distal boundary at 2.2 Mb from the telomere, and cleft palate at 2.5 Mb (Zollino et al., 2003).

The majority of scientific reports consider the phenotype associated with 2 Mb deletions as mild and the cardiac involvement in these cases is either absent or represented by ASD. In our cohort, however, the patients with limited deletion present VSD, valvular insufficiency and, in case 3, pulmonary valve stenosis, which, as discussed previously, poses serious risks because of the important obstruction of ventricular blood flow.

These findings support the hypothesis that genes found within the 2Mb intervals play an important role in either cardiac function or development, something that will require further studies into genotype influence.

Moreover, as stated earlier in this paper, genes typically involved in septal configuration are localized on chromosomes 5 (*NKX2.5*) , 6 (*CITED2*), 7 *(TBX20*) , 8 (*GATA4*) , 12 (*TBX1*), 14(*MYH6),* 15(*ACTC1*) and 20 (*TBX5*) and those responsible for normal pulmonary valve development on chromosomes 7 *(BRAF*) , 11 *(HRAS*), 12 (*KRAS* and *PTPN11*). The association between deletions on chromosome 4p and cardiac defects has not been explained through gene function studies. The theories currently in circulation are based on animal models, particularly on mouse experiments. However, the mouse gene arrangements on chromosome 4 differs from the human one, making these studies indicative, but without proper justification of the pathogenesis in humans. In our study, MLPA analysis revealed haploinsufficiency of *FGFRL1* gene in all seven cases, and all but one presented mild cardiac defect. *FGFRL1* gene was stated as responsible for the cardiac pathogenesis in WHS in humans based on mouse studies. Corroborating this information, we can consider FGFRL1 as not having a definitive role in pathogenesis.

Intrauterine growth restriction is a clinical sign present in most cases of WHS (80–90% cases) (Battaglia et al, 2015). In our study, IUGR was detected in cases with deletions larger than 2 MB, and postnatal failure to thrive was a constant feature presented in all reported patients.

Cardiac congenital heart diseases have long been implicated as a cofactor for developmental delay in patients. The relationship between these two phenomena is bidirectional. Factors acting *in utero* can lead to IUGR which in turn will affect cardiac morphogenesis. Babies born at term with fetal growth restriction usually catch up growth within 2 years of life, depending on the severity of the FGR. The presence of a CHD can impede the process of the growth process, closing a vicious circle.

In our cohort, the patients with the larger deletions had the more serious cardiac involvement and therefore the question of who acted first rises: was the cardiac impairment in utero, caused by complex gene interaction that led to IUGR or the other way around? It is important to answer this question in order to establish a clear management direction for fetuses diagnosed with cardiac defects.

I.2.5. Conclusions

Wolf-Hirschhorn syndrome is a rare genetic disorder characterized by complex clinical manifestations and a severe prognosis. The clinical spectrum of this disease causes difficulties in diagnostic and management, particularly from a cardiac and neurological point of view. Also, the carcinogenetic risk in these patients is insufficiently analyzed. Therefore, cytogenetic, and molecular tests are imperative in these patients. Despite the fact that 85% of cases are de novo mutations, in 15% of cases the derivative chromosomes generated by a meiotic mis-segregation of a balanced translocation present in one of the parents were identified. Hence, different genetic investigations (CMA, MLPA, FISH, karyotype) are requested in order to complete the diagnosis

in patients with WHS, as well as the chromosomal analysis of the parents becoming mandatory for assessing the risk of occurrence of another case in family.

I.3. Primary prophylaxis of congenital heart disease

I.3.1. Background

Being an important public health problem, congenital heart diseases benefit from rigorous screening both prenatal and postnatal. Ultrasonography can tell early if the fetus has a structural heart abnormality, but the detection rate depends on gestational age, fetal position, and type of heart defect. Pulse oximetry is a non-invasive, easy-to-perform examination method that has proven useful as a postnatal screening method. It is performed in the first 48 hours after birth, a positive test being considered one in which SaO2 <90%, in which case it is no longer necessary to repeat it and the echocardiographic investigation of the newborn is indicated (Harold, 2014).

A negative result does not rule out the presence of heart disease. A saturation below 95% or a difference greater than 5% between the level of peripheral oxygen saturation measured on the right hand and one of the lower limbs is considered borderline pathological. Screening is a method of secondary prophylaxis, in the sense that it can no longer prevent the occurrence of heart disease, but early detection improves the quality of life of the patient and his family, while easing the economic and social impact of this category of disease.

Vaccination programs aim at reducing the risk for infectious disease associated with teratogenic effects and cardiovascular anomalies. Maternal immunization is a safe strategy to ensure primary prophylaxis of certain CHD as well as protective neonatal antibiodies levels. Inactivated vaccines for influenza, pertussis and tetanus can be administered in pregnant women (Robl-Mathieu, 2021). The MMR vaccine (Measles, Mups, Rubella) should be given at least a month before conception.

A balanced diet and the periconceptional use of vitamins, especially folic acid and vitamin B12, by both the mother and father may reduce the risk of neural tube defects, abdominal wall malformations, and heart malformations, according to several studies (Feldkamp et al., 2011, Botto et al., 2002, Ionescu-Ittu et al., 2009).

The efficacy of folic acid in preventing the occurrence of omphalocele or neural tube defects has been demonstrated, but the preventive effect of this treatment is increased if folic acid is taken three months before conception and continued throughout the first pregnancy trimester (Van Allen Mi, 2002). Since this is not always feasible at the population level, especially in low-income countries, where more than half of the total number of pregnancies are unplanned, another method that has already begun to be implemented is industrial food enhancement using folic acid Food enhancement using folic acid may prevent about 46% of neural tube defects and reduce the neonatal mortality rate due to visible birth defects by 13% (Blencowe H, 2010).

Good health and nutrition before conception are central to a mother's ability to meet the nutrient demands of pregnancy and breastfeeding and are vital to the healthy development of the mother's embryo, fetus, infant, and child. Many women and adolescent girls are malnourished because of the inadequacy or imbalance of their diets, leading to malnutrition and micronutrient deficiencies or, conversely, overweight and obesity (Koletzko et al., 2019). Particular attention should be paid to the intake and status of some micronutrients in women of reproductive age, especially folate, but dietary supplementation with iron, zinc, vitamin D, vitamin B12, iodine, and others may also be recommended for women at risk of the poor supply and insufficiency of these

micronutrients. The importance of vitamin D for fetal skeletal development is well-known, and maternal deficiency results in a low birth weight, increased risk of neonatal hypocalcemia, cardiac failure, osteopenia in the newborn, and childhood rickets (Cooper et al., 2016). Pregnant women should consume a balanced diet and should not increase their dietary energy intake during late pregnancy by more than about 10% above the recommended energy intake for non-pregnant women to avoid obesity (Koletzko et al., 2019). Healthy pregnancy outcomes are more likely if the woman who enters pregnancy is physically active, has a healthy diet, does not smoke, avoids alcohol, and has a normal body mass index.

There are a multitude of individual or social circumstances that translate into risk factors for the generation of congenital heart defects. Some of them are modifiable and are the subject of analysis in this section. Whether we are talking about elements proven to be involved in the pathogenesis of cardiac developmental abnormalities, or about widespread factors that could be the cause of minor abnormalities, for primary prophylaxis both categories require attention.The interest for identifying risk factors for CHDs is reflected in the articles:

1. **Luca**, **A.C.;** Tarca, E.; Rosu, S.T.; Cojocaru, E.; Trandafir, L. ; Rusu, D.; Tarca, D.. Socio-epidemiological factors with negative impact on infant mortality, and the occurance of birth defects, Healthcare, 2021**,** pp.1-12, **IF 2.645**
2. **Luca**, **A.C.**; Braha, E. Pre-existing maternal disease-risk factor for congenital abnormalities in the newborn, *Ro J Pediatr.* 2016;66(2), pp.141-144.

I.3.2. Material and Methods

We conducted a thorough literature review using PubMed as a reference database and infant mortality, morbidity, socio-economic status, genetic anomalies and as keywords. We compared the results with data provided by the Institute of National Statistics regarding causes of infant mortality in the last 20 years in Romania. Lastly, we matched the information with our patients pathological and familial history from the archive of the cardiology department to establish and interpret correlations between maternal history and the risk for congenital heart disease.

I.3.3. Results

Teratogens are any agent (medicine, drug, chemical, physical agent, external agent, infections, maternal diseases) which causes a permanent anomaly in its structure the function of the embryo or fetus affects growth or causes his death (Covic et al., 2017). Typically, in the first two weeks of pregnancy, the teratogens destroy the embryo and lead to miscarriage. Pregnancy-associated plasma protein A appears to be a biomarker for maternal diabetes and newborn with heavy birth weight (Springer, 2019). Some studies note a correlation between levels of HbA1c and the incidence of major birth defects in the newborn of a mother with diabetes (Perloff, 2012; Mossmann, 2015). HbA1c levels higher than 11.5% are associated with congenital anomalies in 66% of offspring: transposition of large vessels, ventricular septal defect, and dextrocardia are the most common CHD, anencephaly, spina bifida and hydrocephalus are major malformations of the central nervous system (Suuronen et al., 2015).

Some results show significant differences in terms of IQ for children of mothers who have presented undiagnosed subclinical hypothyroidism during pregnancy, the child depending entirely on maternal thyroid hormones in the first 12 weeks pregnancy until the fetal thyroid appears (Da Cruz et al., 2014). Children of mothers with untreated hypoparathyroidism and low serum calcium may have parathyroid hyperplasia and transient hyperparathyroidism in the fetus or neonatal. Congenital hyperparathyroidism should be considered in newborns with respiratory distress, thoracic deformities, bone demineralization, and periosteal resorption along the long bones, pulmonary artery stenosis, ventricular septal defect and muscular hypotonia (Nevado et al., 2020; Mekkawy et al., 2021).

Folate deficiency can lead to congenital abnormalities (neural tube defects, cardiac abnormalities), anemia and miscarriages, preeclampsia and intrauterine growth retardation Preconception supplementation with folic acid (400 μg / day) prevents neural tube defects (Tautz et al., 2010).

However, some experimental studies support the idea that folic acid administered during the first weeks of pregnancy is related to epigenetic modifications in fetal DNA, leading to increased risk of developing allergies and asthma in childhood. Maternal iron deficiency has an indirectly influence the neonatal iron reserves and birth weight. This deficit can lead to cognitive and behavior problems in childhood. Calcium deficiency is associated with preeclampsia and intrauterine growth restriction.

DNA polymorphism-diet-cofactor-of-development hypothesis claims that schizophrenia is due to fetal brain abnormalities, determined by the interaction between gene mutations maternal and nutritional co-factors (e.g. cobalamin or pyridoxine) that are deficient in the maternal diet (Kumar & Elliot, 2018). It is well known that both vitamin A excess and deficiency leads to birth defects including eye malformations such as rudimentary eyelashes, microphthalmia, exophthalmia, ear abnormalities, cardiovascular anomalies (cardiac hypoplasia, persistence of the arterial canal, large vessel transposition), or diaphragmatic hernia (Saad et al., 2010; Gatzoulis et al., 2017; Kohler et al., 2018).

According to our research, the socio-economic status of parents greatly affects the probability of a congenital malformation in the newborn as well as augmenting the risk for miscarriage. The link between these two aspects is represented by the higher exposure to toxic products, inadequate eating habits, and increased alcohol consumption. Perinatal mortality is also far greater in cases where access to professional medical supervision during pregnancy and medical assistance at the time of birth are absent.

Regarding CHDs, we found that the canonical maternal risk factors remain the most important culprits.

I.3.4. Discussions

Maternal conditions usually investigated by the TORCH test are the subject of primary prevention. Vaccination against rubella at least 6 months before conception is an easy measure to prevent congenital heart disease associated with rubella infection, namely pulmonary stenosis, patent arterial duct, tetralogy of Fallot.

Among the clearly identified teratogens is maternal pregestational diabetes. It is associated with lateral heart defects, conotruncal or atrioventricular septal defects **(**Gatzoulis et al., 2017; Kohler et al., 2018). The levels of glycated hemoglobin have a prognostic utility, seeing as levels exceeding 11,5% are linked with 66% chances of CHD. There are opinions that folic acid treatment

can also help reduce the impact of maternal diabetes on fetal development, in addition to the effect of preventing neural tube defects.

Phenylketonuria is a condition diagnosed at birth through neonatal screening. Female patients were submitted to a treatment based on a diet low in phenylalanine up until puberty, and developed normally. However, patients with phenylketonuria who wish to have children of their own should engage in secondary prophylaxis measures comprising of restrictive diet for phenylalanine at least 2 years before procreation and the maintenance of this diet throughout pregnancy. Sapropterin dihydrochloride (Kuvan®) is a drug which allows the control of the level of phenylalaninemia during pregnancy and is a viable alternative (Gatzoulis et al., 2017).

Exposure to retinoic acid, common as a result of its widespread use in dermatocosmetic skin care products is related to r\ complex conotruncal heart defects associated with extracardiac abnormalities. Eretetinate, used to treat psoriasis, and isotretinoin treatment of severe acne, are derivatives of retinoic acid, with a teratogenic effect similar to it **(**Gatzoulis et al., 2017).

Obesity, smoking and caffeine consumption, all widespread elements among women of childbearing age, are points of interest for the future analysis of their implications in the pathogenesis of congenital heart malformations. At present, studies have not found a statistically significant risk of developing any type of heart abnormality in the context of the action of the aforementioned factors. On the other hand, severe chronic alcohol consumption has well-known teratogenic effects, causing heart defects in the heart. However, it is not known whether moderate consumption has any impact on fetal development.

Maternal autoimmune disorders, such as Lupus erythematosus, increase the risk for cardiac conduction disorders, endocardial fibroelastosis and cardiomyopathies. Hypothyroidism and hypoparathyroidism cause neonatal instability manifested by cardiac failure, tachycardia exophthalmia and bone demineralization, VSD, pulmonary valve stenosis, respectively.

I.3.5. Conclusions

In conclusion, knowledge of human teratogens offers the possibility to prevent some types of congenital malformations. This study demonstrates the continuing need to assess health status of the mother when we are dealing with newborn congenital anomalies. Identification of high risk pregnancies as well as discussions about teratogenesis and the provision of appropriate documentation aim to reduce the teratogenic potential of maternal diseases.

I.4. Pompe Disease

I.4.1. Background

Cardiac involvement is a critical component of a high range of inherited metabolic disorders that typically manifest as a multisystem disease.

The diagnostic is usually miscellaneous and the cardiovascular signs and symptoms do not always present themselves from the onset of the disease.

There are three major categories of metabolic disorders, classified according to the main pathological event (Harold, 2014):

- Diseases caused by a metabolic block: the accumulation of intermediary metabolites leads to intoxication, as is the case in propionic academia
- Disorders provoked by energy metabolism malfunction: the heart is a major energy consumer and in this type of anomalies there is a faulty energy chain provider that leads to impared cardiac function; the liver, striate muscles and brain tissue may also be affected;

- Diseases impacting complex molecules: they arise from an incomplete or absent anabolism or catabolism of complexe molecules and usually have an insinuating onset

Pompe disease or type 2 glycogen storage disease is a rare neuromuscular disorder caused by a mutation in the gene encoding alpha glucosidase acid (GAA) located on chromosome 17q25.2-q25.3(Kohler et al., 2018). A study conducted on the European population in 1998 showed an incidence of 1:100,000 for infant form (early onset and 1: 60,000 for the juvenile and adult form (Llerena et al., 2016).

The transmission of type 2 glycogenosis is autosomal recessive, which means that the carriers (individuals who have a normal and a defective allele) will not be affected. The incidence of carrier is 1/138,000 for classic infantile disease and 1/57,000 for late-onset disease (Mechtler et al., 2012).

GAA has the role of cleaving glycogen from lysosomes into glucose 1 phosphate. In the absence of this enzyme, glycogen will accumulate in the lysosomes from skeletal muscle, heart and liver leading to the lysosomal membrane rupture and accumulation of hydrolytic material in the cytoplasm (Adeva-andany et al., 2016). Muscle reshaping causes various clinical manifestations, depending on the affected tissue.

The infant form evolves most severely and is diagnosed in children younger than 12 months. The enzyme replacement therapy is the only available option for preventing premature death.

Patients present with hypertrophic cardiomyopathy, respiratory failure and progressive loss of ability to breathe independently, generalized hypotonia, delayed or absent neuromotor acquisitions, myopathy, macroglossia, difficulty feeding (Owens et al, 2018). In the atypical (or nonclassical) infant form, patients do not have cardiomyopathy (Kohler et al., 2018).

The juvenile and adult form are diagnosed in both children aged 1-18 and adults. The clinical manifestations are less severe and have a slower progression. However, patients will declare axial muscle weakness or pelvic and shoulders girdle and may progress in the final stages to the complete inability to mobilize independently, dysarthria, dysphagia, dysphonia, eyelid ptosis, macroglossia, respiratory failure, sleep apnea, aneurysms, hepatomegaly, chronic diarrhea, osteoporosis and scoliosis (Kohler et al., 2018).

The treatment of Pompe disease consists of ERT with recombinant human alpha-glucosidase administered intravenously every two weeks. This treatment has appeared on the pharmaceutical market since 2006 and is not curative but improves the evolution of the disease. This line of therapy poses the risk of developing anti-GAA antibodies after initiating treatment higher in CRIM-negative patients (cross-reactive immunological material; patients in whom no endogenous AGA protein was detected following Western blot analysis) than in CRIM-positive patients (patients in whom the endogenous AGA protein was detected by Western blot analysis) (Tarnopolsky et al., 2016).

The interest for this specific spectrum of diseases is reflected in the articles below:

1. **Luca, A.C.**; Diaconescu, S.; Rusu, C.; Bozomitu, L; Vlad, E.; Matei-Ciobanu, O.L.; Babici, R.; Donea, L.; Strat, S. Pompe disease a late-onset misleading form of diagnosis in a patient with persistent hepatic cytolysis syndrome, *Ro J Pediatr.* 2020;69(2), pp.162-165.
2. **Luca, A.C.**; Braha, E. Cardiovascular involvement in Pompe Disease, *Ro J Pediatr.* 2017;66(1), pp.8-11.

I.4.2. Material and Methods

We conducted a literature review and compared the data with the cases diagnosed in our Cardiology Department. We then presented the cases of a 15 years old boy and his brother, both diagnosed by molecular testing with Pompe disease. We detailed and discussed the multidisciplinary approach necessary for diagnosing and treating our patients.

I.4.3. Results

The initial family anamnesis uncovered that the adolescent patient had 3 siblings, one of which died 3 days after birth without being diagnosed. His other two siblings and his parents were apparently healthy. No other congenital anomalies, genetic diseases, intellectual disability were present in his family.

Our patient had a normal psychomotor development. There were no pathological aspects identified at the clinical examination. The objective neurological examination was normal, with no signs of central deficit or intracranial hypertension. However, the patient complained of decrease in exercise capacity. The ECG revealed sinus bradycardia and left ventricular hypertrophy (Fig. I.4.).

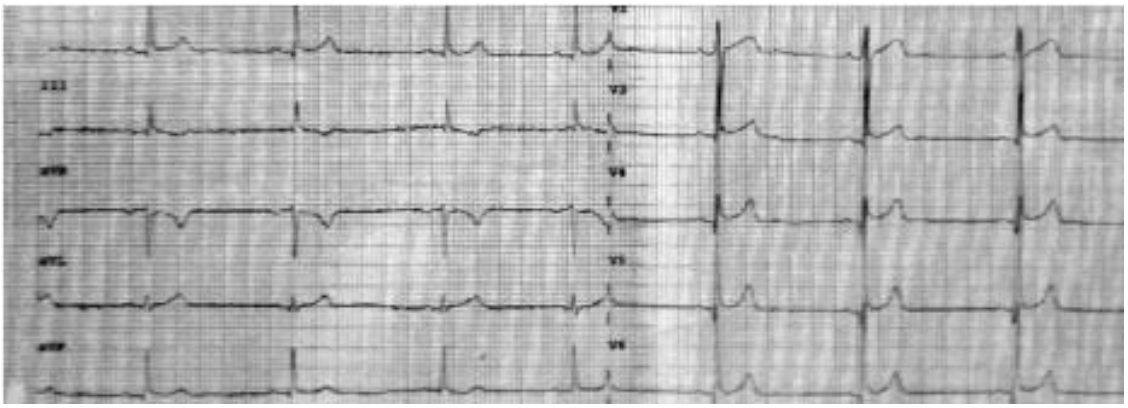


Figure I.4. ECG showing HR of 50 bpm, Sokolov-Lyon index greater than 35 mm, short PR segment and repolarization abnormalities

Biological investigations identified , hyperammonemia (63.73 ng/dl, NV = 11-51 µmol/l), hepatic cytolysis (AST = 227 U/l, ALT = 148 U/l, 3xNV), rhabdomyolysis (CPK = 659 U/l, NV = < 308 U/l, LDH = 659 U/l, NV = 300-600 U/l), negative viral serology for hepatitis B and C, negative lupus cells. Anti-LKM antibodies, and ANA panel did not support an autoimmune disease diagnostic. Restrictive respiratory dysfunction was not identified during functional tests.

The echocardiography diagnosed mitral valve prolapse, with grade I-II mitral regurgitation (Fig. I.5.).

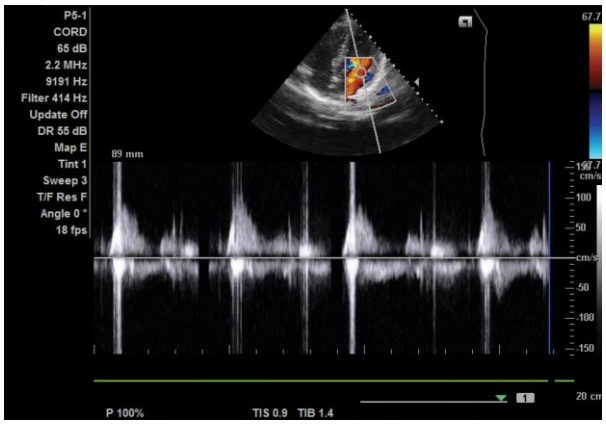


Figure I.5. 2D TTE, Apical 4 chamber view; Doppler mode- shows Mitral regurgitation

The investigations for Pompe disease were positive: enzymatic test (No. 192007415–EMPO) - alpha 1.4 glucosidase activity): 0.5 µmol/l/h (reference value > 2), molecular test - DNA extraction, PCR amplification, sequencing (No. 192012026 EMPG/11.09.2019): 2 pathogenic variants in the GAA gene (compound heterozygous status) c.[-32-13T>G]; and [406T>A].

Following thorough genetic investigation, the parents were identified as healthy carriers (heterozygous status), and an apparently healthy brother was confirmed with the diagnosis of Pompe disease (Fig. I.6.).

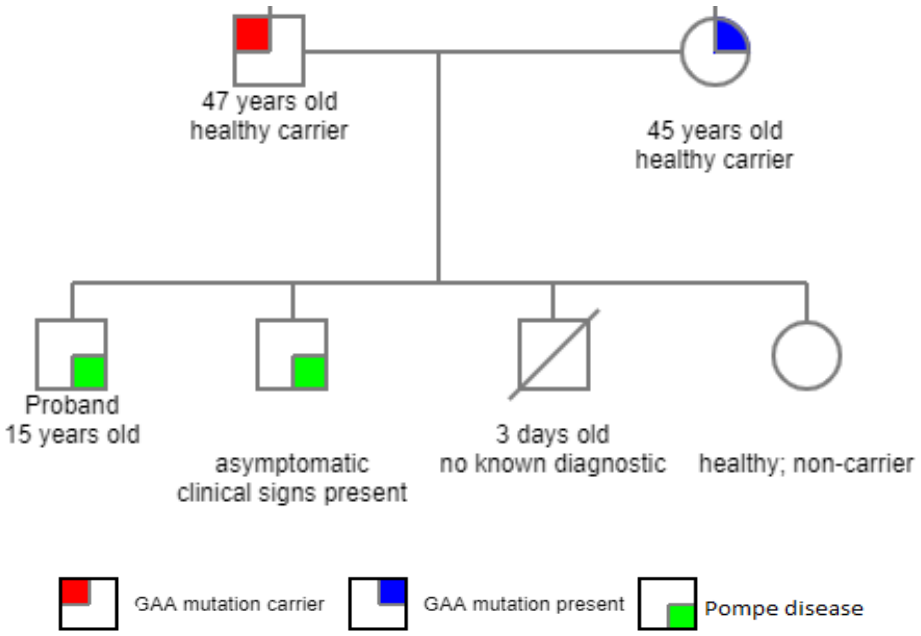


Figure I.6. Pompe disease pedigree showing carrier status of the parents and the affected individuals.

The medical investigations of the second proband uncovered no signs of impaired muscle strength, but upon palpation of the calf muscles a “orange pulp”-like feeling was detected, a possible sign of glycogen stores. Laboratory data revealed a minor hepatocytolysis syndrome (2 x NV), discrete hyperbilirubinemia based on the direct fraction and rhabdomyolysis (CPK = 5 x NV).

TTE revealed anterior mitral valve prolapse, minimal mitral reflux, grade II-III tricuspid reflux, small ASD *ostium secundum* type, without PH. The ECG detected sinus bradycardia with no other modifications.

No restrictive changes were present during respiratory function tests. Thoracic X-ray examination revealed accentuated perihilar and bilateral hilio-basal lung pattern, and normal appearance as for the rest. The exercise stress test was not altered.

Abdominal ultrasound showed homogeneous hepatic echostructure with moderate hepatomegaly.

The enzymatic and molecular test for the PD revealed the reduced activity of alpha 1,4 glucosidase and the presence of the same c.[- 32-13T>G] and [406T>A] mutations.

Both patients are now receiving Myozime® , an enzymatic substitute therapy. We initiated the treatment with a starting dose of 20 mg/kg/dose, as recommend in the current therapeutic protocols. Liver, cardiac , and respiratory functions are periodically evaluated through biological and imagistic investigations.

I.4.4. Discussions

The classic form of PD is the most severe end of the spectrum with rapidly progressive hypertrophic with or without left ventricular outflow tract obstruction and EKG anomalies, alongside respiratory distress, hypotonia and feeding difficulties (Li et al., 2020). In terms of EKG modifications, PR prolongation, LVH signs, atrioventricular blocks and Wollf-Parkinson-White syndromes have been noted.

The late onset form of Pompe disease is generally difficult to diagnose, due to the miscellaneous and insidious characteristics of symptoms. Although general rules apply, meaning that the warning signs consist of progressive myopathy affecting skeletal muscles, cardiac involvement varies in its manifestations. Cardiomyopathy is rarely seen, probably because of a residual enzymatic activity. Electrical disturbances are present in the form of shorter PR interval, sinus bradycardia and WPW syndrome. The accumulation of glycogen in the cardiac conducting tissue classifies patients in the group at high risk of sudden death, especially under stress, in an infectious context or during anaesthesia, which is why these patients are approached in a multidisciplinary team. Excess glycogen in the media of blood vessels may lead to aneurysm of the basilar and internal carotid arteries or dilation of the ascending aorta. The patients could be asymptomatic or have unspecific neurologic symptoms.

Studies also showed a link between the expanding spectrum of *GAA* mutations and the phenotypic variability of PD, with special emphasis on the cardiovascular type of anomalies. For instance, a tudy conducted by Jia uncovered a family with PD, for which two novel mutations, a missense mutation c.2238G>C (p.Trp746Cys) from the father and a frameshift mutation c.1388_1406del19 (p.Arg463fs) from the mother where responsible. Evaluating gene activity, the team discovered that, compared to the missense mutation, the consequence of frameshift mutation was more serious, leading to almost complete loss of enzyme function. Moreover, metagenomics analysis revealed the important interplay between gene mutations responsible for PD and mutations that determine autophagy impairment, both phenomena contributing to the appearance of cardiovascular anomalies otherwise not associated with PD. It also seems that certain mutations of the GAA gene are more likely to cause an overall biochemical imbalance, leading to exceeded variability of the disease manifestations (Jia et al., 2020).

This findings raise questions regarding the appropriate management plan for patients diagnosed with PD. From the cardiologist's perspective, this means that solely EKG and 2D echocardiography are no longer enough to supervise disease manifestations and progression.

For example, *El-Gharbawy H.A et al*, found dilated arteriopathy involving the thoracic aorta in a patient diagnosed with Pompe, a novel vascular complication, which was not previously associated with this disease, indicating that screening tools for aortic aneurysms should be incorporated in the long-term evaluation protocol (El-Gharbawy et al., 2011).

In terms of treatment, despite very good results rendered by enzyme replacement therapy, the adverse effects, namely anti-GAA antibodies in CRIM-negative patients as well as the goal of improving compliance and quality of life among patients fueled researches into novel therapeutic strategies. Systemic delivery of AAV vectors encoding *GAA* is an attractive alternative to ERT as is targeted intramuscular treatment (Salabarria et al., 2020).

I.4.5. Conclusions

Pompe disease leads to advanced disability status, affecting muscular function, while also leading to CNS and cardiac abnormalities, a situation in which existing treatments are ineffective. Early diagnosis ensures survival and preservation of the quality of life by maintaining mobility, preserving muscle strength and preventing complications, provided that the appropriate therapy is initiated. The advent of new gene therapy techniques might prove useful in finding tailored management and ultimately curing the disease itself.

Routine cardiac evaluation in PD is a essential and should include extensive imagistic studies and surveillance of the cardiac electric activity to prevent sudden cardiac deaths, a far too common event among affected patients.

I.5. Endocardial fibroelastosis

I.5.1. Background

Endocardial fibroelastosis is a process consisting in collagen and elastin deposition in the endocardial layer of the heart, leading to changes of both structural and functional nature in the left ventricular endocardium (Zhang et al., 2017).

Multiple mechanisms have been implicated as causes of PEF, including obstruction of subendocardial lymphatic drainage, blockage of Thebesian veins, premature closure of the foramen ovale. Primary endocardial fibroelastosis tends to manifest in the first year of life, most often having a sudden onset, precipitated by a respiratory infection.

Secondary fibroelastosis is associated with congenital heart malformations characterized by an incompetent left ventricle with an obstructed ejection tract.

Corroborating evidence from echocardiographic and anatomo-pathological aspects led *Seki et al.*, to conclud that primary endocardial fibroelastosis has morphopathological characteristics distinct from those of dilated cardiomyopathy, as shown by studies on tissue samples and imaging data from a cohort of 52 patients aged 3 months to 17 years who had undergone a heart transplant for dilated cardiomyopathy (Seki et al., 2013).

Of these, 14 presented what the authors considered to be typical aspects of primary endocardial fibroelastosis - diffuse thickening of the left ventricular endocardium, elevation of papillary muscles and thickening of the free edge of the mitral valve; specific aspect of the organization of elastic fibers at microscopic examination (Seki et al., 2013).

The clinical examination reveals tachypnea, fatigue, decreased appetite, diminished apex shock, deafening heart sounds, ventricular gallop, both mitral regurgitation and aortic stenosis murmur, which are variable in intensity and disappear from day to day. There are signs of heart failure. The electrocardiogram detects left ventricular hypertrophy, isoelectric or negative T waves in precordial leads, R wave > 20 mm in V6, S > 20 mm in V1, normal or discrete left QRS axis (Xu et al., 2015).

Treatment consists in administering digoxin 60-80 μg / kg loading dose, then 15-25μg / kg along with diuretics and oxygen as needed. A particular case is of PEF occurring in newborns with mothers suffering from autoimmune diseases. In these cases, the administration of intravenous immunoglobulin G together with glucocorticoids and angiotensine conversion enzyme inhibitors had favorable results (Yan et al., 2016).

The interest for this specific spectrum of diseases is reflected in the articles below:

Luca, **A.C.**; Lozneanu, L.; Miron, I.C.; Trandafir, L.M.; Cojocaru, E.; Paduret, I.A.; Mihaila, D.; Leon-Constantin, M.M.; Chiriac, S.; Iordache, A.C.; Tarca, E. Endocardial fibroelastosis and dilated cardiomyopathy – the past and future of the interface between histology and genetics, *Rom J Morphol Embryol* 2020, 61(4), pp.999-1005, **IF 1.033**

I.5.2. Material and methods

We presented the case of a 7-month-old patient admitted for an upper respiratory tract infection associated with signs of cardiac insufficiency. We detailed and discussed the multidisciplinary approach necessary for diagnosing and treating the patient.

I.5.3. Results

The initial anamnesis revealed a female patient born naturally, at 40 weeks of pregnancy, weighing 3150 g at birth, with no pregnancy-related , intrapartum or postpartum pathological aspects. During clinical examination we observed expiratory dyspnea, signs of respiratory distress and systemic hypoperfusion, tachycardia and hypotension.

The electrocardiogram showed sinus tachycardia, with a frequency of 200 bpm, QRS axis at 65 degrees, P wave axis at 60 degrees, duration of PR / QRS / QTc: 0.12 / 0.08 / 0.40 sec, aspect of left ventricular hypertrophy based on voltage criteria and left atrial hypertrophy.

Echocardiographically, the appearance of subendocardial fibroelastosis was detected, with a dilated left ventricle (Fig. I.7.) ejection fraction of 10% and a shortening fraction of 4%, tricuspid and mitral reflux grade II (Fig.I.8.), minimal pericardial reaction.

The patient was admitted to the intensive care unit, where she was treated with Digoxin 0,15 mg intravenously bolus then 0,075 mg iv, Furosemide 5 mg every 12 hours, along with Acetylcysteine 100 mg per day and Dexamethasone 1 ml every 12 hours. Initially, the patient maintained peripheral oxygen saturation over 97%, tachycardia and polypnea. Twelve hours after presentation, she developed fever (38.5 degrees C), with cold and “marbled” extremities, blood pressure of 77/35 mm Hg, respiratory rate of 50 per minute. She was intubated and mechanically ventilated.

The patient had a cardiorespiratory arrest with initial response to resuscitation, but whose recurrence did not respond to resuscitation maneuvers.

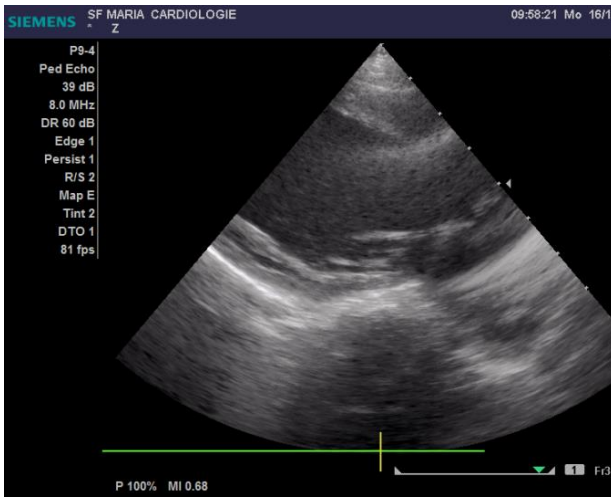


Figure I.7. TTE Parasternal long axis view. Dilated left ventricle

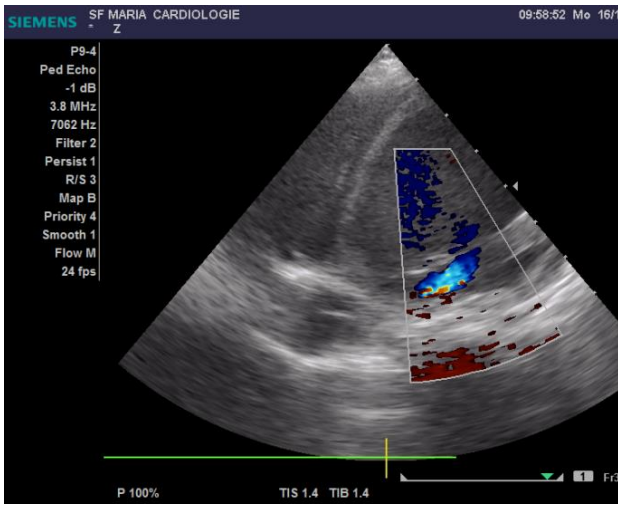


Figure I.8. Apical four chamber view. Mitral insufficiency and hyperechoic endocardium

At autopsy, morphological examination described cardiomegaly caused by fibroelastosis, with thickened endocardial deposits, proximal insertion of the papillary muscles, damage to the edge of the mitral valve (Fig.I.9.).

Microscopically, aspects of myocytolysis in the subendocardial area have been described along with important fibroelastic tissue in endocardium and subendocardium (Fig.I.10.).

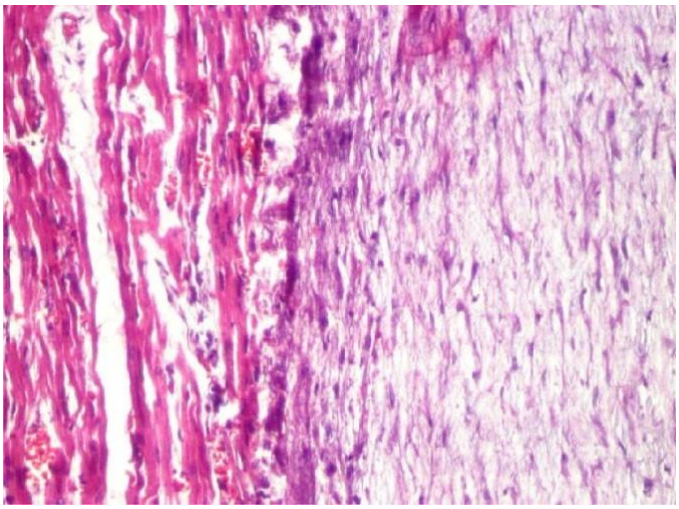


Figure I.9. – Abundant fibroelastic tissue in subendocardial layer and myocardium with vascular congestion (HE staining, ×400). HE: Hematoxylin–Eosin.

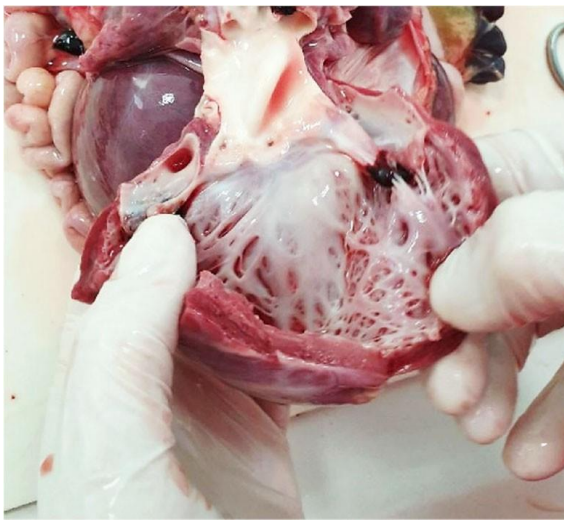


Figure I.10. – Macroscopic heart feature: ventricular and atrial fibrous thickening of endocardium.

The family history did not reveal the existence of other cases of fibroelastosis and did not raise the suspicion of a hereditary syndromic pathology. However, this line of investigation needs to be followed in any type of cardiomyopathy, including in those related to PEF.

I.5.4. Discussions.

PEF usually manifests in the first month of life. While the secondary form is related to other cardiac abnormalities or systemic diseases, the primary form is often confused with idiopathic DCM (Alperi et al., 2019).

As stated before, the "gold standard" for PEF diagnosing is the histological examination, followed by genetic studies that may help to establish the natural course of the disease and to communicate prophylactic measures to family members of the affected child.

Cardiac CT identifies aspects of apical calcifications in fibroelastosis, while MRI allows the detection of cardiac fibrosis, assessment of ventricular wall thickness, quantification of overall ventricular function and regional contractility, intracavitary thrombus formation, all known as important prognostic factors (Raman et al., 2005).

Echocardiography and electrocardiography are useful in differential diagnosis, but MRI and/or CT in conjunction with the typical features of microscopic examination provide vital information.

PEF may present in the form of premature ventricular contractions and non-sustained ventricular tachycardia that may easily change into life-threatening conditions that require fast identification and prompt intervention (Arya et al., 2019).

The literature describes cases of children having conduction abnormalities or frequent premature ventricular contractions. While initial ECGs and echocardiographic aspects seem to be normal, recurrent examinations and detailed investigations helped reveal the true nature of the underlying disease as EFE, allowing successful treatment courses.

Ina et al. conducted a retrospective study on 52 patients aged eight months to two years diagnosed with fibroelastosis, assessing the specificity and sensitivity of clinical and echocardiographic criteria and negative prognostic factors. The major clinical signs were dyspnea, gastrointestinal disorders, fever, weight loss and hypotonic neurological disorders.

Clinical examination revealed systolic murmur of mitral regurgitation and ventricular galloping sounds. ECG signs of left ventricular hypertrophy were present, with ST segment and T-wave inversion in V5 and V6, as well as left atrial or biatrial enlargement (Ino et al., 1988).

An unfavorable prognosis was found among patients with persistent ECG changes despite appropriate drug therapy, associated with an ejection fraction less than 33% and a cardiac index of less than 3.5 L/min/m^2. In this group, mortality at four years was 100%, suggesting that the identification of the previously mentioned negative prognostic factors would be an indicator for early heart transplantation.

In the group with a four-year follow-up, the persistence of electrocardiographic abnormalities was associated with an increased risk of death, suggesting the need for long-term patient monitoring (Ino et al., 1988).

Our case exhibited canonical aspects of PEF. Since the family history revealed no potential indicators of *in utero* harm, genetic syndromes, intellectual disability or any other signs of an autoimmune disorder, the only plausible explanation is that the patient had one of the many mutations associated with PEF (Table I.5.).

Table I.5. Genotype-phenotype correlation in patients with PEF

Affected gene/ gene location	***Protein name and role***	***Phenotype***	***Heart histopathology***
TAZ/ Xq28[20]	Tafazzin; transacylase involved in mitochondrial cardiolipin maturation [20].	DCM+EFE, proximal myopathy, growth retardation, organic aciduria, neutropenia [20], [21].	Myocardial fibrosis and cardiomyocyte apoptosis; sarcomere Z-lines normal morphology; A bands and M lines were not delineated poorly delineated; disorganized mitochondrial cristae [21].
CSRP3/11p15[22]	Muscle-specific LIM-protein; myogenesis and sarcomere assembly [22].	Fatigue, DCM+EFE, congestive heart failure; Defective neuromuscular transmission [22].	Disruption of cardiomyocyte cytoarchitecture; irregularly arranged myofibrils [22].
ACTN2/1q43 [23]	α actinin 2; cytoskeletal protein localized in the Z disc [23].	DCM; LVNC; Congenital myopathy [23].	Diffuse fibrosis; immunohistology-diminished signal for plakoglobin [23].
NEXN / 1p31.1 [14]	Nexilin F-Actin Binding Protein; filamentous actin-binding protein [14].	rapidly progressive dilated cardiomyopathy; diminished cardiac contractility [14].	Collagen-positive mural masses ; absence of elastic fibers and presence of fibrin; dissrupted connections between the SR and sarcolemma; absent sarcolemmal invaginations [14].
NEBL/ 10p12.31 [25]	Nebulette; involved in cardiac myofibril assembly[25].	DCM+EFE; HCM; [25]	Endocardial thickening, deposition of elastic tissue and collagen, progressive Z-line abnormalities; enlarged and deformed mitochondria; abnormal lysosomes and mitochondrial remnants; accumulation of lipids in cardiomyocytes [25].
LDB3 / 10q23.2 [26]	LIM Domain Binding 3; cytoskeletal protein binding and muscle alpha-actinin binding[26].	LVNC;DCM-Association with mitral valve prolapse; Myofibrillar myopathy [26].	Z-disk streaming & disintegration; Disorganized sarcomeres & myofibrils; Immunocytochemistry: Aggregates may contain Myotilin, Desmin, αB-Crystallin, Dystrophin [26].
PRDM16/ 1p36.3 [27]	PR domain containing 16; Binds DNA and functions as a transcriptional regulator; Functions as a repressor of TGF-beta signaling [27].	LVNC; EFE; DCM; myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML) [27].	Myocardial thickening with a compacted and noncompacted area, Myocyte disarray with staghorn-like spaces, in the noncompacted layer [27].

I.5.5. Conclusions

Fibroelastosis is a rare condition with a relatively poor prognosis. The suspicion of fibroelastosis can rise from the prenatal period and can be managed using echocardiographic criteria associated with the fetal cardiovascular score. Early detection allows the initiation of neonatal treatment as soon as possible, thus preventing further complications. The proper management should be established considering negative prognostic factors, involving early transplantation, drug therapy and long-term follow-up.

CHAPTER II. PEDIATRIC CARDIAC AND VASCULAR TUMORS

Introduction

Primary cardiac tumors represent a rarely encountered pathology in the pediatric group and most of them are benign. Nevertheless, they associate a significant degree of hemodynamic instability and/or arrhythmias. Malignant tumors of the heart are rarely seen, most of them being metastases (90%) and only 10% considered primary malignant cardiac tumors. The estimated incidence of primary cardiac tumors is 1:100000 cases and autopsy series established the prevalence at 1:2000, as opposed to secondary tumors, with an estimated prevalence of 1:100 (Rosario et al., 2019).

In adolescents, myxomas are the most frequent benign cardiac tumors, whereas in neonates, infants and children, rhabdomyomas account for 45% of the cardiac tumors and myxomas are found in only 10% of cases (Basso, 2013). Cardiac hemangiomas are rarely diagnosed in children (4% of cases), they are usually clinically silent, can be identified in any heart segment and can sometimes be associated with extracardiac lesions.

Myxomas account for 6% of tumors diagnosed in pediatric cases. Histological studies characterized them as cellular cords on paucicellular myxoid background, with small vessels, surrounded by lymphocytes and hystiocites (Allen et al., 2021). Malignant myxomas are rare and usually invade the adjacent tissue, exhibiting intense mitotic activity and a significant degree of local recurrence. Patients develop symptoms similar to mitral or tricuspid valve stenosis due to the frequent obliteration of the valves by a mobile myxoma, arising either in the right or left atrium (Palaskas et al., 2018). Myxomas can occur in a familial context, as a result of a mutation in *PRKAR1A* gene, alongside ephelides, mucocutaneous myxomas, lentigines, or naevi, a constellation known as Carney complex, an autosomal dominant syndrome (Pitsava et al., 2021).

Fibromas are congenital cardiac masses, associated with Gorlin or Gardner syndrome. They usually have large dimensions and present as intramural, unique masses placed in the interventricular septum, the left ventricular free wall, or apically. Histologically, fibromas are rich in fibroblasts, collagen fibers and present few of elastic fibers (Palaskas et al., 2018). They are usually associated with ventricular arrhythmias and cardiac arrest (Carreon et al., 2019).

Malignant primary tumors of the heart are exceedingly rare in children. They are represented by sarcoma (40%) non-Hodgkin lymphoma and teratoma (12%) (Allen et al., 2021).

TTE is valuable for determining the location, morphology, mobility, and density of a cardiac mass. Through continuous and color Doppler the hemodynamic impact of a tumor can be evaluated, while speckle tracking helps in establishing the contractility of masses (Wu et al., 2019). TEE is especially useful in evaluating atrial tumors and renders superior results to cardiac magnetic resonance imaging in valvular masses (Li et al., 2020).

Cardiac computed tomography and magnetic resonance have proven useful in a preoperative scenario. CT detects calcifications and cardiac valve masses with a superior accuracy compared to CMR, which makes it the go-to imagistic tool for reconstruction planning. When a differential diagnostic is necessary, CMR is indicated, especially in pediatric cases (Aggeli et al.,2020). Positron emission tomography is used when differentiating between the malign and benign nature of a tumor is difficult. Increased metabolic activity is a pathognomonic sign of a neoplasm, although false positive results may be registered in inflammatory and infectious conditions (Meng et al., 2020). The main imagistic criteria for the differential diagnosis of primary cardiac tumors are listed in Table II.1.

Tabel II.1. Characteristics of cardiac tumors (modified from : Li X et al, 2020)

Tumor type	Clinical Manifestations	ECG	TTE/TEE	CT	CMR	Biomarkers	Differential diagnosis[36]	Therapy
Myxoma	Flow Obstruction; Emboli; Systemic symptoms;	Left atrial hypertrophy; Ventricular tachychardia;	Narrow stalk; Hyperechoic mass in characteristic location; Calcifications; Dynamic tumor;	Low-attenuating heterogeneous mass compared with myocardium; Pulmonary infarcts; Intratumoral calcification;	T1 hypointense, T2 hyperintense Heterogeneously enhancing isointense or hyperintense on delayed imaging;	CD31+ CD34+ Calretinin+ CD68- Cytokeratins-	Left atrial thrombus; Metastatic carcinoma; Myxoid sarcoma; Papillary fibroelastoma; Fibroma;	Surgical excision
Fibroma	Heart murmurs; Congestive heart failure; Arrhythmias; Sudden death;	T-wave abnormalities; Ventricular tachychardia; Atrioventricular block;	Large, solid, heterogeneous mass that is noncontractile	Central calcification within a discrete mass ; Non-specific low attenuation mass;	Encapsulated mass; Delayed enhancement; Hypointense to isointense in T1; Hypointense in T2;	Vimentin+ Ki-67- CD34- S100- HMB45-	Cardiac rhabdomyoma; Myxoma; Teratoma; Lipoma; Hemangioma; Hypertrophic cardiomyopathy; Metastatic disease	Amiodarone and/or beta-blockers; Surgical excision; Single ventricle palliation; Cardiac transplant;
Rhabdomyoma	Flow obstruction; Heart failure; Arrhythmias; Decreased peripheral pulses and/or cyanosis	Extrasystoles; Ventricular Tachycardia; Supraventricular tachycardia; Wolff–Parkinson–White syndrome	Solid, hyperechoic, avascular mass; Focal abnormality of cardiac wall motion	Hypodense compared with adjacent myocardium	T1 isointense/slightly hyperintense ; T2 hyperintense; No fat suppression	Myoglobin+ Actin+ Desmin+ Vimentin+ S100-	Glycogen storage disease; Granular cell tumor; Lipoma;	mTOR inhibitors; Surgical excision if located in the left ventricle;
Rhabdomyosarcoma	Systemic illness; Syncope; Arrhythmias; Sudden death; Pericardial disease or tamponade; Embolic phenomena	Ventricular arrhytmias	Solid, hyperechoic mass with irregular borders	Hypoattenuating mass involving any cardiac chamber; Smooth or irregular borders	Heterogeneous mass with high signal intensity in T2	Myogenin+ MSA+ MYOD1+ Desmin+	Angiosarcoma; Fibrosarcoma; Osteosarcoma; Leiomyosarcoma; Liposarcoma; Lymphoma; Intrapericardial pheochromocytoma; Metastatic disease	Surgical resection; Heart transplantation; USA chemotherapy: Vincristine + Actinomycin-D + Cyclophosphamide; EU chemotherapy: Ifosfamide + Vincristine + Actinomycin-D

II.1. Infantile hemangioma

II.1.1. Background

Infantile hemangioma is the most common vascular tumor of the child, with an incidence of 5–10% at the end of the first year of life (Schupp et al., 2011).

Premature birth, low birth weight, female gender, Caucasian race and also multiple gestations, increased maternal age, in vitro fertilization, pre-eclampsia and placental anomalies are all risk factors (Tiemann & Hein, 2020).

Depending on their depth, IHs can be superficial, mixed or deep. If only the superficial dermis is affected, the color is typically bright red, whereas deeper IHs have a blue hint. Morphologically, IHs can be localized or segmental or exhibit an indeterminate phenotype (Pandey et al., 2018).

Medical or surgical treatment for IH is reserved in cases where complications such as obstruction, bleeding, ulceration, or superinfection occur .

The clinical aspects of hemangiomas, as well as therapeutic interventions and management of risk factors have been discussed in the article :

Luca, A.C.; Miron, I.C.; Trandafir, L.M.; Cojocaru, E.; Paduret, I.A.; Trandafirescu, M.F., Iordache, A.C.; Tarca, E. Morphological, genetic and clinical correlations in infantile hemangiomas and their mimics, *Rom J Morphol Embryol* 2020, 61(3), pp.687–695, **IF 1.033**

II.1.2. Material and methods

In the wake of the novel coronavirus disease 2019 (COVID-19) pandemic, the Hemangioma Investigator Group (HIG) released a classification of IH based on size and location useful in evaluating the probability for complications, establishing the optimum moment for initiating conservative treatment and directing the patient monitoring techniques.

We elaborated on these recommendations taking into consideration our own experience in diagnosing and treating IH.

II.1.3. Results

Histopathological features of IHs

IHs have a two- stage rhythm of development, with a proliferative phase in the first year of life, more intense during the first three months, followed by the involution phase. During the proliferative phase, blood vessels are disorganized and composed of immature non-permeable ECs, possibly due to alpha-smooth muscle actin (α-SMA). In the involution phase of the tumor, the blood vessels mature and grow in size, but are reduced in number. The vascular tissue is replaced with fat, connective tissue, and fibroblasts (Drolet et al., 2013).

Investigations on glycolysis metabolism in hemangioma-derived endothelial cells (HemECs) unveiled that glucose consumption and ATP production were higher in HemECs, while lactate production was lower. Also, hypoxia did not significantly alter glycolytic metabolism in these cells; instead, genes coding for proteins involved in glycolysis experienced an increased expression.

From a histological point of view (Fig. II.1., Fig. II.2.), hemangiomas in their proliferative phase present lobules with a high density of capillaries-like vessels, displaying plumped ECs, and a thin basal layer surrounded by a layer of pericytes (Tarca et al., 2019).

Immunohistochemical markers used to identify hemangioma cells are GLUT1, CD31, CD34, α-SMA, Ki67 (Greenberger & Bischoff, 2013).

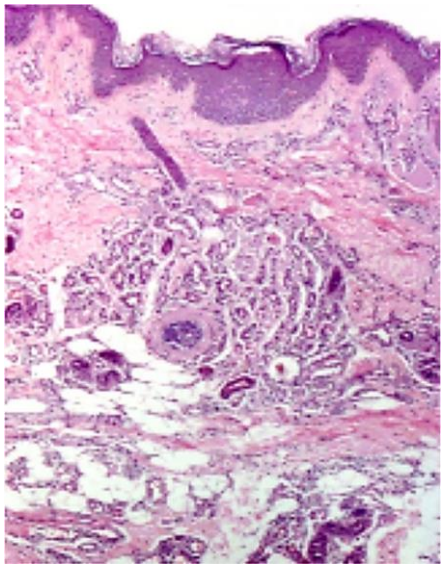


Figure II.1.– Hemangioma: general view [Hematoxylin– Eosin (HE) staining, ×40

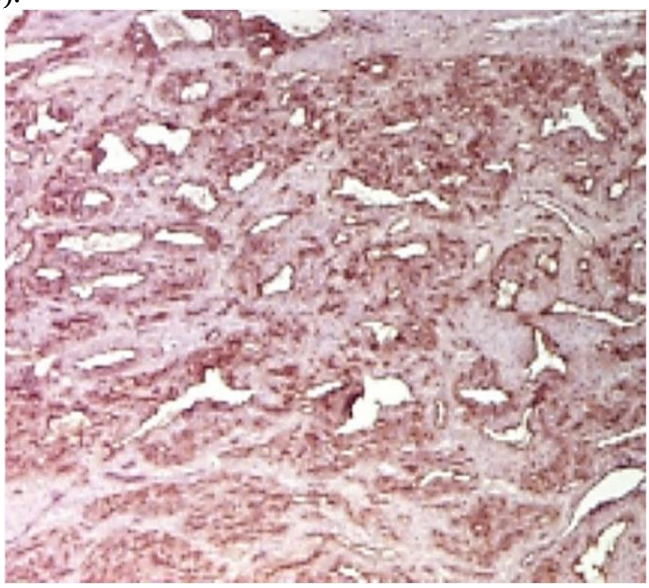


Figure II.2. – Endothelial cells positive for anti-CD34 antibody (Anti-CD34 antibody immunostaining, ×40).

Differential diagnosis

Ultrasonography, histological studies and clinical aspects are used as tools for differentiating hemangiomas from other soft tissue tumors. Imaging techniques are an important source of diagnostic information and required to properly classify and treat a vascular tumor. Although magnetic resonance and computer tomography- based investigations gained a lot of attention, ultrasonography (US) and Doppler analysis are still useful, cost-efficient and readily available tools for any diagnostician in the quest to categorize and surveille most of the vascular malformations, including infantile hemangiomas and their mimics (Krowchuk et al, 2019).
During the proliferative phase, the US findings in IH are a heterogenic mass, with high density vascularization (>5 vessels/cm^2) a high Doppler shift, and low flow resistance (Luo & Zhao, 2011). In the involution phase, the vessel density decreases. However, there is no pathognomonic lesion for IH, and careful analysis is required.

US characteristics of the most frequent differential diagnosis for IH are summarized in TableII.2. and Table II.3**.** for the malign and benign tumors, respectively.

Table II.2. - US characteristics of malignant tumors

MALIGNANT TUMORS	**US CHARACTERISTICS**
METASTATIC NEUROBLASTOMA	-blood flow consistent with localized deep hemangio - peak velocity between 10-20 m/sec
EXTRASKELETAL EWING SARCOM	- hypoechoic - increased Doppler flow - marked internal vascularity

Table II.3. - US characteristics of the frequent benign tumors

Benign lesions	US characteristics
Pyogenic Granuloma	- ill-defined, oval, - hypoechoic , marked central and peripheric vascularization with feeders vessels **or** - decreased peripheric vascularity -venous and arterial flow of low resistive index.
Syringocystadenoma Papilliferum	- US findings are similar to those of a typical hemangioma hemangioma.
Wart Versus Eccrine Poroma	**Wart** -fusiform -hypoechoic focal increase in the arterial dermal flow. **Eccrine poromas** - well-defined -hypoechoic -avid post-contrast enhancement on MRI
Aneurysmal Benign Fibrous Histiocytoma	-well-defined, rounded, -mainly hypoechoic, posterior acoustic enhancement, -significant arterial and venous flow
Paronychia	- diffuse, periungual thickening - areas of increased echogenicity interposed with hypoechoic areas
Cranial Fasciitis	-small, superficial, -hypoechoic, marked internal vascularity; venous and arterial low resistance arterial flow
Hemangioma	-well defined, ovoid -hypoechoic, high vessel density -high Doppler Shift -low flow resistance

Therapeutic strategies and risk classification

IH can be harmless, which is why conservative measures are recommended, but in rare cases when complications such as obstruction, bleeding, ulceration or superinfection occur, therapeutic intervention is required (Callahan & Yoon, 2012). Roughly for every 10 cm^2 increase in lesion size, there is a 4% increase in the need for treatment, and a 5% increased chances for complication (Cheng & Friendlander, 2016).

The new risk stratification score proposed by The Hemangioma Investigator Group allows the initiation of medical therapy according to the probability for complications (Table II.4.).

Corticosteroid therapy

Corticosteroid (CS) therapy has been widely used in the treatment of IH. Systemic therapy using Prednisolon 2–3 mg/kg/day every morning had a 33% success rate, with only one third of lesions regressing, one-third remaining stable, and the last third showing no response (Sethuraman et al., 2014).

Growth delay, skin hypopigmentation, hyper- trichosis, Cushingoid appearance, behavior alteration, irritability, glaucoma, cataract are all complications associated with systemic administration of CSs (Schultz et al., 2019).

Angiotensin-converting enzyme inhibitors

Angiotensin-converting enzyme inhibitors (ACE) have been studied as potential therapeutic agents, with a small study comparing results from Propranolol with Captopril (Zaher et al., 2016). Studies have demonstrated a likely relationship between IH development and the renin– angiotensin system (RAS) function, which prompted the investigation of ACE inhibitors, such as Captopril, as possible treatment alternatives. Overall, the β-blocker therapy showed better results, but further investigation is needed.

β-Blocker therapy has been used since 2008, when two infants treated with Propranolol for heart disease also had spectacular remission of IH (Barwe et al., 2009). Possible administration options include systemic therapy with Propranolol or Nadolol and topical applications with Timolol (Guo et al., 2017).

Table II.4. Risk stratification depending on IH morphological characteristics, number of lesions, location according to The Hemangioma Investigator Group

Highest	High	Medium	Low
• >5 cm or facial /scalp • Large or segmental lumbosacral or perineal • Multifocal IHs (≥5) and abdominal hemangiomas (US) • Periocular IH with potential impact on visual axis	• Large IH on trunk/ extremities • facial IH ≥ 2 cm (> 1 cm if ≤ 3 mo of age): • Nasal tip or lip IH even if < 1 cm • Oral • Neck or scalp IH > 2 cm during growth phase • Breast • Ulcerated hemangioma	• Localizes perineal IH without ulceration • Trunk of extremity IH > 2 cm especially in growth phase or if abrupt transition from normal to affected skin	• IH < 2 cm on trunk of extremities in areas easily covered by clothing. • IH on trunk of extremities > 2 cm if gradual transition from normal to affected skin.

Nanotechnology applied in the field of drug therapy helped design nanostructures that enhance drug administration efficiency. Mesoporous silica nanoparticles (MSNs) have an important role in cancer therapy and, propranolol loading into MSNs, seem to have a cumulative effect of apoptosis of hemangioma stem cells and cell proliferation suppression (Wu et al., 2020).

Nadolol has been suggested as an alternative to Propranolol because of a lower rate of complications, since Nadolol does not cross the brain barrier and does not exhibit membrane stabilizing effects. Nadolol has been cited in a case report of a 10-year-old girl who, after being started on a therapy course for IH, died within seven weeks of treatment, with a postmortem concentration of Nadolol of 0.94 mg/L (McGillis et al., 2020).

The infant had no bowel movements 10 days before her death. The authors of the case report suggest monitoring bowel movements in pediatric patients receiving systemic therapy with Nadolol, since this particular molecule is eliminated in the feces and, in the case of discontinued bowel movements may present increased absorption. Timolol is indicated alone or in combination with laser therapy, in the case of superficial, smaller IH or in residual telangiectasias after treatment with Propranolol (Chambers et al., 2012).

Laser therapy is an option for superficial, early detected hemangiomas or as an adjuvant therapy for refractory or ulcerated lesions. Pulse dye laser (PDL) photocoagulates targeted vessels while leaving the overlying skin intact (Asilian et al., 2015).

Follow-up

To summarize the *AAP Guidelines*, imaging of lesions is not necessary unless the diagnosis is uncertain, there are more than five cutaneous or there is a high suspicion of anatomic anomalies. Also, in case of uncertain diagnosis, US should be the first imaging diagnostic tool used. MRI should be performed when structural abnormalities are suspected.

The *European Guidelines* recommend echocardiography whenever large hemangiomas are identified, as this is a high risk for high-output cardiac failure. For this group of patients and for those with intrahepatic hemangiomas, thyroid hormones monitoring is important as hypothyro- idism may occur. MRI angiography is useful in evaluating patients with head and/or neck hemangiomas, in order to detect further arterial anomalies.

II.1.4. Discussions

In our unit, we manage IH cases in agreement with the available guidelines. Conservative therapy consisted of long-term daily administration of β-blocking medication and / or intralesional injection of Bleomycin (1 session / 4 weeks, for 6-8 consecutive months), depending on the size and possible complications of IH, as well as responsiveness to treatment.

Propranolol is administered at a dose of 1 mg / kg body weight / day, for 7-10 days, then increased to 2-3 mg / kg body weight / day, orally for a minimum of 6 months. Timolol is given topically, either as a gel, solution or ointment in superficial, small IH.

In the case of large IH or complicated IH we opt for interventional treatment or surgical excision, along with intralesional injections with Bleomycin, in combination or not with β - blocker medication.

Although the literature shows that Propranlol can also be used successfully in intralesional injections, in periorbital IH we only administered it orally. Before initiating the β-blocker treatment and subsequently periodically, the cardiological examination evaluates the patient's tolerability to medication by means of blood pressure, heart rate monitoring, EKG and echocardiography.

Parents are instructed to avoid prolonged fasting between meals in order to prevent hypoglycemia and also not to administer a double dose of Propranolol.

During the entire treatment course, all patients have been monitored weekly by the primary care physician (BP, HR, blood glucose) and periodically by the attending physician (dose adjustment based on weight and clinical and biological reevaluation).Among the biological constants observed in patients with IH, hypoglycemia, known as an adverse effect of active therapy on β2-adrenergic receptors, led to the cessation of propranolol treatment in one of the patients.

The association of IH with anemia, rickets, protein-caloric malnutrition or hypotrophy is difficult to interpret as having clinical significance. The significant incidence of hepatocytolysis syndrome may indicate low hepatic tolerance in pediatric patients receiving oral propranolol daily, which is why the development and widespread accessibility of β-blockers releasing liposomes is an interesting and promising therapeutic approach.

II.1.5.Conclusions

The widespread occurrence and distribution of infantile hemangiomas can mimic other benign or malignant disorders. The main purpose of the clinician is to obtain a complete history and appropriate imaging in order to make an accurate diagnosis.

The treatment of IH is established considering the location and extent of the tumor, number of lesions and their stage and distribution, presence of systemic involvement and/or local complications. Evaluation of complex systemic disorders such as PHACE and LUMBAR syndromes is warranted to avoid possible iatrogenic complications. For this purpose, imagistic studies are useful, especially MRI, but US can be used as a first evaluation step for multiples cutaneous tumors, considering the fact that multiple localizations are usually associated with visceral involvement. Thyroid function can also be impaired , often in cases with large cutaneous hemangiomas, which is why thyroid hormone levels should be tested before treatment initiation. Before initiating the β-blocker treatment and subsequently periodically, the cardiological examination should evaluate the patient's tolerability to medication by means of blood pressure measurements, heart rate monitoring, EKG and echocardiography and should exclude pathologies like bronchial asthma.

The management of IH cases should be performed in agreement with the available guidelines. The choice between systemic or topical administration, surgery and laser therapy should be made after careful prognostic evaluation and should take into account patient compliance. Newer strategies of drug delivery may prove to be an attractive alternative to invasive measures, as more studies evaluate safety profile in direct correlation with patient's characteristics.

II.2. Extrarenal rhabdoid tumor

II.2.1. Background

Rhabdoid tumors are rare and aggressive neoplasms developing mainly in the kidney, but also in the cerebral and extra-cerebral tissues (Bartelheim et al., 2014). Anatomical regions such as the heart and mediastinum remain an exception, but immunohistochemistry evaluation that help detect INI-1 loss in affected cells as well as sequence analysis and gene targeted analysis for *SMARCA4* or *SMARCB1* mutations helped to reveal the rhabdoid type in tumors diagnosed in these tissues (Mohamed et al., 2020).

Due to the very few cases available for studies, rhabdoid tumors in children have yet to offer the possibility for a therapeutic consensus. According to the EU-RHAB registry, total resection, standard chemotherapy, intrathecal methotrexate (MTX), and high-dose chemotherapy with Carboplatin and Thiotepa and radiotherapy over 18 months of age can be used even in youger children, associating significant survival rate improvement, albeit burdened by multisystem adverse effects (Seeringer et al., 2014). Multiple phase I and II studies are evaluating a series of inhibitors targeting histone deacetylase, histone methyltransferase DNA methyltransferase, Aurora kinase A, Hedgehog pathway in order to establish new therapeutic regimens that may help decrease the chemotherapy dosage and improve prognostic (Beck et al., 2021).

The genetic, clinical and therapeutic aspects of cardiac rhabdoid tumor have been discussed in the article:

Cardiac rhabdoid tumor – a rare foe – case report and literature review **– *Children in publishing***

II.2.2. Material and methods

We present the case of a patient diagnosed with malignant extrarenal rhabdoid tumor, highlighting the particularities of this rare neoplasia and the importance of molecular diagnostic. Initial symptoms, differential diagnosis and criteria for the positive diagnostic have been detailed.

II.2.3. Results

A 2 years old male patient was admitted for productive cough, drowsiness that alternated with psycho-motor agitation, fever, and bilateral sero-mucosal otic secretions. The patient was born at term, naturally, with an APGAR score of 8; he had a normal neuromotor development in the first year of life. Afterwards, the patient's records show multiple hospital admissions for bronchopneumonia, diagnosed with chest radiographies and treated with antibiotics and also primary pulmonary tuberculosis and tuberculous pericarditis treated with quadruple drug association in a 7/7 regimen.

Our clinical examination revealed weight deficit (weight = 9 SD, height = 26 SD), pale skin, serous ear secretion, vesicular murmur present on both lung areas, peripheral oxygen saturation of 92% and heart rate of 118 beats per minute. The chest radiography showed left lung opacity with blurred edges, with unaffected left lung tip and left costodiaphragmatic sinus, and another opacity that occupied the anterior mediastinum.

Abdominal ultrasound revealed liquid in the peritoneal cavity with a thickness of 1.2 cm. The soft tissue ultrasound described right cervical adenopathy of 37/19 mm, positioned 5 mm subcutaneously, posterior to the sternocleidomastoid (SCM) muscle, in the lower cervical floor , with apparently present Doppler signal. Despite multiple antibiotic courses, the patient's condition worsened, so we decided to perform a contrast thoracic CT. The results were more than concerning: a tumor mass of 8.57 / 10.37 / 9.42 cm (anteroposterior / transversal / craniocaudal dimensions) with native soft tissue densities (18-30 HU), moderately iodophilic, nonhomogeneous, straight polycyclic contour, located anteriorly in the upper and middle mediastinum, fully occupying the retrosternal space with an important left paramedian extension.

The tumor exerted a mass effect on the left main bronchus, reducing its caliber up to the lobular bifurcation, sleeved two thirds of the right intermediate bronchus posteriorly, and encompassed the trunk of the pulmonary artery, the right and left branch of the pulmonary artery, the ascending aorta, the aortic cross and the branches emerging from it, with preservation of the vascular lumen and moved the trachea posterolaterally to the right. There was also adenopathy located cervically inferior to the right, with dimensions of 1.5 /1.9 / 1.86 cm (AP / T/ CC), located posterior to the right SCM muscle, imprinting it and moving it anteriorly. Pericardial effusion with variable thickness between 1.46 cm and 2.11 cm and axillary localized infracentimetric lymph nodes could also be seen.

Based on the initial CT, a suspicion of lymphoma arose. Additional lymphoma investigation refuted this diagnostic.

Flow cytometry revealed no atypical lymphocytes in the pleural fluid sample. The malignant hematological disease monitoring test identified large, mature monocytes with the following phenotype: CD45 HLA / DR + ; CD34-; CD117-; CD64 +; CD36 +; CD14 +; IREM-2+. Pleural fluid cytological examination described numerous mesothelial cells and monocyte-macrophages; lymphocytes and polymorphonuclear cells in approximately equal proportions. The medullary puncture revealed no bone marrow infiltrations, and nodal biopsy uncovered sinus histiocytosis.

Biologically, the patient presented leukocytosis with neutrophilia, moderate anemia, low fibrinogen levels, significantly increased C-reactive protein, high D-dimers levels, severe hypoproteinemia, hyponatremia, and metabolic acidosis.

After two weeks of medical treatment the patient's health continued to depreciate, he developed upper body edema, oxygen desaturation, and psycho-motor agitation and was transferred to the intensive care unit. The CT was repeated and showed a tumor evolution pattern. The mediastinal formation described above had dimensions of 8.22 / 11.30 / 11.20 cm, and

changed its CT appearance, exhibiting a non-homogeneous structure, with necrosis area inside, contrast setting at the level of solid components.

The tumor encompassed the left common carotid arteries, left subclavian artery, right brachiocephalic arterial trunk, pulmonary artery trunk, right and left branches of the pulmonary artery and the SVC, which presented a parietal thrombus (0.81 / 0.73 cm) in the middle to the inferior third of the SVC, which extended without obliterating the lumen of the vessel, in addition to complete obstruction of the left brachiocephalic venous trunk. The left ventricular wall showed nonhomogeneous contrast. There were also round infracentimetric lymph nodes, with post-contrast constants , located at the left supraclavicular level and bilaterally cervical. There was also adenopathies located at the superior mediastinum with dimensions of 2.03 / 1.43 cm and a nonhomogeneous structure.

The patient's condition deteriorated, he developed acute liver failure and superior vena cava syndrome. He was intubated and mechanically ventilated. 27 days after hospitalization an irreversible cardio-respiratory arrest installed. The patient died and the necropsy was performed in the Pathological Anatomy and Prosecution Service.

The macroscopic evaluation revealed stasis, meningeal and cerebral edema with discrete hydrocephalus, a malignant cardiac tumor (Fig.II.3.) that infiltrated the pericardium with lung metastases, pulmonary collapse, liver stasis with massive steatosis, congestive-erosive gastro-entero-colitis, renal stasis, extensive thrombosis of the superior vena cava, left brachiocephalic vein and left internal jugular vein thrombus , as well as generalized edema.

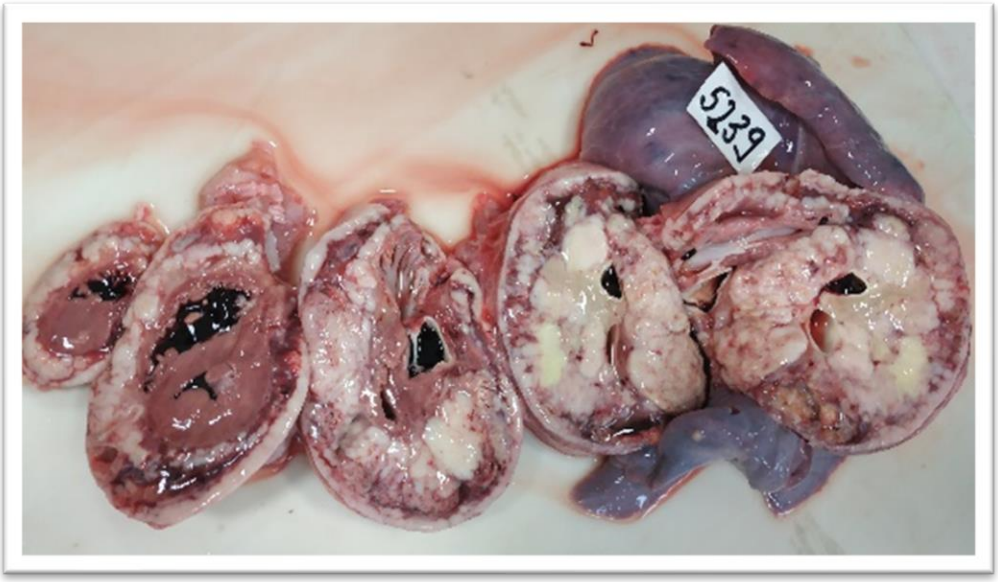


Figure II.3. Macroscopic aspects of malignant cardiac tumor

The microscopic examination could not establish the exact type of the tumor and so immunhistochemistry tests were required**.** Immunoperoxidase studies were performed on paraffin-embeded tissue samples with antibodies to INI-1 and it was absent in the cells. Therefore, a diagnosis of rhabdoid extrarenal malignant tumor was made.

II.2.4. Discussions

Rhabdoid tumors are rare and aggressive neoplasms developing mainly in the kidney, but also in the cerebral and extra-cerebral tissues (Bartelheim et al., 2016). Anatomical regions such as the heart and mediastinum remain an exception, but immunohistochemistry evaluation that help detect INI-1 loss in affected cells, as well as sequence analysis and gene targeted analysis for *SMARCA4* or *SMARCB1* mutations, helped to reveal the rhabdoid type in tumors diagnosed in these tissues (Mohamed et al., 2020).

Rhabdoid tumors describe a more complex clinical entity now known as rhabdoid tumor predisposition syndrome (RTPS). Such diagnostic should be suspected if a patient presents with any of the following:

1. Atypical teratoid/ rhabdoid tumor (rhabdoid tumor affecting the central nervous system)
2. Rhabdoid tumor of the kidney;
3. Rhabdoid tumors of the heart, liver, mediastinum, retroperitoneum, bladder, and pelvis;
4. Small cell carcinoma, the hypercalcemic type, of the ovary (Fruhwald et al., 2021).

Histologically, rhabdoid renal and extrarenal tumors share common characteristics. The cells are polygonal, with eccentric, vesicular nuclei, and prominent nucleoli, eosinophilic cytoplasmic inclusions (Ng et al., 2019). The tumor has an infiltrative behavior, with a non-homogenous cellular repartition, evidence of necrosis and high proliferative index (Dongyou, 2020). The immunohistochemistry tools of investigation facilitate differential diagnosis by underlining INI-1 loss of expression, usually accompanied by germline mutations in the *SMARCB1* gene. When INI-1 is still expressed in the affected tissues, a rhabdoid tumor with *SMARCA4* mutations should be considered (Hasselblatt et al., 2011).

The incidence of Atypical teratoid/ rhabdoid tumor (AT/RT) in children younger than 1 year is estimated at 5.4: 10 (Aggeli et al., 2020). Carriers of the *SMARCB1* mutation have the RTPS 1 type and those carrying the *SMARCA4* mutation are diagnosed with type 2 RTPS. The inheritance pattern is autosomal dominant, although the penetrance is yet to be established. Additional loss of function or missense mutations as a "second hit phenomenon" have been implicated in the occurrence of different types of syndromes associated with *SMARCB1* and *SMARCA4* germline mutations (Foulkes et al., 2017).

Given that the age of symptoms onset is approximately 2 years and the 5-year survival rate is 10%, surveillance of the proband and familial studies are paramount. The surveillance strategies rely on physical examination every 2-3 months and imagistic studies with a frequency dictated by age. Whole-body MRI, abdominal ultrasound, and soft tissue US should be performed every 2 months between 0 months- 5 years, and every year afterwards (Fruhwald et al., 2021).

Due to the very few cases available for studies, rhabdoid tumors in children have yet to offer the possibility for a therapeutic consensus. According to the EU-RHAB registry, total resection, standard chemotherapy, intrathecal methotrexate (MTX), and high-dose chemotherapy with Carboplatin and Thiotepa and radiotherapy over 18 months of age can be used even in younger children, associating significant survival rate improvement, albeit burdened by multisystem adverse effects (Seeringer et al., 2014).

Multiple phase I and II studies are evaluating a series of inhibitors targeting histone deacetylase, histone methyltransferase, DNA methyltransferase, Aurora kinase A, and Hedgehog pathway to establish new therapeutic regimens that may help decrease the chemotherapy dosage and improve prognostic (Beck et al., 2021).

Our patient had a previous history of lung disease, a pathology that was unresponsive to multiple antibiotic regimes. When admitted to our unit, heart involvement was severe and the signs and symptoms were consistent with the patterns associated with rhabdoid tumors of the

mediastinum, represented by dyspnea, cough, and respiratory distress. Also, our patient was male, a gender that seems to be slightly more affected by this type of neoplasm (Tomlinson et al., 2005).

Initially, after the histological examination, a suspicion of epitheloid sarcoma raised. It is a difficult differential diagnosis because the epithelioid sarcoma cells have a rhabdoid likeness, INI-1 expression is also lost, while epithelial and mesenchymal markers are positive (Hollmann et al., 2011). Both epithelioid sarcoma and malignant rhabdoid tumors show immunopositivity for keratin and EMA, with occasional staining for desmin and CEA. CD34 is as a differential tool, since epithelioid sarcoma is CD34 positive in 50% of cases, while rhabdoid tumors are always negative (Hollmann et al., 2011).

The identification of such a young patient with a malignant rhabdoid tumor meets the criteria necessary to suspect a predisposition syndrome. The next step is the family investigation, which should be aimed at identifying all cases with a diagnosis of rhabdoid tumor and/or multiple *SMARCB1* or *SMARCA4* deficient tumors. These tumors may be metachronous or synchronous. Both *SMARCB1* and *SMARCA4* gene mutations have been incriminated for Coffin-Siris syndrome and *SMARCB1* for schwannomatosis, an important aspect to remember when conducting family clinical investigation (Nemes et al., 2017). The next step in the case of positive family history, is the identification of a germline mutation in either *SMARCB1* or *SMARCA4* gene through molecular testing. It seems rational to test for *SMARCB 1* mutations first, since the vast majority of rhabdoid tumor predisposition syndrome cases are caused by variants of this gene and those cases have a more reserved prognosis, hampered by the recurrence of tumors, their synchronous presence in different sites and the high probability of developing masses in the central nervous system, if it was not initially affected.

The treatment of a rhabdoid tumor is multimodal. Good results were obtained with induction therapy employing Cyclophosphamide, Cisplatinum, Etoposide, Methotrexate, and Vincristine, followed by consolidation therapy with Carboplatinum and Thiotepa (Reddy et al., 2016). A consensus does not yet exist. Radical surgery has been suggested as the best option, however, the tumor size and location seldom allow for such interventions. The European Rhabdoid Registry recommends high dose chemotherapy, Methotrexate, and radiotherapy (Bartelheim et al., 2016).

Unfortunately for our patient, his definitive diagnostic came too late, significantly lowering the already slim chances of survival.

In any case, provided prompt and early diagnostic is made and the patient is responsive to the multimodal treatment scheme, surveillance will be necessary. The guidelines evoke the importance of genotyping to establish steps in long-term surveillance. *SMARCB1* mutations associate a higher risk for abdominal neoplasm, which is why an MRI every 5 years and an ultrasound every 3 months are appropriate. *SMARCA 4* mutations are more likely to cause small cell carcinoma of the ovary, thus prompting abdominal ultrasound every 6 months as a wise course of action.

II.2.5. Conclusions

Rhabdoid malignant tumors are exceptionally aggressive neoplasms, though rare. The old paradigm of kidney and CNS involvement is now obsolete, as genetic studies shed light on the ancestry of tumors found in various other locations. Rhabdoid predisposition syndrome is caused by a germline mutation in either *SMARCB4* or *SMARCA1* genes and should always be considered when dealing with a patient diagnosed with a RMT.

The only way to increase even slightly the chances of survival is by fast recognition and prompt positive diagnostic, facilitated by immunohistochemical and molecular studies.

Management guidelines are still subjective, the only common ground being aggressive, prompt, and multimodal therapy. Newer targeted therapies are currently under development to minimize the long-term associated sequelae. The mediastinal and pulmonary sites are still rarely involved in these malignancies, but in such cases the prognostic is dismal.

CHAPTER III. CARDIAC RHYTM DISTURBANCES

Introduction

Congenital heart malformations are usually accompanied by ECG modifications, often very useful in assessing the extent of heart damage, possible complications and proper medical and/or surgical management. When interpreting an electrocardiogram, it is important to be aware of the normal aspects in the pediatric group , depending on age, sex, race and body habitus.

P wave

The normal values regarding amplitudine and duration do not significantly change with age. The depolarization vector is oriented down and forward for the right atrium, and to the left and downward for the left atrium. This means that P wave is generally monophasic, with an axis located in the lower left quadrant. The best leads to evaluate its morphology and duration are leads II and V1. Biphasic P waves can be seen in inferior leads and a negative P wave in lead I is suggestive of ectopic rhytm or dextrocardia. The normal amplitude is 2,5 mm, regardless of age, with the exception of newborns, in which case the threshold is 3 mm. Altered P wave morphology can be caused by atrial enlargement, dextrocardia, or chest deformities (Bronzetti , 2018**).**

The normal cardiac frequency gradually decreases from 145 BPM in resting state in newborns to 85 bpm in adolescents. The PR interval may be shorther in newborns, without any pathological significance. In other age groups, short PR can be an indicator of storage disease and subsequent hypetrophic cardiomyopathy. Preexcitation syndromes can only be diagnosed in the presence of a delta wave. On the other hand, PR lengthening can be associated with channelopathies, electrolyte imbalance, or increased vagal tone.

QRS complex

In newborns, the QRS axis is oriented to the right, and then gradually shifts to the left, in the elderly. As a general rule, a normal AQRS should not exceed 100 degrees in children and adolescents (Bostaca, 2002). Left axis deviation is a clear signs of CHD such as atrioventricular canal, or LVH, aortic stenosis, in older children. Right axis deviation accompanies anomalies such as TOF, pulmonary stenosis, TGA, ASD.

The duration and morphology of QRS complexes is an indicator of conduction abnormalities. Normal variations can be encountered in small children due to right ventricular dominance manifested on the ECG through tall R waves and deep S waves in right precordial leads; as they advance in age, R waves decrease in the right leads and increase in the left ones.

The criteria for ventricular hypertrophy are related to the amplitude of the R and S waves in V6 and V1, respectively, and to the presence of Q waves in the DIII, aVF, V5 and V6 leads.

T wave

At birth, the T wave is a positive deflection, but after a few hours it becomes negative in right precordial leads and may maintain this aspect until the age of 8; afterwards, between 8-12 years , V1 is the only lead in which a negative T wave is acceptable. In left precordial leads, DI ,

and aVF, T wave should always be positive; it can be negative in aVR and it varies in DIII and aVL (Bostaca, 2002).

ST segment

In children, ST segment abnormalities are associated with congenital ischemic conditions, such as ALCAPA and ACAOS anomalies (Angelini, 2007), or with acquired anomalies, like Kawasaki disease. In newborns, an ST segment depression of 1-2 mm in the precordial leads is considered normal, as is the ST elevation in inferior leads and left precordial. In children and adolescent, ST segment depression is a better indicator of ventricular hypertrophy than the voltage criteria (Bronzetti, 2018).

Frequent types of tachy-arrhytmias

Sinus arrhythmia is defined as variation in the sinus rhythm in which the P-P interval is 25% longer or shorter than the normal limits. It is quite common in the pediatric population as it is associated with the respiratory pattern- the rhythm increases during inspiration and decreases during expiration. Sinus bradycardia and tachycardia are related to specific morphologic, metabolic, hematologic and infectious causes (Iordachescu, 2019).

Atrial fibrillation is characterized by desynchronized, fast and irregular atrial activity, which can be transmitted to the ventricles in a 4:1, 2:1 or, in the worst scenario in a 1:1 rate. Diagnostic criteria include the absence of P waves which are replaced by fibrillator waves (f) and irregular ventricular rhythm. The differential diagnosis should take into consideration atrial flutter and atrial tachycardia.

Supraventricular arrhythmias originate above the atrioventricular node, the QRS complexes are usually narrow, as long as an intraventricular conduction disorder is not present, and they are preceded by P waves. In the first year of life, the majority of them are due to an accessory conduction pathway. SVTs can also be the result of a reentry mechanism within the atrioventricular node, a mechanism usually found in children and adolescents. Lastly, ectopic atrial beats may result in supraventricular arrhythmias in 5% of cases, usually in infants.

Ventricular arrhythmias such as long QT syndrome, Brugada syndrome, and short QT syndrome have a genetic basis implicating ion channels. Ventricular tachycardias are defined by the presence of at least 4 ventricular depolarization stimuli with aberrant QRS complexes. Sustained VT usually lasts longer than 30 minutes and the incessant forms tend to be discontinued. If long QT syndrome is also present, there is an increased mortality risk.

Ventricular flutter and ventricular fibrillation consist of disorganized ventricular electrical activity with a ventricular rate of 200-400 bpm. In ventricular flutter, the rhythm is regular and the frequency is around 200 bpm. In ventricular fibrillation, because of the chaotic electrical activity, the dysrhythmia evolves into cessation of the heart's mechanical activity

The etiology is based on severe organic heart disease, such as cardiomyopathy, ischemic heart disease, mitral valve prolapse with mitral regurgitation, penetrating and non-penetrating chest injuries, cardiac catheterization or surgery, heart attack, marked hypotonia, some medicines (digitalis, quinidine, procainamide, antidepressants) as well as some electrolyte imbalances (major hyperkalemia, hypokalemia, hypercalcemia).

Diagnostic criteria in ventricular flutter are as follows:

- ventricular sinusoidal waves
- indistinguishable isoelectric line
- no distinction can be made between QRS, ST and T, the sinusoid representing continuous depolarization and repolarization of the ventricles
- the frequency of sine waves exceeds 200 bpm and the rhythm is constant, regular
- the amplitude of the waves is higher than 15 - 20 mm

Usually, the episode is short and reversible; if, however, lasts more than 1-3 minutes it can cause syncope and sudden death (Luca AC, 2011).

III.1. Supraventricular paroxysmal tachycardia

III.1.1. Background

Rhythms that originate from the atria but not in the sinus node are premature atrial contraction, atrial flutter, atrial fibrillation and supraventricular tachycardia. Atrial flutter and fibrillation are rarely seen in children, whereas supraventricular tachycardia is common in this age group (Iordachescu, 2019). The incidence is estimated at 1:250 cases, with a peak in infancy and late childhood (Tester et al., 2021).

Supraventricular tachycardia may occur in the normal heart, based on automatic atrial foci, accessory conduction pathways, or atrioventricular reentry mechanism, the last two being the most frequent mechanisms in children. SVT can also be caused by congenital malformations, inflammation, acute conditions or medication (Bassareo et al., 2018).

Symptoms that accompany an episode of SVT are palpitations with an abrupt start, polypnea or dyspnea , abdominal pain, appetite loss / poor feeding, dizziness/ syncope, or signs of congestive heart failure in severe cases (Rotes et al., 2020). Coumel's tachycardia is a particular supraventricular rhythm disturbance, caused by an accessory pathway located in the right posterior septal portion, in the vicinity of the tricuspid ring, that leads to a permanent junctional reciprocating tachycardia usually diagnosed when tachycardiomyopathy signs become apparent. It is found in infants and children and is usually refractory to medical treatment (Ilhan et al., 2020).

Recurrent episodes of tachycardia can result in cardiac, neurological, or developmental sequelae in the context of cerebral and cardiac hemodynamic flow disorders, particularly in children and adolescents (Tester et al., 2021).

Diagnosing a tachycardia requires much more than assessing the heart rate. ECGs are necessary in order to identify the underlying cause and sometimes electrophysiological studies are warranted.

The pathogenesis, natural history, clinical aspects and management of supraventricular tachycardia in the pediatric age group were detailed in the article listed below:

Luca, A.C.; Curpan, AS; Miron, I; Horhota, E.O.; Iordache, A.C. Paroxysmal Supraventricular Tachycardia in Wolff-Parkinson-White Syndrome in a Newborn-Case Report and Mini-Review, *Medicina*, 2020; 56(11); pp.1-6, **IF 2.430**

III.1.2. Material and methods

We present the case of a 13-day-old female patient diagnosed with SVT, underlying the mechanism, investigation protocol and treatment course. A literature review was conducted in order to emphasize the case particularities and establish possible options for improving the current set of guidelines and protocols.

III.1.3. Results

Our patient was a 13-day-old female born through C-section during the 37th week of pregnancy. The personal pathologic history revealed that she was admitted in the ED due to an elevated HR during echocardiography (250 BPM) and a systolic murmur grade II out of VI detected at birth.

At that time, the anamnesis and clinical examination revealed a history of poor feeding, hypotony, drowsiness, jaundice, peripheral cyanosis, tachycardia (HR =260–280 bpm), subcostal retractions, polypnea.
The EKG (Fig. III.1.) showed supraventricular tachycardia.

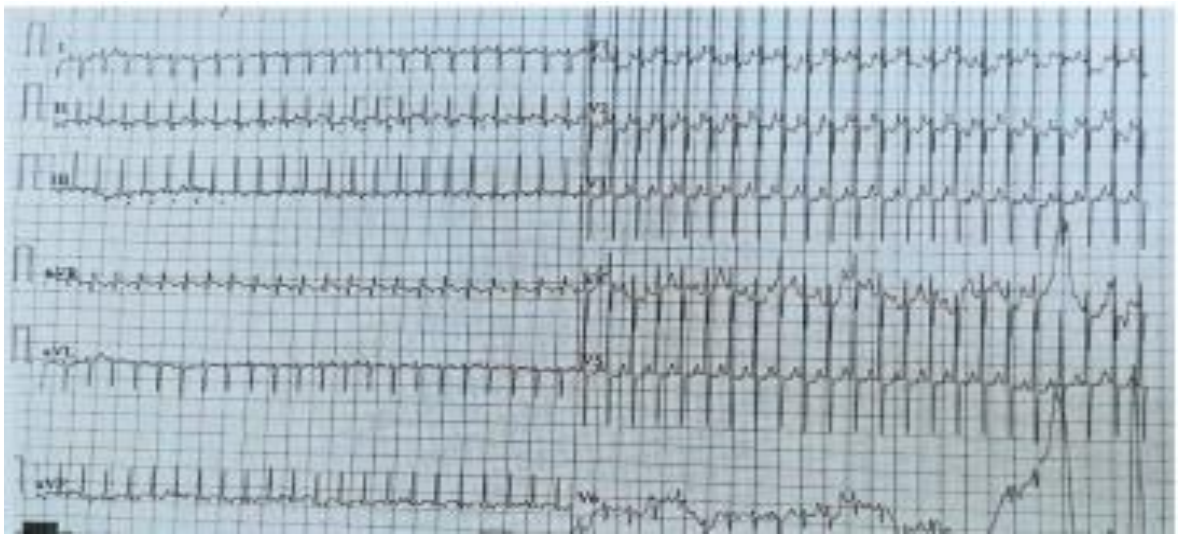


Figure III.1. EKG in crisis. Sinus rhythm, HR = 270 BPM, supraventricular tachycardia, right axis deviation, right ventricular hypertrophy, and widespread ST–T abnormality

The oculocardiac reflex test was performed in the ED with no response, leading to adenosine administration (one dose) followed by reconversion to sinus rhythm (HR = 140–160 BPM).

Subsequently, after an hour and a half, the patient became tachypneic, developing signs of respiratory and cardiovascular distress, requiring endotracheal intubation and mechanical ventilation (IPPV). Systemic perfusion remained at a low level (80–95% pre and post ductal SaO2 saturation), with a HR of 180 BPM preductal and 65 BPM postductal. The patient received CPR for a cardiopulmonary arrest and adrenaline (3 doses of 0.3 mL), resulting in her being transferred to the acute therapy unit.

By means of an electrophysiological study, it was possible to diagnose the patient with preexcitation syndrome, lateral left WPW syndrome and electrically reduced orthodromic paroxysmal supraventricular tachycardia (Fig.III.2.).

The interventional cardiologist did not consider radiofrequency ablation necessary at that moment, since the patient was stable under medication and did not present other episodes of PSVT and only recommended adenosine in case of recurrence.

When she was later admitted in our clinic, her clinical profile included orotracheal intubation and mechanical ventilation, icteric teguments, harsh vesicular murmur, RR of 54/min, subcostal recession, tachypnea, gallop rhythm, HR = 150–160 BPM, SaO2= 90%, left parasternal systolic murmur II/VI, gavage feeding.

The laboratory analysis results included leukocytosis, neutrophilia, monocytosis and normochromic microcytic anemia, thrombocytosis, immunoglobulin G (IgG) and immunoglobulin M (IgM) deficiency, hypoproteinemia, hypocalcemia, and elevated levels of bilirubin.

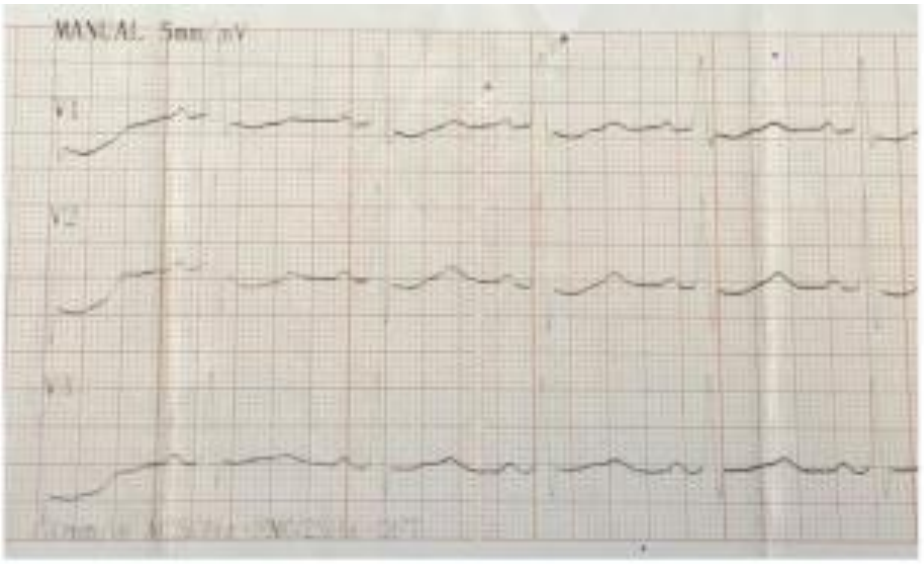


Figure III.2. EKG illustrating delta waves in V1 and V4 as the basis for the diagnostic of WPW syndrome

The chest X-ray showed an infiltrated pulmonary hilum. In the tracheal aspirate (collected one day after her transfer to our unit when she was already intubated), we identified Acinetobacter baumannii.

To the best of our knowledge, the source of infection is unknown (whether she contacted it at birth or during intubation), but we believe that the infection either triggered SVT or worsened the patient's condition.

Echocardiographic examination detected global heart failure, patent *foramen ovale*, patent *ductus arteriosus*, coarctation of the aorta, atrial septal aneurysm , partial anomalous pulmonary venous return, grade II mitral regurgitation, and ventricular diastolic dysfunction.

After 19 h from the first PSVT episode, the patient had a second episode, with a HR of 295 BPM. The therapy, this time, consisted in a dose of Adenosine 0.1 mg/kg (0,4 mg), unsuccessful in restoring the sinus rhythm, followed by 2 more doses of the same volume, unsuccessful as well. Thus, the patient received half a dose of Digoxin 0.04 mg/kg (the other half was divided into halves itself, the first being administered at the 8 h mark and the second at the 16 h mark), which led to HR dropping at 67 BPM, requiring the administration of 0.3 ml of intravenous adrenaline.

In view of the fact that, two hours later, the HR greatly increased again (270 BPM) and there were signs of hepatomegaly (4 cm below the costal rim), we chose to associate the therapy with beta-blockers and follow up with Amiodarone and Dobutamine. Since none of the therapeutic maneuvers was successful, we opted to use cardioversion at 1.25 J/kg (5j) (5 h after the first dose of adenosine was administrated for the second episode), which turned out to be the right choice, as it was able to restore the sinus rhythm (HR = 110 BPM).

The patient did not have another PSVT episode during her hospitalization; she was extubated, and her alimentation switched back to oral feeding. She continued treatment with Ceftazidime, Digoxin, calcium gluconate, magnesium, Furosemide, Spironolactone, Ursofalk, Midazolam, 1/3 vial of albumin for 5 consecutive days, and she also received 50 mL of blood transfusion (O type, Rh+).

At discharge, we recommended the patient to continue treatment with Spironolactone, Furosemide, and Digoxin for another month and return for reevaluation. At reevaluation, she presented in good general condition, with sinus rhythm, HR = 120 BPM and systolic murmur grade II out of VI. To our knowledge, the patient is now 3 years old, is being reevaluated by the pediatrician in her region (she is currently in Germany) and has not presented other episodes of PSVT.

III.1.4. Discussions

The pathways through which the ventricular myocardium can receive partial early depolarization are the Kent James or Manhaim bundles or the fast atrioventricular node pathway.

The electrical impulse can be conducted anterogradely through the atrioventricular node and retrogradely through the accessory pathway, or vice versa.

The Kent bundle normally exists in fetuses and regresses by 20 weeks of pregnancy. It is associated with the classical WPW syndrome, characterized by shortening of the PR interval, widened QRS complex with an initial delta wave, and ST-T segment abnormalities**.**

The ECG aspects vary depending on the localization of the bundle, on the right or left side:

- Left-sided bundle: positive delta wave in V1-V6; R to S ratio > 1 in V1.
- Right-sided bundle: negative delta wave in V1 and V2.

Lown-Levine syndrome is caused by the persistence of James fibers, that connect the atrial myocardium to the superior segment of the AV node. In this case, the QRS aspect is normal and the PR interval is shorter than 0.12 sec (Soos et al., 2021).

Mahaim-Type Pre-excitation is based on bundles that connect the inferior portion of the AV node with the interventricular septum. The ECG typically shows wide QRS complexes with LBBB aspect (Bostaca & Marcu 2002).

In the pediatric age group, the triggering factor can be either a premature atrial contraction or a ventricular one.

The clinical presentation tends to be similar regardless of the pathological substrate, so that sometimes only electrophysiological studies can establish the exact mechanism of tachycardia.

The emergency treatment for SVT's acute stage is first and foremost represented by vagal maneuvers, a method used for our patient as well. If vagal maneuvers are not effective, than iv or io administration of adenosine, in a initial dose of 0.1 mg/kg, 6 mg maximum and a subsequent administration of 0.2–0.3 mg/ (maximum 12 mg) is recommended. In our patient, three 0.1-mg/kg doses were administrated (Richardson & Silver, 2017).

Other antiarrhythmic drugs that might be administrated for converting PSVT are propranolol (in patients with WPW syndrome) as well as other beta-blockers, such as verapamil, digoxin or amiodarone, the efficiency and safety of which was observed in nurslings and may be administrated if adenosine proves to be ineffective (Iordachescu, 2019).

We used digoxin both for antiarrhythmic purposes and for the positive inotropic effect. In the situation where the therapeutic maneuvers described above are ineffective or when the patient is hemodynamically unstable in cardiogenic shock or congestive heart failure, the patient might be subjected to direct cardioversion from 0.5 J/kg of body weight to up to 2 J/kg (Richardson & Silver, 2017).

The management of WPW-related PSVT is different due to its high risk of cardiac arrest or sudden death (which is most frequent in people with high-risk occupation and athletes), in which case, electrophysiological studies and radiofrequency catheter ablation may be curative and the treatment of choice.

III.1.5. Conclusions

Every patient will react in a personal and specific manner to antiarrhythmic therapy based on PSVT cause, as well as other associated conditions. In our case, the newborn responded to adenosine in the first episode of PSVT, but for the second one, cardioversion was required as no other therapeutic option available in our clinic was able to restore the sinus rhythm.

The therapeutic options are various, ranging from medication to cardioversion and ablation and all possible scenarios have to be analyzed before deciding the route of treatment. Ablation is a highly successful approach with a low complication incidence, but with risks high enough to be carefully analyzed before performing it on pediatric patients, as the benefits of this procedure need to outweigh the risks.

As described in this case report, in our 13 day-old patient, treatment with heart rhythm medicine and cardioversion seemed to be effective, but due to the lack of follow-up, it is close to impossible to determine the exact explanation for the clinical improvement.

III.2. Ventricular tachycardia

III.2.1.Background

Ventricular tachycardia is very rare in children, but the impact of this condition on prognosis is extraordinarily high. This is especially important as the higher the heart rate, the more reduced is the ventricular diastole, atrial contribution is missing (atrio-ventricular dissociation), leading to impaired myocardial perfusion and then to sudden death (Bostaca & Marcu, 2002).

Incessant monomorphic ventricular tachycardia is characterized by the presence of ventricular activity sequences with the same morphology becoming permanent, which is impossible to interrupt under medication (Shebani et al., 2015).

It occurs either on a pathological ground (congenital heart disease, ventricular hypertrophy, hypertrophic obstructive cardiomyopathy, arrhythmogenic right ventricular cardiomiopathy, acute myocardial infarction, sarcoidosis, Chagas Disease, Brugada syndrome, long QT syndrome) aggravated under ventricular fibrillation (Chug et al., 2004) or in a normal heart, being very well tolerated, but leading in time to dilated cardiomyopathy (Arya et al., 2007).

By Verekei's algorithm, we can differentiate a ventricular tachycardia from a paroxysmal supraventricular tachycardia by:

- the presence of an initial R in aVR; where initial R or Q duration is over 40 ms in aVR; "notched" view of initial slope of the QRS complex predominantly negative in aVR
- ventricular activation-velocity report (Vi/Vt) ≤ 1 (slope of first 40 ms of the QRS complex of aVR/slope of the last 40 ms of the QRS complex of aVR)

If the patient's condition is stable and is not accompanied by hemodynamic deterioration, after proper diagnosis of ventricular tachycardia, cardioversion is attempted with amiodarone 5 mg/kg intravenously or procainamide 15 mg/kg intravenously (amiodarone and procainamide are never administered together) , or lidocaine 1 mg/kg bolus. If drug cardioversion was not successful, then the synchronous biphasic electrical cardioversion synchronous is used of 0.5-1 J/kg (2 J/kg may be attempted if the initial dose was ineffective); these maneuvers are done only after prior sedation.

After cardioversion, in fascicular ventricular tachycardia, relapse prevention is achieved by administering orally Verapamil (Hiremath et al., 2015). If symptoms reappear, or there is a drug-intolerance, invasive treatment methods may be applied:

- Implantable cardio-defibrillator (if there is an increased family risk of sudden death: long QT syndrome, Brugada syndrome, ventricular tachycardia with severe hemodynamic and ejection fraction <35%);
- Radiofrequency ablation treatment (especially indicated in normal cardiac ventricular tachycardia in certain diseases but also in specific pathologies: fascicular tachycardia, idiopathic tachycardia, arrhythmogenic right ventricular dysplasia); ablative treatment by other forms of energy: cryoablation, microwaves, laser and ultrasound (Calkins et al., 2000).

The general aspects, diagnostic challenges, therapeutic maneuvers, drug therapy and prognosis have been the scope of the articles listed below:

1. **Luca, A.C.**; Iordache C.; Holoc A.S.; Toma, C.M.; Grecu M. Diagnostic challenges in child ventricular tachycardia, *Romanian Journal Of Pediatrics*, pp.161-165, 2015.

III.2.2. Materials and methods

We presented the case of a 6 years old patient diagnosed and treated for VT in our department, highlighting the latest guidelines in managing this rhythm disturbance in pediatric patients.

III.2.3. Results

Patient B.B., aged 6 years and 5 month is admitted for respiratory symptoms: expiratory productive coughing and dyspnea, treated at home for 5 days with clarithromycin and Fenspiride. From previous medical history, we discovered multiple upper airways infections that did not require hospitalization.

Our physical examination of the patient revealed:

- Altered mental status; height = +1.23 SD, weight = +0.87 SD, CC= +1,5 SD;
- Pale skin, throat congestion;
- Lungs – rough vesicular murmur, bilateral basal rales ,RR=32/min, SaO2(-) = 99%;
- Heart – rhythmic tachycardia HR = 214 BPM;
- The laboratory data have been within normal range.

Due to increased heart rate, we decided to perform an electrocardiogram (Fig.III.3.) which indicated HR= 211 BPM, regular rhythm, QRS axis of - 90 degrees; monomorphic QRS complexes, with a duration of 0.13 sec and regular succession; atrio-ventricular dissociation and the aspect of right bundle branch block associated with left anterior hemiblock.

Stage diagnosis based on the electrocardiogram was difficult to establish. Therefore, the following have been excluded:

• *Paroxysmal supraventricular tachycardia anomalously led*: despite RS complex in V1-V6, the duration of RS is over 100 ms in precordial leads, atrio-ventricular dissociation in V1 and the initial R wave in aVR > 40 ms support the diagnosis of VT.

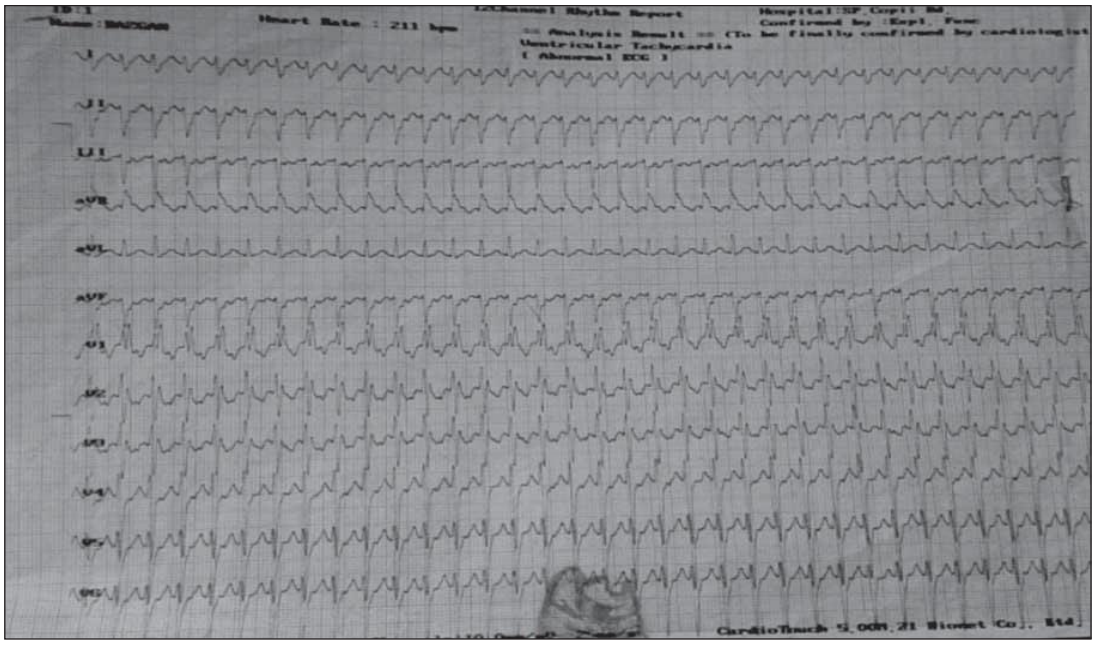


Figure III.3. Electrocardiogram: anterior fascicular ventricular tachycardia

- *Atrial tachycardia with wide QRS complexes and ventricular fibrillation*: disorganized ventricular rhythm

•*Ventricular flutter*: sinusoidal aspect through which ORS seems symmetrical on both sides of the isoelectric line.

•*Polymorphic VT*

- *Accelerated ideoventricular rhythm*: it is a monomorphic ventricular tachycardia with frequency in most cases below 120 BPM, repetitive by accelerated pathological automatism.

Cardio-thoracic radiography showed bilateral infiltrations in the pulmonary hilum and mild cardiomegaly. TTE showed a ventricular diastolic dysfunction, tricuspid regurgitation, *patent oval foramen*, ventricular septum contraction asynchrony, ejection fraction 60%, shortening fraction 31%.Structural changes of the heart do not represent a starting point of ventricular tachycardia but rather a consequence of electrophysiological changes.

The final diagnostic was anterior fascicular ventricular tachycardia with atrioventricular dissociation, right bundle branch block with left anterior hemiblock. The patient received antibiotic treatment, symptomatics, adenosine 2.5 mg initially, then 5 mg, amiodarone 350 mg x 2/day. Tachycardia persisted for 24 hours under anti-arrhythmic medication. We decided that cardioversion should be performed as the functional condition of the child worsened progressively. Under general anesthesia with 1mg iv Midazolamum and Revafil 20 mg intravenously, 50 J biphasic external electric shock in sinus rhythm was performed. We continued daily monitoring of ECG and the patient received Verapamil 40 mg/day. Under medical treatment, the child's general status remained good, maintaining a heart rate of 100 BPM.

ECG at discharge showed sinus rhythm 100 BPM, QRS axis: +100 degrees. PQ = 0.14 sec. RBBB, negative T waves in DII, DIII, aVF and in left precordial leads (suggesting a slight decrease in cardiac output due to ischemia).

At check-up admissions, the patient's condition was good, heart rate of 90 BPM, right bundle branch block persisted (Fig.III.4.).

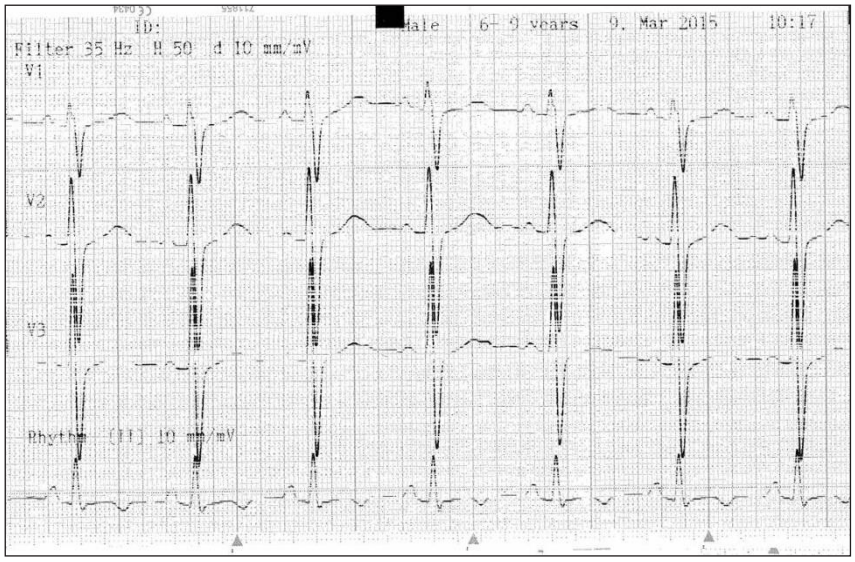


Figure III.4. EKG at discharge: FC:100/min, RBBB

III.2.4. Conclusion

VT in children is rarely associated with ischemic heart disease, like in adults. Instead, it is more frequently caused by postsurgical sequels for TOF, cardiac tumors or ARVD. Episodes of ventricular tachycardia may very well occur on an otherwise healthy heart. It was once the general opinion that amiodarone and beta-blockers should be the first line of therapy. However, recent scientific advances opt for electrical cardioversion if anything else fails. In our case, good tolerance of symptoms and lack of cardiac abnormalities that could have triggered ventricular tachycardia determined us to opt for anti-arrhythmic medication, but the therapeutic approach was not successful in this particular case. Electrical cardioversion restored the normal electrographic path with a long-term favorable outcome.

CHAPTER IV. LIFESTYLE, EPIGENETICS, AND CARDIAC MALFUNCTION

Introduction

Children's heart conditions go beyond the classic categories of heart malformations and rhythm disorders. In adults, lifestyle changes translate, from a cardiac point of view, into clinically "noisy" manifestations, such as acute myocardial infarction and hypertension. In children, the same changes cause heart damage that can often go undetected and viceversa, the cardiovascular insult caused by a variety of "silent" factors that will be detailed further in this chapter may imprint on other functions , particularly on neurodevelopment.

Epigenetics has also gained a lot of recognition lately as a major player on the field of cardiovascular diseases, particularly in the pediatric population. The causal relationship oftentimes is miscellaneous.

For instance, epigenetic factors determine a series of neurodevelopmental disabilities which, in turn, negatively influence the normal development and function of the heart. Autism spectrum disorder has been intensively researched and latest developments suggest that vitamin and mineral deficiencies caused by whimsical appetite negatively impact cardiovascular activity by direct mechanism or associated neuromotor abnormalities.

Cognitive impairment accompanies a multitude of neurological and psychiatric disorders. Convulsive syndromes may well associate behavioral, growth and nutrition anomalies, as well as overall developmental delay. Dravet syndrome is the epitome of neurological impact over cardiovascular function. In addition to this intuitive connection, there are molecular mechanisms related to the level of oxidative stress, increased in neurological and psycho-psychiatric disorders.

Serum BDNF levels are altered in children with cognitive impairment and/or neurodevelopmental delay, irrespective of any association with AD. Alterations of BDNF levels in serum have been reported in epilepsy, various neurodegenerative and psychiatric disorders, such as Alzheimer's dementia, and schizophrenia. It has also been proven that BDNF levels negatively impact the risk for stroke in children, as well as adults Seeing as obesity leads to decreased BDNF levels, it becomes clear how this particular pathology can impact the cardiovascular function through multiple pathways (Karantali et al., 2021).

Last, but not least, the surgical and interventional procedure used to correct CHD associated with periods of hypoxia, respiratory depression, dysregulation in enteral feeding and limitation in parenteral nutrition, increase the risk of impaired cardiac function. Maintaining the balance requires knowledge of dietary guidelines for pediatric patients with heart abnormalities

For the pediatric cardiologist, an eagle-eye view over neurological, environmental, epigenetic and lifestyle factors influencing each individual patient is of the outmost importance, as detailed in the articles below.

IV.1. OBESITY AND CARDIAC DISEASE

IV.1.1. Background

Today, obesity is one of the main health issues impacting children and adolescents. Alongside hereditary factors, lifestyle, social and economic factors that are mainly modifiable represent the aria of focus in the latest scientific research.

In the pediatric group, obesity is defined as the presence of excess adipose tissue that amounts to more than 20% over the normal body weight, leading to + 2 SD or over the 95th percentile for sex and age. Clinically, obesity is defined by means of 2 coordinates: anthropometric measurements and paraclinical evaluation of excess adipose tissue. Weight, height, abdominal circumference, skin fold thickness and BMI are indices used to assess the degree of obesity. Gender-specific weight/height charts can be used up to the age of 5. The CDC issued a recommendation to consider the 85th and 95th percentiles as cutoff points for overweight and obesity, respectively (Negrea et al., 2021).

The World Health Organization standards are summarized in Table IV.1.

Hereditary factors are known culprits in pediatric obesity. Studies linked maternal BMI to higher risk for the offspring to become overweight to various degrees (Linabery et al., 2013). Monogenic disorders caused by mutations in genes regulating the leptin/melanocortin pathway, such as *FTO, INSIG2 MC4R, POMC-ADCY3* have been heavily investigated (Clement et al., 2020). They represent less than 5% of cases, but lead to severe obesity with an early onset (under 5 years old). Usually, eating disorders and endocrine deficiencies are also associated.

Genetic syndromes linked to obesity are Turner, Cohen, Prader-Willi, Temple and Bardet-Biedl syndromes as well as 16p11.2 and 2p deletion syndromes. Early-onset obesity associated with intellectual disability and/or congenital anomalies and a history of poor feeding in the neonatal period should be considered an indicator of a genetic syndrome (Kleinendorst et al., 2020).

Tabel IV.1. WHO definition of obesity based on growth charts

AGE INTERVAL	OVERWEIGHT	OBESE
0-5 YEARS	+ 2 SD above median value	+3 SD above median value
5-19 YEARS	+ 1 SD above median value	+2 SD above median value

The personal contribution related to the study of childhood obesity and the implication it has on the cardiac function was synthesized in the study mentioned below :

1. Lupu V.; Miron I.C.; Lupu, A.; Moscalu, M.; Mitrofan, E.C., Munteanu, D.; **Luca, A.C.** The relationship between gastroesophageal reflux disease and recurrent wheezing in children, *Medicine*, 2021; 100(47), pp.1-4, **IF 1.889**
2. **Luca, A.C.**, Iordache C. Obesity – a risk factor for cardiovascular diseases, *Rev Med Chir Soc Med Nat*, 2013; 117(1), pp.65-71.

IV.1.2. Material and Methods

Direct effect of obesity on cardiovascular function :Our study was conducted on a group of 188 obese and overweight patients that were hospitalized in the Pediatric Cardiology Department, from "Saint Mary" Clinical Hospital of Emergency for Children, Iaşi between 1 January 2006 and 1 January 2011. The patients' evaluation was based on anamnesis, that which included questions related to the patients' age, gender, origins, family history of various diseases, personal physiological history (small weight at birth, food received during the first months of life, diversification) and personal pathological history (genetic syndromes, endocrine diseases, dyslipidemia, diabetes mellitus). Emphasis has been placed on the child's feeding habits, on investigating possible psychological causes of hyperphagia and also on the child's lifestyle. Clinical criteria included anthropometric data, cardiovascular parameters, neurological and cardiac symptomatology. Paraclinical evaluations were based on blood test, ECG, Holter-ECG and monitoring of blood pressure, echocardiogram, ophthalmological, genetic, endocrine, neuropsychiatric, and psychological examination, as well as nutritional disease evaluation.
GERD and obesity- Our study examined the effect of GERD on pulmonary function. Gastroesophageal reflux is commonly associated with obesity, and the two of them create a second hit phenomenon which impacts cardiac and pulmonary function. Gastroesophageal reflux disease occurs when gastric contents flow back into the esophagus and produce symptoms. Recurrent wheezing affects the quality of life for the patient and family. The association of gastroesophageal reflux with recurrent wheezing is suggested by different studies. The purpose of this study was to explore this relationship and to evaluate the outcome after appropriate treatment.

A retrospective study on 85 children with recurrent wheezing, admitted in a pediatric gastroenterology regional center in Romania was performed. 24-hour continuous esophageal pH monitoring was used to evaluate the presence of gastroesophageal reflux and the results were

interpreted using the Boix Ochoascore. All patients with positive score received treatment with proton pump inhibitors and they were evaluated again after 2months. Gastroesophageal reflux was present in 71 children (83.5%), while 14 (16.5%) had a negative score, with a statistic significance (x2=6.88, P=.0086, 95% confidence interval). After 2months treatment with proton pump inhibitors, the Boix Ochoa score was still positive in 15 patients (21.13%).

IV.1.3. Results

The distribution of cases by age group was the following: 51% of patients were aged 7-13 years, 42% were teenagers and only 6% of patients were aged between 2-6 years (Fig. IV.1.). As far as the patients' origin is concerned, we noted that 58% of them lived in urban areas. Study of anthropometric indices (weight, height, body mass index) showed that 63%of patients in the study were obese (BMI greater or equal than percentile 95) while 37% were overweight (BMI between 85ft and 95ft percentiles).

Case distribution per year and gender is showed in Fig IV.2. and Fig.IV.3.

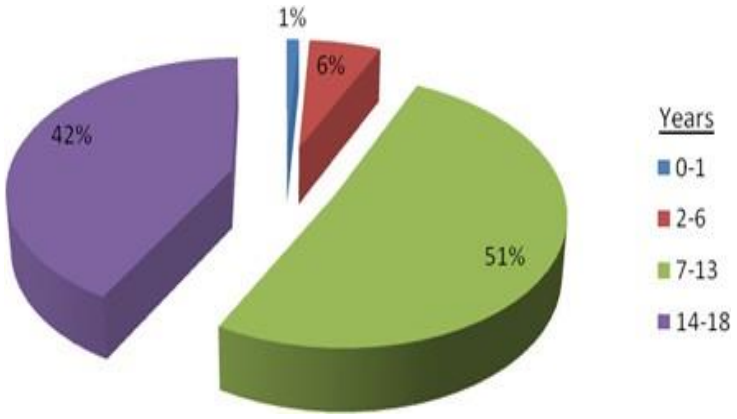


Figure IV.1. Age distribution of obesity

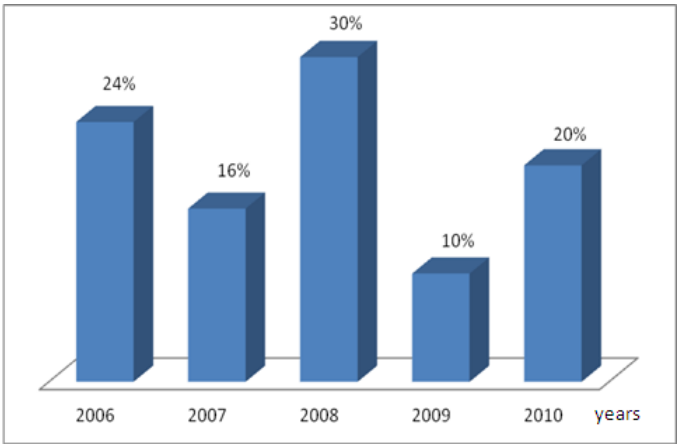


Figure IV.2. Case distribution over the years

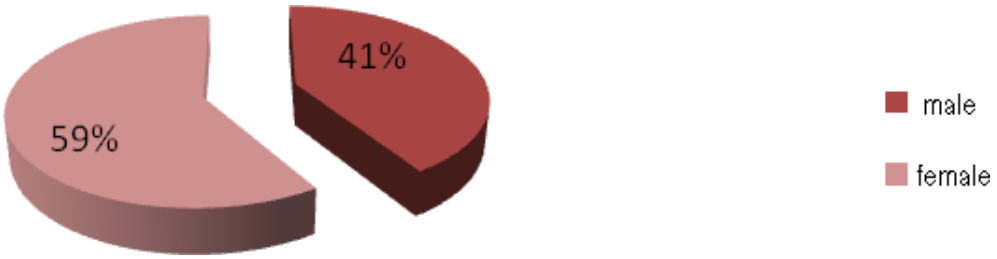


Figure IV.3. Gender distribution

The analysis of the data gathered during anamnesis revealed the 33% of the patients had a personal history of various diseases, and 34% of the subjects had immediate family members suffering from arterial hypertension, diabetes mellitus or obesity. 35% of the patients came to the hospital since they experienced specific symptoms such as dyspnea on exertion, tinnitus, headache, dizziness or high arterial tension values detected in the outpatient clinic.

In the study group, 21% of the subjects had borderline hypertension, while 16% of them suffered from definite hypertension (Fig.IV.4.).

Another criterion considered was ECG modification. Of the 188 patients included in the study group, 10% had sinus tachycardia, 2% had extrasystolic arrhythmia, 5% had sinus arrhythmia, 7% had atrioventricular conduction diseases and 6% had biventricular strain.

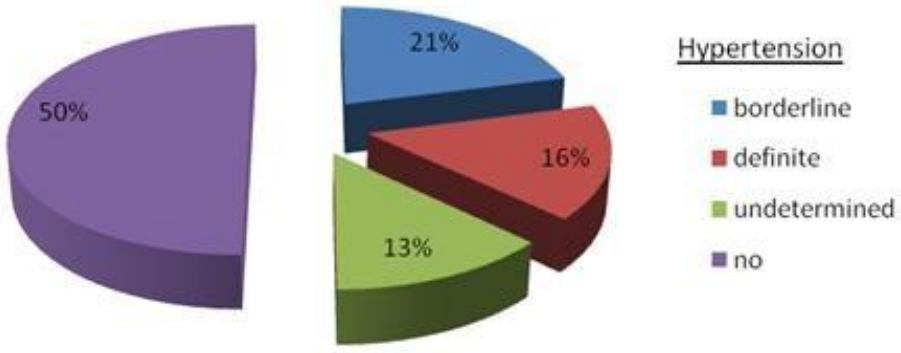


Figure IV.4. Association between hypertension and obesity

The echocardiogram that was performed on all the patients included in the study revealed hypertrophic cardiomyopathy in 12% of them.

8% of patients in the study group associated hypertension and high cholesterol levels, 16% of them had high blood pressure values, yet their cholesterol levels were low and 5% of the patients had high cholesterol levels but no hypertension (Fig.IV.5.). 4% of the children in the study group associated high blood pressure values, high cholesterol levels and hypertrophic cardiomyopathy.

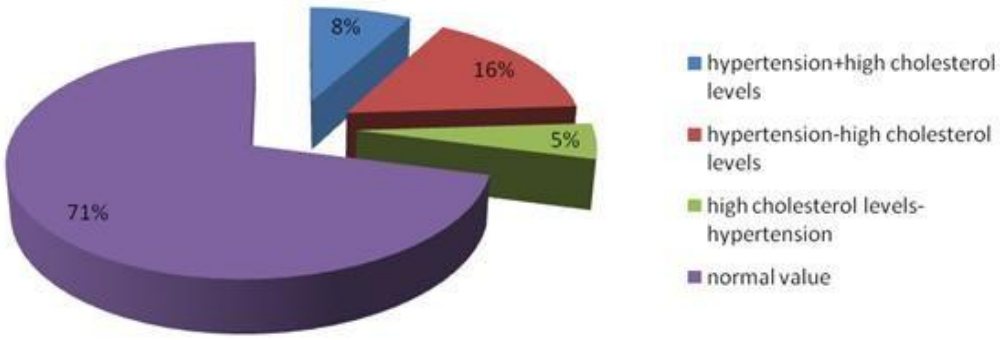


Figure IV.5. Case distribution according to the association between hypertension and high cholesterol levels

The psychological and neuropsychiatric exams carried out revealed that 1% of the 188 studied patients came from over- protecting families, 4% suffered psychological trauma in their personal history(parents' divorce/separation), 2% of the subjects suffered from depression, 18% encountered social and family integration difficulties and 14% had a liminal intellect.

IV.1.4. Discussions

Obesity in childhood is a risk factor for multisystemic complications in adulthood, therefore it is paramount to identify risk factors. With the exception of obesity based on genetic or endocrine causes, in other situations the pathology can be prevented by identifying lifestyle aspects and modifiable socio-economic factors. Some studies suggest the age of 7 as the time when risk factor screening should begin (Raziani & Raziani, 2020).

Alongside eating habits and sedentary lifestyles, the sleep program also has an important influence on maintaining a normal weight. Night-time wake patterns determine besides insufficient rest, changes in neuropeptides levels that help regulate appetite (Raziani & Raziani, 2020).

The obesity rate is higher in pre-school children and adolescents. The infantile obesity rate increased twice in pre-school children aged between 2 and 5 and in adolescents between 12 and 19 years of age, and 3 times in 6 to 11 year-old children, over the last three decades. Although the obesity rate is higher in females, the male sex has a higher risk of cardiovascular diseases (Raj, 2012).

High blood pressure values (AT>95%) were detected in 35.4% of the overweight children by a study conducted in Europe (I'Allemand et al., 2008). In our study, high blood pressure wass identified in 10.10% of the normal weight children, in 17.34% of the overweight children, and in 18.32% of the obese children.

Hypertension and obesity are connected most times and they put double pressure on the left ventricle, thus leading to the occurrence of both dilated and muscular hypertrophy. In left pathological ventricular hypertrophy, the increase is not proportional, the ventricular function is altered, the coronary vessel dilating capacity is reduced, and the process regression is impossible (Maraval & Lurbe, 2012). Obese subjects are 4 times more likely to suffer from compensatory left ventricular hypertrophy associated with low ejection fraction, reduced myocardial contractility and the need of the left atrium to have more force to fill the left ventricle (Chinali et al., 2006). Concentric remodeling was the most common in obese children, whereas concentric hypertrophy was especially characteristic of those who also suffered from hypertension (Dhuper et al., 2011).

In their study, Li et al. performed TTE on 467 young people and reported that a high BMI during childhood, obesity and systolic hypertension during adulthood, as well as the cumulated burden of obesity and systolic hypertension from childhood up to adulthood are predictive factors of left ventricle muscle mass increase in young adults (Li et al., 2004). Ventricular hypertrophy has higher prevalence in obese (33.5%) and overweight (12.4%) children as compared to normal weight children (3.5%). In a study, the patients with borderline hypertension were found to have higher left ventricle muscle mass values than those with normal tension values (Dhuper et al., 2011).

Obesity, hypertension and concentric hypertrophy are independent predictors of diastolic dysfunction. 52% of obese children in our study had hypertension, which means that their body mass index, left ventricle dimensions and left atrium muscle mass index were also high. These subjects also exhibited diastolic function changes. 42% of patients had concentric remodeling (muscular dilation) and 32% of them suffered from concentric hypertrophy, which supports a strong association between hypertension and ventricular remodeling. Previous studies have shown that an excess of body mass is associated with high blood flow values and eccentric hypertrophy. Nevertheless, only 6% of the subjects in our study suffered from such conditions, as most of them exhibited muscular dilation and concentric hypertrophy.

On the other hand, cardiovascular diseases, be they inborn or acquired, cause increased obesity prevalence, given the limited physical activity recommended by the cardiologist or preferred by the family or even the patient. A vicious circle is thus created in which obesity worsens heart conditions and lack of physical activity due to heart conditions accentuates obesity (Nelagi et al., 2007).

IV.1.5. Conclusions

The research conducted on the study group described above enabled us to conclude that high overweight and obesity rates are especially characteristic of the 7-13 years age group. The incidence of obesity is much higher in urban (58%) than in rural areas, which could be due to the unhealthy fast food available in urban areas.

The incidence of obesity is higher in females (59%). 37% of patients suffered from hypertension, 21% of whom had borderline hypertension and 16% suffered from definite hypertension. Changes in the fundus of the eye associated to hypertension were revealed in 5% of patients, while hypertrophic cardiomyopathy was detected in 12% of study subjects. 8% of subjects associated hypertension and high cholesterol levels, and 5% had high cholesterol levels but no hypertension. Only 4% of subjects associated high blood pressure values, high cholesterol levels and hypertrophic cardiomyopathy.

IV.2. Eating disorders- the cost for cardiovascular function

IV.2.1. Background

It is a well-documented fact that eating disorders have a multifactorial etiology and in little children, they have a dismal influence on their overall development and cardiac function, making this pathology extremely important to be assessed and monitored by the pediatric cardiologist.

The interplay between neurological and psychological factors gained more recognition in the research regarding eating disorders. Eating behaviors are based on the balance between norepinephrine and serotonergic mechanisms, thus explaining the association of nervous disorders with eating disorders (Buica, 2019; Pizzino et al., 2017).

Autism spectrum disease and epilepsy associate a significant decrease in cognitive function which, in return, correlates with poor overall function and self-regulating eating habits, leading to obesity and /or macro and micronutrients deficiencies.

From a cardiologic perspective, etiological factors influencing the neurological integrity of pediatric patients with congenital heart defects are: length of hemodynamic and hematologic effects produced by the disease, hypoxic and ischemic lesions secondary to hypothermia used while performing the cardiopulmonary by-pass.

The personal contribution regarding the correlation between physio-pathological, and clinical aspects of eating disorders, their impact on cardiac function and vice versa is exemplified by the articles mentioned below.

1. Curpan A.; **Luca A.C.**; Ciobica A. Potential novel therapies for neurodevelopmental diseases targeting oxidative-stress, *Oxidative Medicine and Cellular Longevity*, 2021, pp.1-13, **IF 6.543**
2. Kazis, D.; Petridis, F.; Chatzikonstantinou, S.; Karantali, E.; Jamali, R.; Chowdhury, R.; Duta, R.; Luca, A.C.; Ciobica, A.; Mavroudis, I. Gray Matter Changes in Juvenile Myoclonic Epilepsy: A Voxel-Wise Meta-Analysis, *Medicina Lithuania*, 2021; 57(1), pp.1-9, **IF 2.430**
3. Robea, MA; **Luca, A.C.**; Ciobica, A. Relationship between Vitamin Deficiencies and Co-Occurring Symptoms in Autism Spectrum Disorder, *Medicina*, 2020, pp.1-12, **IF 2.430**.
4. Ciobica, A.; Padurariu, M.; Curpan, A.; Antioch, I.; Chirita, R.; Stefanescu, C.; **Luca, A.C.**; Tomida, M. Minireview on the Connections between the Neuropsychiatric and Dental Disorders: Current Perspectives and the Possible Relevance of Oxidative Stress and Other Factors, *Oxidative Medicine and Cellular Longevity*, 2020; pp.1-13, **IF 6.543**
5. Lefter, R.; Ciobica, A.; Antioch, I.; Ababei, D.C.; Hritcu, L.; **Luca, A.C.** Oxytocin Differentiated Effects According to the administration Route in a Prenatal Valproic Acid-Induced rat model of autism, *Medicina-Lithuania*, 2020;56(6), pp.1-21, **IF 2.430**
6. **Luca, A.C.**; Holoc, A.S.; Mihalache, I; Luca, F.A. Management of Neuropsychomotor and Economic Cost of Social Insertion of Children with Congenital Heart Malformations, *Journal of Social Research and Intervention*, 2018; 63, pp.379-388 **IF 1.076**

IV.2.2. Materials and methods

Novel therapies targeting oxidative-stress :

We performed an initial literature search in January 2020 and included articles published after 2000 with a focus on the most recent relevant ones. The articles were found by using the keywords under different combinations such as oxidative stress, schizophrenia, vegetal extract, glutathione, schizophrenia, N-acetyl cysteine neurodevelopmental disorders, and attention-deficit hyperactivity disorder. The search was performed by accessing MEDLINE, Hindawi, and Google Scholar databases.

Management of Neuropsychomotor and Economic Cost of Social Insertion of Children with Congenital Heart Malformations :

We conducted a literature review focusing on keywords such as congenital heart disease, stress, neurodevelopment, bypass, cerebral perfusion and included databases found on Google Scholar.

Vitamine deficiencies and autism spectrum disorders:

We summarized several reports that studied the correlation between autism disorders and eating and feeding problems. In addition, we expanded on the deficiency of vitamins in the human body which seemingly could lead in some cases to a dysregulated functioning of the nutritional

status of AD individuals.

IV.2.3. Results and Discussions

Novel therapies targeting oxidative-stress

For the present article, we selected a total of 114 articles, and their division based on the disorder - general aspects, oxidative stress role, NMDAR/glutamate , NAC, and phytochemicals interplay can be observed in Fig.IV.6. Cellular redox status is a key player in various cellular functions and diseases.

The accumulation of oxidants (such as reactive oxygen species, also known as ROS) has long been associated with oxidative stress (Buica, 2019; Pizzino et al., 2017). Reactive oxygen species can be differentiated in two types following one criterion: whether they are free radicals or not. The most encountered free radicals are hydroxyl, superoxide, and nitric oxide, as they are produced during ionizing radiation or environmental toxicology reactions. As for non-radicals, the most well-known are hydrogen peroxide, single toxygen, ozone, and peroxynitrite. Nonradical species are less reactive from a chemical point view, but they can easily become radicals by reaction with macromolecules and cellular metabolites (Armstrong & Stratton, 2016).

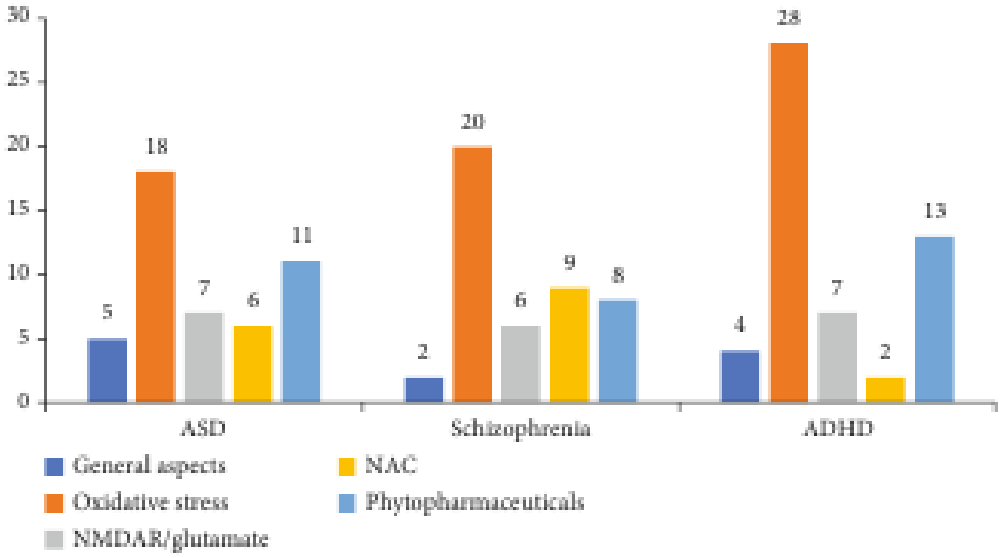


Figure IV.6. *Divison of articles based on disorders , pathological mechanisms and treatment options*

Each cell has a specific threshold of endurance for this species; once that is exceeded, the cell and the organism itself cannot efficiently detoxify the reactive species (Di Meo et al., 2016). It has also been observed that oxidative stress is linked with various physiological changes, implying damage to proteins, nucleic acids, lipids, and others. Therefore, cells must maintain a very precise balance between the benefiting effects of oxidants in signaling, immune responses, and biosynthesis and their downsides. Any sort of disturbance in this equilibrium can cause various dysfunctions and disorders (Mesika & Reichmann, 2019).

Reactive oxygen species target oxygen sensible lipids such as glycolipids, cholesterol, and phospholipids, which can undergo lipid peroxidation. Interesting is that these lipid peroxides are beneficial to the cell in small quantities as they are used for cellular signaling via ligand-receptor interaction mediation using thiol reversible modifications (Higdon et al., 2012). When these lipid

peroxides are generated in a nonspecific manner and they accumulate in membranes, they may lead to perturbations of the membrane bilayered structure affecting its permeability, plasticity, and as a result the ion gradient in cells. When these oxides are found in elevated quantities, 5-hydroxynonenal (4-HNE) and malondialdehyde (MDA) produce lesions to proteins, nucleic acids, and antioxidant molecules, eventually leading to the cell death (Adams et al., 2011; Higdon et al., 2012).

One interesting aspect of reactive oxygen species is that they were proposed as a possible treatment for brain tumors due to their apoptotic effects. At all events, the ROS-based treatment is disputed due to their opposing effects: on the one side, they have a deleterious effect on cancerous cells; on the other side, they contribute to cellular proliferation and tumorigenesis (de Sa Junior et al., 2017).

Similar to schizophrenia, studies on postmortem brain samples acquired from individuals affected by AD indicate the involvement of oxidative stress in the pathology of this disorder. Several studies have reported damage of oxidative nature to lipids, proteins, and nucleic acids and a decreased level of the major cellular antioxidant—GSH. Two studies using, 10 and respectively, 15 individuals and age-matched controls made one common observation—decreased levels of GSH/GSSG in the cerebellum. Similar findings were made by Chauhan et al in the temporal cortex and by Rossignol et al in the Brodmann area. Interesting is that these findings are not correlated with age, but rather as a chronic condition (Chauhan et al., 2012; Rossignol & Frye, 2014).

Damage to lipids is represented as significantly higher levels of hydroperoxides of lipid origin in the temporal cortex and cerebellum and mitochondrial dysfunction in the same areas When it comes to proteins, oxidation at this level was observed by measurements of 3NT levels and was reported to be elevated in the cerebellum and correlated with increased neurotrophin-3 in the same brain area. This protein has a critical role in normal brain growth and differentiation, and its increase may be related to oxidative stress (Rose et al., 2012).

In studies conducted for identifying oxidative damage to nucleic acids, the level of two DNA biomarkers was pursued—8-oxo-deoxyguanosine (Tang et al., 2013) and 8-hydroxydeoxyguanosine (Sajdel- Sulkowska et al., 2009) and was found to be elevated in the temporal lobe and in the cerebellum, respectively, while the one for RNA—8-hydroxyguanosine (Muratore et al., 2013) was higher in the frontal cortex when compared to controls (Rossignol & Frye, 2014).

Mitochondrial dysfunction was also observed in postmortem brain samples of individuals suffering from AD under the form of decreased activity of the electron transport chain (ETC) complex and tricarboxylic acid (TCA) cycle enzyme, as well as discrepancies in gene expression (Rossignol & Frye, 2014).

Management of Neuropsychomotor and Economic Cost of Social Insertion of Children with Congenital Heart Malformations

In the study of Daliento, Mapelli, & Volpe from 2006**,** it was shown that there is a risk of brain lesions even in patients with non-cyanotic congenital heart defects without severe heart failure. Surgical correction of CHD includes protection methods of brain structures, such as: cardiopulmonary bypass and hypothermia induction. Both methods can have neurological side effects caused by: risk of microembolism during bypass, difficulty in obtaining the optimal temperature to induce cardiopulmonary bypass (Daliento et al., 2006).

Risk factors for brain lesions caused by hypothermia are: cardiopulmonary bypass, prolonged especially in younger patients (more than 45-50 minutes), rapid induction of hypothermia (under 20 minutes), severe hypothermia, hyperglycemia before cardiac arrest and during reperfusion, severe cyanosis and blood hyperviscosity before surgery, immediate

postoperative low cardiac output, impaired cerebral blood flow secondary to autoregulation process deficiency.

Postoperative brain dysfunction is a serious complication of pediatric cardiac surgery that prolongs hospitalization; therefore, the early detection of postoperative neurological problems is important for establishing the treatment needed for early rehabilitation. Additionally, preoperative identification of children at risk requires new strategies to reduce the negative neurological impact after cardiac surgery.

Specialized literature reports specific tests used to identify the degradation of cerebral function after cardiac surgery: increased levels of S-100 protein or neuron specific enolase in postoperative patient and changes in EEG. Clinically, the postoperative cerebral dysfunction includes the following symptoms: convulsions, paralysis, choreoathetosis, impaired cognitive or psychomotor development.

In children with pre-existing neurological problems or with chromosomal abnormalities, the neurological worsening was considered to be present if the skills acquired by learning disappeared after surgery.

Trittenwein et al. (2003) report in a study that brain repercussions discovered after pediatric cardiovascular surgery were: cerebral infarction, cerebral bleeding, hydrocephalus and serious cerebral atrophy, diagnosed by CT, MRI or by performing necropsy. The same study represents the following important predictors for postoperative negative neurological impact: young age, complex cardiac defects, metabolic acidosis and increased serum levels of lactic acid. These predictors are plausible in the first year of life; the accelerated growth of the brain makes it more susceptible to the adverse effects of hypoxia. Specifically, in the neonatal period, lactic acid is described as an important predictor of neurological dysfunction due to hypoxia (Trittenwein et al., 2003).

More complex congenital heart defects require a more elaborate surgery leading to a higher risk of intraoperative complications. Generally, most lesions are located in the cortical region of the brain, especially in the visual cortex and in the parietal lobe and cause difficulty in integrating images in space and in developing organizational skills (Griffin et al., 2003).

Intellectually, most researchers agree that patients with CHD are at increased risk of intellectual impairment, the higher the cardiac dysfunction, the more impaired the cognitive function. Studies showed that patients with cyanotic heart disease had lower IQ scores and learning difficulties. Noteworthy is the importance of differentiating between the effects produced by chronic disease in children and the development of cognitive impairment caused by CHD.

Complex mental and behavioral disorders especially in patients with CHD disorders were noted, and classified into two categories: externalizing (attention deficit disorder, aggression) and internalizing (anxiety, depression, somatization) disorders (Marino et al. 2012). In contrast, other studies found that chronological age and not the severity of congenital defects are significant risks in psychological development, noting that children and teenagers with CHD face higher risk of developing internalizing problems , caused also by parental overprotection. Alternatively, brain and hormonal changes that occur during this period, combined with genetic vulnerabilities can enhance behavioral disorders (Karsdorp et al., 2007). Neuropsychological differences are observed not only between children with CHD and healthy children, but also between children with non-cyanogenic and cyanogenic CHD. Gupta et al. (1998) objectified that patients with cyanotic congenital heart disease have an increased risk of developing depression, anxiety and behavioral problems (Gupta et al., 1998). Other researchers noted a link between the total number of surgeries of a child and the induction of hypothermia with a higher rate of behavioral problems. Interiorizing disorders have been associated with an increased number of surgical procedures in which

hypothermia was induced, small gestational age, low oxygen saturation and the age at surgery (Utens et al., 1993).

Most studies showed the emotional and psychological impact of CHD diagnosis on mental and behavioral disorders, impact which translates into high financial costs, especially in patients with complex CHD classified into two categories: externalizing behavior (attention deficit disorder, aggressiveness) and interiorizing behavior (anxiety, depression, somatization). Therefore, in order to reduce financial costs, it is important to diagnose and treat these psychological disorders early , as they may lead to psychological and psychiatric morbidity, the prevalence of major psychiatric diseases being 3-4 times higher in patients with neuro-cognitive impairment than in general population.

Vitamine deficiencies and autism spectrum disorders

Feeding problems are common in autism spectrum disorders and they have been overlooked for a long time in favor of behavioral, social and communication deficits (Hill et al., 2015). These problems are generally reflected through a selective texture of food, a particular presentation or even preference for a certain meal (Schreck & Williams, 2006; Martins et al., 2008).

Because of this situation, there are differences between children with AD and normal children. AD children tend to gain weight more quickly compared with the general population deficits (Bertoglio et al., 2010; Hill et al., 2015; Mousain-Bosc et al., 2010). In one study performed by Hill et al. it was confirmed that overweight or obesity has a higher prevalence in AD children than in non-affected ones. Also, they found evidence that suggests the existence of multiple factors associated with autism, such as sleep and affective problems, parent education, economic status, and even geographical factors.

AD can be identified through symptoms grouped into two categories: the core and the secondary symptoms deficits (Hill et al., 2015; Fakhoury, 2015). Feeding and eating problems can be found under the umbrella of secondary symptoms. Atypical eating behaviors and feeding problems are often reported by the parents of a child with AD (Lonsdale et al., 2011; Marotta et al., 2020; Malhi et al., 2017). Food refusal, preferences for a certain product or foods, an obsessive routine for taking meals, and preference for the color and the texture of a specific kind of foo are th most common (Criado et al., 2018; Mazahery et al., 2019). Because of this, children and adults with AD have deficiencies regarding the nutrients intake (Fig. IV.7.), especially micronutrients, making vitamin supplementation important, as summarized in Table IV.2. (Modabbernia et al., 2017).

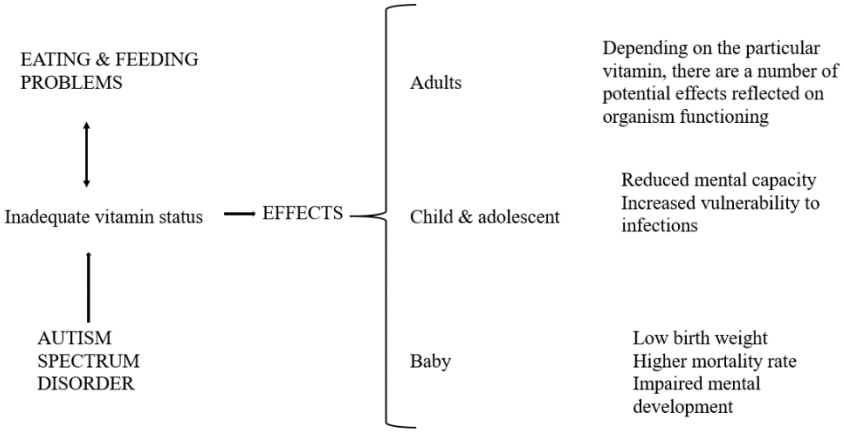


Figure IV.7. Effects of inadequate vitamin intake on AD individuals

IV.2.4. Conclusions

As we demonstrated in the previous chapter, obesity is a significant risk factor for cardiovascular disease, especially in the pediatric age group, where the incidence of this disease has been increasing significantly in the last decade.

In addition to genetic diseases, endocrine abnormalities, and low physical resistance to effort, neuro-psychological conditions and cognitive developmental delays or decline caused by surgery to correct congenital heart defects, are also important factors that need to be premeditated. The amount of evidence supporting the role of oxidative stress in the progression of neurodevelopmental disorders seems to increase and becomes more visible as new evidence is piling up. As these disorders have a very complicated origin and high comorbidity, a trait that could tie together all symptoms and hypotheses has the potential to shed some light on how therapies should be approached in the future. Oxidative stress has always been considered the "blackhole" of science as it seems to be involved in a large repertoire of disorders, without having a focus. As all areas of science have evolved enormously compared with the times when traditional medicine was used, in current times, we can use them as therapies based on their phytochemical components; therefore, they have a big potential as targeted drugs and future studies should focus on the antioxidant properties of plant extracts.

N-Acetylcysteine as a repurposed drug for several times has the potential to improve negative symptoms of schizophrenia, when compared to antipsychotic effects, and to improve irritability and social traits in autism spectrum disorders individuals; hence, future studies should be realized on larger scales and in association with different types of medications; it would be also interesting to test its abilities right after the onset of these disorders.

Thus, in ameliorating the AD symptoms, vitamin supplementations are recommended as alternative therapy. In this mini review, we have gathered several studies in which the effects of vitamin B1, B6, B12, A and D intake on autistic individuals were highlighted. Beside these aforementioned vitamins, there are also other vitamins or combinations between micro- and macronutrients which need to be explored. Despite the positive effects recorded for vitamin intake, recommended supplementation is required from medical specialists. In conclusion, further research is needed to provide safety assurance and evidence of efficacy in vitamins administration

Table IV.2. Studies evaluating the dosage and effects of vitamin supplementation in AD patients.

Vitamin	Participants	Dosage	Time of Administration	Type of the Study	Effects	Side Effects	Observations	References
B_1	10 children	50 mg TTFD	Twice a day / 2 Months	PS	P.E.	-	↑clinical symptoms ↑Pb, Hg, Cd, As levels	147
	141 children	20 mg	Days 1–4: 1/6 of fd Days 5–8: 2/6 of fd Days 9–12: 3/6 of fd Days 13–16: 4/6 of fd Days 17–20: 5/6 of fd Days 21 and later: fd	RCT, DB, P	P.E.	diarrhea, constipation	↑ATP, sulfation, NADH, NADPH, GSH ↑clinical symptoms ↓ OS	148
B_6	33 children	6 mg/kg Mg and 0.6 mg/kg vit. B_6	6 months	RCT	P.E.	-	↑social interactions, communication, abnormal functioning, stereotyped restricted behavior ↑Erc-Mg values	149
	141 children	40 mg	Days 1–4: 1/6 of fd Days 5–8: 2/6 of fd Days 9–12: 3/6 of fd Days 13–16: 4/6 of fd Days 17–20: 5/6 of fd Days 21 and later: fd	RCT, DB, P	P.E.	diarrhea, constipation	↑ATP, sulfation, NADH, NADPH ↑clinical symptoms ↓ OS	148
B_{12}	30 children	0.06 mg/kg	12 weeks	PS, DB	N.E.	-	↓ OS ↑GSH no behavioral improvements	150
D	122 children	0.0075 mg/kg	3 months	RCT	P.E.	skin rashes, diarrhea and itching	↑core symptoms	151
	73 children	0.049 mg/kg	1 year	RCT	P.E.	-	↓ irritability and hyperactivity	152

↑ rise/ improvement; ↓ decrease; As: arsenic; ATP: plasma adenosine-5'-triphosphate; Cd: cadmium; DB: double-blind; Erc-Mg: intraerythrocyte Mg^{2+}; fd: full dose; GSH: glutathione; Hg: mercury; Mg: magnesium; N.E.: no effects; NADH: nicotinamide adenine dinucleotide; NADPH: nicotinamide adenine dinucleotide phosphate; OS: oxidative stress; P.E.: positive effects; P: placebo; Pb: lead; PS: pilot study; RCT: randomized controlled trial; TTFD: thiamine tetrahydrofurfuryl.

IV.3.Optimal nutrition parameters

IV.3.1. Background

The evaluation of nutritional status and growth in children is based on WAZ (weight for age), WHZ (weight for height) , and HAZ (height for age) parameters . A cut-off Z-score < -2 classifies malnutrition into underweight (low WAZ), stunting (low HAZ), and wasting (low WHZ) (Mirzaaghayan et al, 2020). Children with multiple anthropometric deficits have a heightened risk of mortality, more so those with congenital heart anomalies. Stunting is associated with cyanotic heart disease and PHA, while acyanotic heart anomalies are usually accompanied by wasting (Arodiwe et al, 2015). The appearance and progression of malnutrition in these patients is dictated by the hemodynamic impact of heart lesions, the occurrence of heart failure, de-layed surgical repair, prolonged intubation and feeding intolerance.

Pulmonary hypertension (PH) has the strongest association with pre-operative malnutrition (Zhang et al, 2020). The underlying mechanisms of PHA are mainly related to hypoxia-induced adaptive responses and increased pulmonary flow. In CHD with left to right shunt, there is excessive pulmonary blood flow which leads to endothelial dys-function, vascular remodeling, and progressively higher pulmonary vascular resistance (PVR). Hypoxic pulmonary vasoconstriction is the physiologic response to regional de-crease in oxygen availability and it is crucial for matching ventilation and perfusion, albe-it causing an increase in PVR (Lumb & Slinger, 2015). Once installed, PHA causes right ven-tricular failure, which in turn leads to gastrointestinal edema, malabsorption, altered mi-crobiome and fluid retention (Kwant et al, 2019). Drugs used to prevent fluid retention in this scenario, such as diuretics, cause vitamin B1 deficiency , a micronutrient involved in carbohydrate and branched-chain amino acid metabolism (Hiffler et al, 2016).

As a response to hypoxic conditions, Na+/K+-ATPase activity is limitated in order to prevent unnecessary ATP expenditure (Bogdanova et al, 2016). Studies showed that Na pump inhibition is related to malabsorption of nutrients and electrolytes (Nepal et al, 2021).

Hypoxia also induces antioxidants depletion and modifies the expression of genes coding enzymes involved in glucose metabolism. HIF-1 (hypoxia induced factor) promotes lactate dehydrogenase A which turns pyruvate into lactic acid in the final stages of glycolysis (Zheng et al, 2021). In chronic hypoxia, glucose and lipid storages are depleted (Kwant et al, 2019).

Mitochondrial role in negative outcomes in patients with CHD has also been investi-gated, and the imbalance between biogenesis, fission, fusion and mitophagy was indicated as the main mechanism behind mitochondrial dysfunction (Ma et al., 2020). Mitophagy can be triggered by exogenous substances and their use in increasing cardio protection is still under investigation (Liu et al., 2020; Morciano et al., 2020).

Once diagnosed, in the case of a positive prognosis, patients with CHD either immediately undergo a surgical repair or are subjected to pharmacological therapy until the surgical intervention is possible, which makes building a trust relationship between par-ents and doctors of utmost importance (Luca et al., 2015). Meeting the perioperative needs of children scheduled for a cardiopulmonary bypass has an important impact on the postoperative clinical outcome. Ensuring up to 170kcal/kg calories and 3-4 g/kg/day was suggested as an appropriate strategy to improve postoperative prognosis (Alakeel et al, 2021).

Similarly, patients reaching puberty before surgical correction face the risk of heart failure, because of the metabolic changes that normally occur in the heart tissue. The glucose-dominant metabolism active during prepuberty switches to fatty-acid dominant me-tabolism during puberty.

This switch is impaired in hearts affected by chronic hypoxia, as is the case with a congenital heart malformation. In this scenario, the outcome of these pa-tients could be improved by pioglitazone administration during puberty (Liu et al., 2021; Morciano et al., 2020).

Enteral and parenteral feeding have standardized parameters in patients with CHD before and after surgery. Minor cardiac anomalies with insignificant hemodynamic and systemic impact fall under the general principles of feeding, while complex cardiac anomalies require personalized regimens.

IV.3.2 Material and methods

We conducted a thorough scientific research using PubMed and ScienceDirect as reference databases and neonatal feeding, congenital heart disease nutrition, pediatric nutritional requirements, and supplements in pediatric patients as queries. General aspects were detailed based also on available guidelines for neonatal and pediatric nutrition. In addition, we used the authors' experience in managing the dietary requirements of patients with CHD before and after surgery to underline aspects of tailored nutritional regimes depending on factors other than the presence of a congenital heart malformation.

IV.3.3. Results

General principles for enteral feeding

During the neonatal period, enteral feeding (EN) should be initiated as soon as possi-ble, with breast milk or formulas that ensure the energy and macronutrients requirements. A term ill newborn initially needs 40-60 kcal / kg / day, with a gradual increase to 90-120 kcal / kg / day. The daily requirement of carbohydrates is between 9-14 g / kg / day (40-50% of total calories), proteins - 1.8-2.2 g / kg / day (7-16% of total calories) and lipids - of 4-6 g / kg / day (34-35% of total calories). In neonates with a CHD, the daily caloric intake should be up to 50% higher, but without exceeding 150 ml/kg/day liquid volume and daily protein requirements can be as high as 3 g/kg/day. Human milk as well as formulas available on the market have a measurable level of calorie, lipid, glucose and protein concentrations.

The evolution of newborns with heart abnormalities associated with patent ductus arteriosus (PDA) is burdened by a high risk of necrotizing enterocolitis (NEC), due to de-creased intestinal blood flow, especially during diastole (Cognata et al., 2019). Secondary measures of prophylaxis of NEC in this category of patients are based on the initiation of parenteral nutrition, in a sufficient volume to ensure the support of the maturation process of the intestinal mucosa, without a high risk of energy waste. It is considered that 10-20 ml / kg / day is a sufficient amount for enteral administration (Martini et al., 2021). Concen-trated feeding formula, with fortified human milk or high calorie formula is a way to de-liver more calories and nutrients when fluid intake volume is limited (Pillai et al., 2018). Breast milk is considered the ideal source of nutrition for all infants, providing important quantities of immunoglobulin A, proteins and amino acids and by doing so lowering the risk of respiratory and autoimmune diseases (Tsintoni et al., 2020). Human milk has also been proved to reduce the chances of NEC and other gastrointestinal malfunctions (Specht et al., 2020; Tsintoni et al., 2020) as long as the mother meets optimal daily nutritional values and is neither malnourished nor overweight, otherwise alimentation should be en-riched with vitamins (Tarca et al., 2021).

Research shows that breastfeeding can be an option for CHD infants, albeit persistent concerns regarding the energy expenditure, which traditionally was considered to be greater than bottle feeding (Hollowell, 2015; Martini et al., 2021). The frequency and dura-tion of feeding as well as the preparation method must be assessed to avoid complications that may arise from

incorrect preparation. Both over-dilution and excessive concentration can result in poor growth, electrolyte imbalance and gastrointestinal impairment (Herridge et al., 2021).

Feeding and crying are activities that require energy consumption. Feeding sessions are the only ones that can be controlled in terms of running time, the current recommenda-tions being to limit them to a maximum of 30 minutes / feeding episode (Vichayavilas et al., 2014).

In preterm babies, gavage feeding via nasal or oral tubes, using intermittent bolus feeding (iBF) or continuous feeding (CF) techniques is the best option to ensure enteral administration of food. Widespread infusion methods for preterm neonates are established (Goswami & Alshaikh, 2017):

1) iBF (10-20 minutes infusion every 2-3 hours),

2) slow infusion intermittent feeding (30-120 minutes infusion every 2-3 hours),

3) CF (continuous infusion over 24 hours),

4) Semicontinuous feeding (feeding every 15 minutes throughout the day with one-fourth of the hourly feed volume)

In choosing the appropriate infusion type, one must acknowledge the maturity of en-teric neuroregulation. The migrating motor complex in term infants follows the classical 3 phased pattern (Berseth, 1996), whereas in preterm infants, phase 3, which is supposed to ensure the passage of the intestinal bolus has low-amplitude non-propagating pressure waves (Goswami & Alshaikh, 2017). Slow infusion of food in preterm infants results in postprandial response similar to that of term infants and helps with the maturation of the neuromuscular function. The characteristics of fasting motor activity mature within 10 days to resemble that of term infants if enteral feeding is commenced, irrespective of the gestational age (GA) at birth (Goswami & Alshaikh, 2017).

The hemodynamic aspects of splanchnic circulation are also critical in choosing the best feeding method. CF ensures a constant blood flow in the mesenteric circulation, but over time the circulation via the superior mesenteric artery (SMA) becomes unable to adapt to higher demands that come with higher feeding volumes or concentration (Bozzetti et al., 2012). iBF ensures a postprandial response similar to adults and is therefore more physiological.

Gut endocrine function and the balance between anabolism/catabolism differs in de-pendence of the feeding regime chosen. Insulin and amino acids concentration increases in feeding-induced cycles, CF ensuring a lower stimulation than iBF. Muscle protein syn-thesis is two times lower in CF compared to iBF (Wang et al., 2020). Continuous protein supplementation rather inhibits protein synthesis and induces AAs catabolism. If, however, CF is required, especially in neonates and infants who are scheduled for a blood transfusion, offering a supplement of leucine can boost the AAs synthesis. CF is more en-ergy efficient, and diminishes behavioral stress, albeit inhibiting protein gain and nega-tively interfering with gut paracrine function. Therefore, iBF is the better option, and slow infusion intermittent feeding with fasting intervals encourages better gastric emptying and paracrine function, while leading to calcium and lipids loss, more so than gravity feeding (Goswami & Alshaikh, 2017; Mann et al., 2021; Wang et al., 2020).

In gavage-fed newborns, monitoring the gastric residue before each feeding is not necessary (Tume et al., 2017). Some studies suggest that a gastric residue above 25% of the volume of the previously administered meal determines the need to deduct an equal volume from the amount of milk to be administered. However, ESPNIC challenges the inaccurate measurement of GRV, and the level of acceptability for the gastric residue and ad-vises against the routine measurement of GRV (Tume et al., 2021).

Gavage feeding may also be initiated in ill term neonates that can not be breast or bot-tle fed but have a functional digestive system. Monitoring the effectiveness of enteral feeding involves daily weight gain evaluation, aspect and frequency of stools, diuresis and weekly measurements of height and head circumference.

General principles of parenteral feeding

Parenteral feeding meets the nutritional needs by administering nutrients via venous pathways. Parenteral nutrition can be total or partial, depending on the newborn's ability to tolerate enteral feeding. The venous routes can be peripheral or central, and choosing them means taking into account the complications that may occur depending on the type of venous approach.

The IV fluid rate for hospitalized children follows the 4:2:1 rule- 4 ml / kg / h for the first 10 kg of weight, 2 ml / kg / h for the next 10 kg and 1 ml / kg / h for every kg past 20 kilograms. Fluid intake in term infants is 60–70 mL / kg on day 1 and increases to 100–120 mL / kg by days 2 or 3. Premature infants may receive 70–80 mL / kg on day 1 and slowly advance to 150 mL / kg / day (Andropoulos & Gregory, 2020).

Choosing the right IV fluid is paramount. The neonate renal function is unable to balance sodium secretion/excretion. Therefore, IV fluids must contain sodium. Consider-ing the normal range of plasma osmolality as 275-290 mOsm/kg, normal saline solution (0,9% NaCl) is slightly hypertonic and Lactate-Ringer solution is isotonic and hypo-natremic. Hypotonic solutions should not be used, due to their risk of inducing hypo-natremia. According to The European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) electrolytes can be administered in infants under 5 kg from day 1, depending on the blood levels (see Table IV.3. for dosage recommendations regarding electrolytes). When calculating the total caloric intake, macro and micronutrients kcal val-ues per gram should be taken into account. 1 g of protein provides 4 kcal and the amount of protein should provide 10-15% of daily calories.1 g of lipids provides 9 kcal and lipids should account for about 30-35% of total daily calories.1 g of glucose provides 4 kcal and the amount of carbohydrates should provide 60-65% of daily calories (Andropoulos & Gregory, 2020).

Table IV.3. Electrolytes requirements according to ESPGHAN.

	Infants under 5 kg (values in mmol/kg/day)				Infants 5-10 kg (mmol/kg/day)	Children >10 kg (mmol/kg/day)
	Day 1	Day 2-3	Day 4-7	Past Day 7		
Sodium	0-2	1-3	2-3	2-3	2-3	1-3
Potassium	0-3	2-3	2-3	1,5-3	1-3	1-3
Calcium	0,8-1,5	0,8-1,5	0,8-1,5		0,5	0,25-0,4
Chloride	0-3	2-5	2-5	2-3	2-4	2-4

The total fluids and nutrients for the ill newborns who require total or partial paren-teral nutrition are calculated using birth weight in the first 3 days of life, then daily weight. The amount of fluid received by enteral feeding or other administered fluids (eg fluids used to dilute medication, blood products) should be deducted from the total fluid calcuated for 24 hours.

Fluid intake should be increased by 10-20 ml / kgc / day and reach a maximum 150 ml / kgc / day during the first week, provided that no significant fluid losses occur.

The infusion rate of the glucose solution should initially be 2.5-5 mg / kgc / min and it can be increased daily by 1-2 mg/kgc/min to a maximum of 12 mg/kgc min, while monitoring blood glucose levels. Peripheral vein acces is suitable for glucose solutions of 12,5%, whereas a central vein access allows administration of 25% glucose solutions.

The minimum amount of protein to avoid a negative nitrogen balance is 1.5 g/kg/day, and the maximum intake of amino acids should not exceed 3 g/kg/day. The amount of amino acids should be reduced to 1.5 g/kg/day in situations requiring fluid restriction, renal impairment, acidosis, hyperammonemia and hepatic impairment asso-ciated with prolonged parenteral nutrition (van Goudoever et al., 2018).

The nitrogen ratio, calculated according to the formula [RCN = non-protein calories (glucose + lipids) / 0.16 x protein (g)] ensures adequate caloric intake for the amount of protein administered. Values above 250 indicate that protein intake may be increased as needed. Values below 150 indicate the need to increase non-protein calories or decrease protein intake (Bolisetty et al., 2020)..

Lipid administration should be initiated from day 1-2 of life, starting with 0.5-1 g / kgc / day, increasing the amount administered by 0.5 g / kgc / day to a maximum of 3 g / kgc / day. Lipid emulsions are calculated from the total volume of liquids. The lipid solu-tion is infused undiluted for 18-24 hours at a rate of 0.5-1.5 ml / hour. The lipid emulsion is administered on a separate venous line or in a triple route with a brown connection (Bolisetty et al., 2020).

The syringes and tubing used for parenteral administration of lipids must be protect-ed from light, because under the action of light and especially phototherapy, lipids can peroxide, generating toxic compounds that can damage tissues.

Parenteral administration of lipids is contraindicated in patients with severe sepsis, severe pulmonary disease, high pulmonary vascular resistance, high bilirubinaemia, that indicates the necessity for exsanguination transfusion (Vlaardingerbroek et al., 2013).

All of the above mentioned enteral and parenteral optimal nutrients for newborns and neonates as well as the special considerations can be visualized in Table IV.4 .

Special considerations in patients with congenital heart disease

Patients with CHD often exhibit failure to thrive and poor nutrition. Hypermetabolic state, swallowing difficulties, upper respiratory tract infections, gastroesophageal reflux (GERD), malabsorption and genetic syndromes are important etiological factors (Arslan, 2019). The hormonal imbalance caused by significant stress together with a metabolic shift toward fatty-acid oxidation and poor carbohydrate use lead to impaired use of nutri-tional resources (Karpen, 2016).

The European Society of Paediatric and Neonatal Intensive Care opines in favor of starting enteral nutrition within 24 hours from admission (Martini et al., 2021), as long as gastro-intestinal anomalies, vomiting, diarrhea, NEC or lactic acidosis are not present. Further-more, introducing EN in neonates and infants on mechanical and/or pharmaceutical he-modynamic support was associated with lower mortality, as research showed that that EN improves gut paracrine function, did not alter intestinal barrier, or increase risk of sepsis. These results apply also to older children. For neonates and children who are stable on vasoactive drugs and/or after cardiac surgery, early EN is also recommended. There is still much debate on whether trophic enteral nutrition (TF) is useful in preventing intestinal tract complications, especially after hypoplastic left heart syndrome (HLHS) corrective procedures. Recent studies showed that TF may improve the clinical outcome of these patients by shortening mechanical ventilation periods and allowing earlier enteral feeding. Patients with ductal dependent-CHD develop systemic desaturation, decreased abdominal blood flow associated with hypoperfusion during diastole, all of which lead to mesenteric ischemia and a theoretical higher risk for NEC. However, Becker et al, argue that, according to their cohort study results, NEC is not significantly associated with pre-operative enteral feeding in patients with ductal-dependent CHD, naming only single- ventricle heart defects, mainly HLHS, as a factor linked to higher percentage of NEC. Mortality rates were higher in patients with NEC, and amongst

them, 7 out of 10 were premature neonates. Neonates should receive EN while being monitored for systemic and gut perfusion abnormalities (Becker et al., 2015).

Table IV.4. Optimal nutrition parameters for term ill newborns and preterm neonates with CHDs by means of enteral and parenteral feeding

	Enteral feeding	Parenteral feeding
Term ill newborn	**Concentrated formula or breast milk**	**IV fluid**
	Start 40-60 kcal/kg/day increase to 90-120 kcal/kg/day	60-70 mL/kg on day 1 increases to 100-120 mL/kg by day 2 or 3
	Carbohydrates 9-14 g/kg/day (40-50% of total calorie intake)	4:2:1 rule
	Proteins 1.8-2.2 g/kg/day (7-16%)	4 mL/kg/h for the first 10 kg weight 2 mL/kg/h for the next 10 kg 1 mL/kg/h past 20 kg
	Lipids 4-6 g/kg/day (34-35%)	
Preterm	**Gavage feeding (nasal/oral)**	**IV fluid** – 70-80 mL/kg on day 1 and slowly advance to 150 mL/kg/day
	iBF (10-20 min infusion every 2-3 hours)	Must contain sodium, but avoid hypotonic solutions
	Slow infusion intermittent feeding (30-120 min every 2-3 hours)	Electrolytes (Table 1)
	Continuous infusion over 24h	Proteins – 10-15% of the total calorie intake (1g protein=4 kcal)
	Semicontinuous feeding (every 15 min throughout the day with ¼ of the hourly volume)	Lipids – 30-35% (1g lipids=9kcal)
		Carbohydrates – 60-65% (1g glucose=4 kcal)
Special considerations	Start within 24 hours from admission as long as no gastrointestinal anomalies, vomiting, diarrhea, NEC or lactic acidosis are present	Glucose – 2.5 mg/kg/min (3.6 g/kg/day) in the acute phase -5.0 mg/kg/min (7.2 g/kg/day) in the recovery phase 10% glucose solutions are preferred
	Formulas rich in protein and energy, but the osmotic load should not exceed 450 mOsm/kg water	Proteins – 1.5 g/kg/day for infants and 0.8 g/kg/day for children -in critical CHD – 2-3 g/kg/day for ages 0-2 years, 1.5-2 for ages 2-13 years, 1.5 for 13-18 years old
		Lipids – 0.5 mg/kg/day intralipid is enough to prevent lipid deficiency -should not exceed 3g/kg/day
		Pharmaconutrients – Zinc and vitamin D should be adm whenever a deficiency is documented
		Electrolytes (Table 1)

Intraoperative fluid requirements in children, depending on the type of surgery, rang-es between 1 ml/kg/h and 15 mL/kg/h, and premature babies may receive up to 50 mL/kg/h. A general rule states that for every ml of blood lost during surgery, 1,5 ml of isotonic crystalloid solution should be infused (Argent et al., 2017).

During the first 12h after surgery, fluid IV intake falls under de 2:1:0,5 rule, that is 2 mL / kg / h for children weighing up to 10 kg, 1 mL / kg / h for the next 10 kg and 0,5 kg / h for every kg exceeding 20, using isotonic fluids (Mathew & Rai, 2021). If enteral nutrition can not be initiated after 12 hours postoperatively, then hypertonic fluids should be ad-ministered according to the 4:2:1 rule.

Regarding specific nutritional parameters, ESPNIC recommends that energy intake in ill patients during the acute phase should not exceed the resting energy expenditure. Resting energy expenditure (REE) in these patients is significantly increased and negative-ly impacts cardiac output and inflammatory responses. In ICUs, indirect calorimetry can be used to assess the energy needs. The formulas used are: REE (kcal/day)=[(VO2 x 3.94) + (VCO2 x1.11)] x1,440 min / day and the respiratory quotient (RQ) = VCO2/VO2 ; RQ is nor-mally within the range of 0,67-1,3. Eligible for applying these measurements are patients with weight <5 percentile or > 85 percentile for age, those with > 10% variation in weight during ICU stay or patients who can not be weaned from respiratory support (Justice et al., 2018). If indirect calorimetry can not be used, then the Schofield equation for age and gen-der, using weight, proved to be the least inaccurate in determining REE, and is therefore a good substitute (Veldscholte et al., 2020). After the acute phase, energy intake should be based on REE, physical activity, rehabilitation, and growth.

Glucose intake

The balance between hyper and hypoglycemia in critically ill patients with CHD is difficult to maintain. The risk for hypoglycemia is higher in neonates, children with endo-crinopathies and those over-feed, as well as in patients who undergo a certain period of fasting before general anesthesia. Mild hypoglycemia combined with hypoxia and/or is-chemia determines severe neurological impairment, measurable up to 18 months of fol-low-up, and increases the chances of a negative outcome for patients in the Pediatric In-tensive Care Unit. Hyperglycemia, on the other hand, resulting from an impaired glucose metabolism, leads to cellular death, electrolyte imbalance and neurological impairment. In the acute phase, endogenous glucose production and a certain level of insulin re-sistance cover most of the glucose requirements, tilting the scales towards hyperglycemia. During the recovery phase, an equilibrium installs, allowing for more glucose to be ad-ministered. A parenteral glucose intake of 2.5 mg / kg / min (3.6 g / kg / day) in the acute phase and 5.0 mg / kg / min (7.2 g / kg / day) in the recovery phase is recommended, 10% glucose solutions being preferred (Joosten et al., 2019).

Protein requirements

A protein intake which avoids a negative protein balance is standard for healthy pa-tients. However, those with CHD exhibit a higher degree of protein breakdown concomi-tant with a higher positive protein balance achieved by up to 3.1 g/kg/day protein intake. In direct dependence of protein intakes, endogenous glucose and lipolysis levels increased in these patients. Administering 1.5 g / kg / day for infants and 0.8 g / kg / day in children seems to be the best approach for these cases. Higher levels do not produce a positive out-come mostly due to anabolic resistance, according to ESPNIC.

The American Society for Parenteral and Enteral Nutrition guidelines offer a different view. Suggestions for protein supplementations in critical CHD's are : 2–3 g / kg / day for ages 0-2 years, 1.5–2 for ages 2-13 years and 1.5 for those between 13–18 years of age (Herridge et al., 2021; Zhang et al., 2019).

Lipid intake

Lipid intake should not exceed 3 g / kg / day and doses should be modified by moni-toring triglycerides levels. Composite lipids emulsions could be a better option than other available solutions, particularly pure soy oil lipid solutions, due to their antioxidative ef-fects, ability to

improve cholestasis and liver dysfunction. Intralipid, a 20% IV fat emul-sion along with parenteral nutrition allows reaching the daily caloric requirement, while maintaining an adequate osmotic load. 0.5 mg / kg / day intralipid is enough to prevent lipid deficiency (Lapillonne et al., 2018).

Formulas

Most children with CHD have a diminished feeding capacity and require fluid re-striction. A formula rich in protein and energy should be used to provide larger amounts of nutrients, while balancing the osmotic load carefully to prevent osmotic diarrhea. In that regard, the osmotic load of formulas should not exceed 450 mOsm/kg water. Hydro-lyzed peptide formulations, whey and soya protein hydrolysates can be used in disaccha-rides or whole protein intolerance (Andropoulos & Gregory, 2020; Steele et al., 2013).

Pharmaconutrients

Supplementation of vitamin C, Zinc and selenium arginine, glutamine, omega 3 fatty acids were not found to significantly impact secondary infections, duration of mechanical ventilation, length of stay or mortality rates. However, zinc and vitamin D should be ad-ministered whenever a deficiency is documented (Shaw, 2020).

Electrolytes

Patients with CHD usually require pharmacological intervention in order to properly manage renal function, hypertension, and cardiac failure. Diuretics modify the electrolytes profile in these patients and therefore careful monitoring is required. Other than that, the dosage recommendations follow the aforementioned guidelines for age and weight (Table IV.4.).

In the United Kingdom, a study employing Pediatric Dietitians from all the pediatric cardiology surgery centers and using a Delphi process for a consensus based nutritional guidelines, developed 3 possible nutrition plans, depending on the nutrition risk, infant's growth and digestive tolerance. Plan A (lower nutritional risk) allows for normal energy and protein requirements, similar to those of a healthy child and a non-restrictive fluid in-take, plan B involves 10% extra energy requirements, 30-50% increase in protein intake, while plan C (high nutritional risk) allows up to 20% extra energy requirements and 50% increase in protein intake. Starting with 17-26 weeks of life, all plans involve adding com-plementary food, based on protein rich meals, ½-1 teaspoon of a nut butter or finely ground nuts (plan B and C) and vitamin D supplementation (Marino et al., 2018; Shaw, 2020).

Depending on the CHD, each plan is more likely to be followed. Patients with small septal defects, total anomalous pulmonary drainage or coarctation of the aorta are more likely to benefit from plan A, while those with pulmonary or tricuspid atresia, prostaglan-dins-dependent lesion , tetralogy of Fallot, severe septal defects, HLHS, Ebstein' anomaly or double outlet right ventricle are considered candidates for plan C.

IV.3.4.Discussions

Most guidelines and protocols for proper nutrition of newborns with congenital heart defects are based on observational or retrospective studies, which have the limitation of non-standard criteria for assessing the needs of each case, therefore posing a risk of initiat-ing inappropriate nutrition due to overestimation or underestimation of a CHD.

Newborns with congenital heart abnormalities have difficulty initiating and sustain-ing an efficient enteral diet, both due to a waste of energy and to neurological, motor, gas-trointestinal, endocrine and renal developmental problems that occur in association with complex cardiac malformations.

Fluid requirements are particularly difficult to assess, especially in cases where vaso-active and diuretic agents are required. It is necessary to maintain a balance between the minimum enteral

diet that has proven its benefits in preventing gastrointestinal complica-tions both pre and postoperatively and parenteral nutrition, which must take into account the daily fluid requirement and electrolyte balance, while also avoiding fluid overload in the context of a malfunctioning heart.

Parenteral nutrition in newborn and infants can prove to be a major source of oxidants (Karthigesu et al., 2021) several studies suggesting higher arginine and cysteine in-take, along with decreased iron and copper concentrations might help decrease the oxi-dant load. The range of products used for parenteral nutrition must be strictly controlled in terms of storage conditions and period, exposure to the sun and oxygen, and the tem-perature of administration. The consumables used can also influence the chemical interactions, leading to the accumulation of oxidants, hepatotoxic and carcinogenic molecules in neonates. For instance, Loff et al reported a spike in DEHP concentration in lipid solu-tions from 0.06 µg / mL to 2 µg / mL when using venous catheter made from PVC–DEHP (Loff et al., 2008).

Macronutrients, vitamins, and electrolytes administration require great care and a thorough understanding of the general rules of neonate physiology and the pathogenesis of cardiac defects.

IV.3.5 Conclusions

Adequate feeding protocols tailored to meet the requirements of patients with congen-ital heart defects help improve short and long term outcomes. Nutritional and metabolic changes are age-dependent and since this is a high-risk population, guiding enteral and/or parenteral feeding is difficult. Sources of increased metabolic demand in CHD in-clude increased REE, higher cardiac workload, pulmonary hypertension and increased catecholamine secretion. Infants and children that receive diuretic therapy experience losses of electrolytes and minerals. Hyponatremia, hypochloremia and metabolic alkalo-sis are common disturbances that lead to anorexia, poor weight gain, and impaired wound healing. The available scientific literature and guidelines, if applied to the letter, may help improve nutritional status and outcomes in patients with CHD. The current best practices both in Europe and America have been summarized in this article, albeit miss-ing information regarding the management of complications that may arise in conjunc-ture with heart defects pre and postoperatively.

CHAPTER V. CARDIAC INFECTIOUS DISEASES

Introduction

Infectious diseases are a challenge for practitioners, even in the age of antibiotics. MDR microorganisms especially, pose a threat in matters of therapeutic management and are the main cause for on going research regarding novel antibiotic agents. Whatever its location, an infectious process may prove difficult to manage, but infections of the heart are particularly troublesome, mainly because systemic drug delivery hampers their effect. Targeted antibiotic treatment is a new exciting aria of research.

Myocarditis has been described during and after a variety of viral diseases (Coxakie virus,cytomegalovirus, HIV, adenovirus, arbovirus, echovirus, Ebstein-Barr virus, influenza virus, syncytial virus respiratory, poliomyelitis, rubella, measles), bacterial (Streptococcus, Staphylococcus, Pneumococcus, Meningococcus, Haemophillus, Gonococcus, Brucella, Salmonella, Koch's bacillus, Diphtheria bacillus), spirochetes(leptosis, Lyme disease, syphilis), fungal (aspergillosis, actinomycosis, blastomycosis, candidiasis, cryptococcosis, histoplasmosis),

parasitic (cysticercosis, toxoplasmosis, schistosomiasis, trichinosis,visceral migrating larvae), ricketsian (typhoid fever, Q fever), or autoimmune processes. Autoimmune myocarditis occurs by hyperstimulation of the immune system, with an abnormal relationship between helper T lymphocytes and natural killer lymphocytes, the abnormal expression of the molecules of the majorhistocompatibility in cardiac tissue and the formation of circulating autoantibodies, which lead to necrosis or damage of the myocyte (Luca A, 2022).

Pericarditis is the consequence of acute or chronic inflammatory processes located in the pericardium. It can be idiopathic or caused by a multitude of factors, including infectious agents, drugs, neoplasia, or connective tissue diseases (Luca A, 2011). The normal volume of liquid between the pericardium layers in children is 10 cm^3. The fluid build-up leads to increased cardiac chamber pressure which in turn causes decreased cardiac output, hypotension, tachycardia and peripheral vasoconstriction. Cardiac tamponade is an acute compression of the heart by rapid liquid accumulation and / or a large amount of fluid in the non-extensible pericardial sac. . The effect is more pronounced on the right heart (especially right atrium): diastolic collapse of right chambers with blood flowing back into the systemic circulation. The paradoxical pulse involves a decrease in systolic blood pressure during inspiration by 10 mmHg and a decrease or even the disappearance of the pulse in inspiration (Luca A, 2011)

Infectious endocarditis is a redutable foe, even more so in children with complex CHD that associates anemia, immunological impairment and other organ injury. In these cases, promptly identifying the pathogen and choosing the appropriate antibiotic, while having the best delivery system literally saves lives.

V.1. Infectious endocarditis

V.1.1.Background

Infectious endocarditis (IE) is an inflammatory process consisting of colonization and invasion of the endocardium by a pathogenic microorganism, causing the formation of vegetation. Infection most frequently affects the heart valves (native or prosthetic),but it may also occur in the reduced pressure section of the ventricular septum (site of a defect), in areas of the endocardium damaged by abnormal blood jets or foreign bodies, or on intracardiac devices (Ginghina 2017).

The analogous process that affects arteriovenous shunts,arterioarterial shunts (patent arterial duct) or areas of aortic coarctation is called infective endarteritis (Loscalzo, 2011). The clinical picture of IE includes a wide range of symptoms, which are mostly due to: virulence of the etiological microorganism, persistence of bacteremia, extent of tissue damage and hemodynamic consequences of the resulting valvulopathies, perivalvular extension of the infection, septic pulmonary and systemic circulation embolisms, and consequences of circulating immune complexes (Garcia, 2007). Due to the complexity of this condition and its high morbidity and mortality rates, it is imperative to quickly set the diagnosis, begin effective treatment and recognize possible complications. The diagnosis of IE is based on the presence of positive blood cultures and evidence of the presence of intracardiac vegetation.

Medical imaging methods, mainly echocardiography, play a key role in both the diagnosis and follow-up of patients with IE. The usefulness of echocardiography may be extended, so it has become the method of choice for evaluating the prognosis of IE patients, for their follow-up antibiotic therapy, intra- and postoperatively. There are three major echocardiographic criteria for the positive diagnosis of IE: vegetation, abscess and new dehiscence of a prosthetic valve. However, the new guidelines recommend the inclusion of other medical imaging techniques:

multi-slice computed tomography (MSCT), magnetic resonance imaging (MRI) and positron emission computed tomography (PET-CT) with 18F-fluorodeoxyglucose.

Another basic pillar in setting an IE diagnosis is microbiological diagnosis. Blood cultures are positive in about 85% of all IE cases, except for blood culture-negative IE (BCNIE) which is generally caused by prior antibiotic therapy. In these cases, antibiotic therapy must be discontinued, and blood cultures must be repeated. Such cases require serological testing, immunological techniques, molecular biology or histological techniques.

Duke criteria based on clinical, echocardiographic and microbiological data are used in current practice. Despite the usefulness of these criteria, they should not replace clinical judgment. Corroboration with the findings of additional medical imaging techniques, such as MSCT, MRI and PET-CT, is useful in detecting silent vascular phenomena and endocardial lesions, and contribute to improving the sensitivity of Duke criteria. Thus, the ESC Guidelines Committee (2015) suggested the implementation of three new diagnosis criteria (Habib et al. 2015):

1. Identification of paravalvular lesions by heart CT (major criterion).

2. In case of suspicion of valve prosthesis IE, detection of abnormal activity at the prosthesis implantation site (only if the prosthesis was implanted more than 3 months before) by 18F-FDG PET/CT or SPECT/CT with radio-traced leukocytes (major criterion).

3. Identification of recent embolic events or infectious aneurysms only by medical imaging techniques (silent events) (minor criterion).

My interest in this subject, as well as part of my experience in treating infectious endocarditis are summarized in the articles listed below:

1. **Luca**, **A.C.**; Curpan, A.S.; Adumitrachioaiei, H.; Ciobanu, I.; Manea, R.S.; Vlad, E.; Surguci-Copaceanu, A. Difficulties in diagnosis and therapy of infective endocarditis in children and adolescents - case series, *Healthcare*, 2021, pp.1-9, **IF 2.645**.
2. **Luca**, **A.C.**; Begezsan (Loghin), I.I.; Iordache, C. Particuliarities in diagnostic and treatment for infectious endocarditis in children, *Rev. Med. Chir. Soc. Med. Nat,* 2012; 116(4), pp.1028-1032.
3. **Luca, A.C.**; Iordache, C. Infectious endocarditis with five localisations- case reports, *Rev. Med. Chir. Soc. Med. Nat,* 2014; 118 (1), pp.81-86.

Also, the knowledge acquired in the management of heart infections served as a basis for writing the relevant chapters on this topic in the BDI papers mentioned below.

1**.** Luca, A.C.; Iordache, C. Pediatric Book, Gr.T.Popa, 2011
2**.** Luca, A.C.; Paduret, I.A. Miocarditis in Essentials in Pediatric Cardiology, Cinteza E, Nicolescu A, Editura Medicala, 2022

V.1.2. Material and methods

We conducted a retrospective case study in the Pediatric Cardiology Department of 'St. Mary' Clinical and Emergency Hospital for Children in Iasi between February 2007 and February 2020, a period in which we evaluated a total of 45,258 pediatric patients, from which we included the 54 children (33 males vs. 21 females) diagnosed with infectious endocarditis, giving an prevalence of 0.11% in our hospital. The patients were chosen based on the European Cardiology

Society guidelines following Duke inclusion criteria. The present study was conducted according to Romanian research law no. 206/27.05.2004 as well as the European laws. The parents and children were informed about the study, what it involved and what information was going to be used, and approval from the Ethics Committee was obtained with the registration number 14,355/14.05.2021.

We analyzed the epidemiological data, the main clinical and paraclinical manifestations, their incidence in the surveyed pediatric population, the main complications and therapeutic approaches in order to reach the final results and draw the final conclusions.

V.1.3. Results

In the case of our study, we had a higher prevalence of male patients (33 males vs. 21 females) and of patients from rural areas (32 rural vs. 22 urban). The clinical picture of patients during their hospitalization was: heart murmurs (100%), followed by fever (83.33%), fatigue (61.11%), reported loss of appetite (57.40%) and dyspnea (51.85%). Other clinical manifestations presented were weight loss (46.29%) and skin manifestations such as petechiae, Janeway lesions and Roth spots (44.44%), followed by cough (20.37%), headache (14.81%), vertigo (12.96%) and myalgias (9.25%) to a lesser extent, as shown in Fig. V.1.

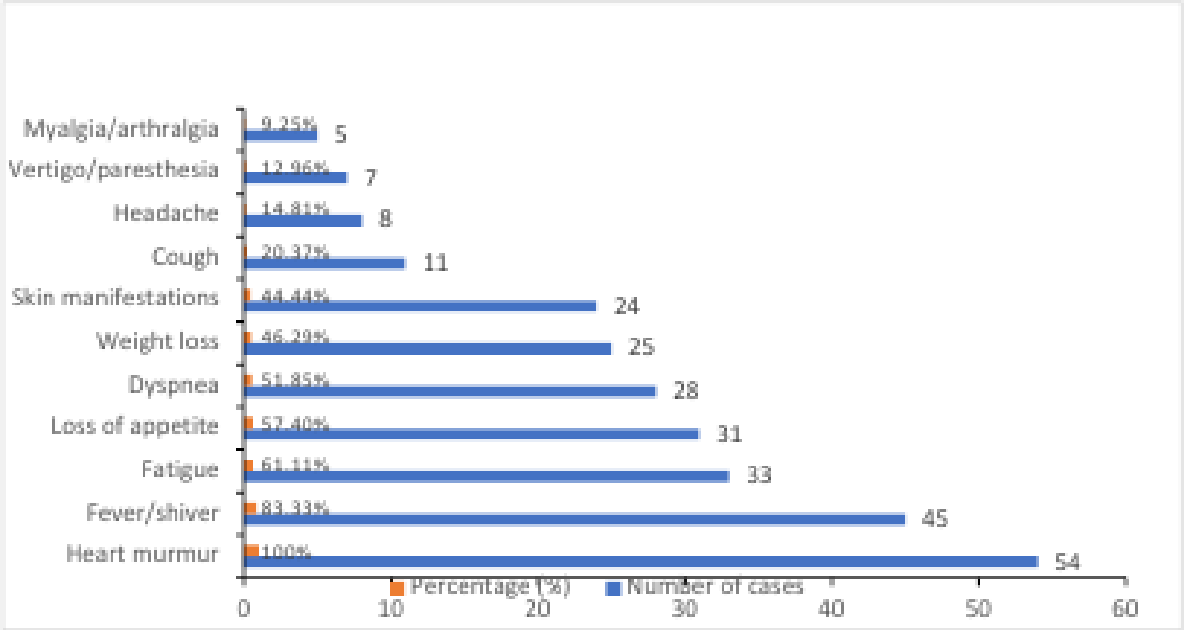


Figure V.1. Clinical manifestations at admission

In about 85% (46 cases) of patients, infectious endocarditis was secondary to a preexisting heart condition, especially valve damage (mainly mitral and aortic valve injury) and congenital heart malformations (more commonly ventricular septal defect, atrial septal defect, patent ductus arteriosus, coarctation of the aorta). All of our patients were examined by TTE, which revealed major criteria for diagnosis of IE. The dimensions of the vegetations identified through echocardiography varied (Table V.1.). In terms of injury localization, the mitral valve (33.33%)

and the aortic valve (29.62%) were most commonly impaired, followed by the tricuspid valve (16.66%) and the pulmonary valve.

Table V.1. The dimensions of the identified vegetations in association with incidence, both in number and percentage, in our studied cases

Dimension (cm)	Number of Cases	Percentage (%)
1–4.9	32	58.82
5–9.9	16	29.41
>10	6	11.76

Besides the abovementioned clinical manifestations, we also observed a series of several complications during the progression of IE, the most common of which were heart failure (51.85%) and valve regurgitation (48.14%), followed by thromboembolic conditions (27.77%), and, with a lower incidence, pericarditis and conduction disorders, which occurred in 10 of the total complicated cases (18.51%) (Fig. V.2.).

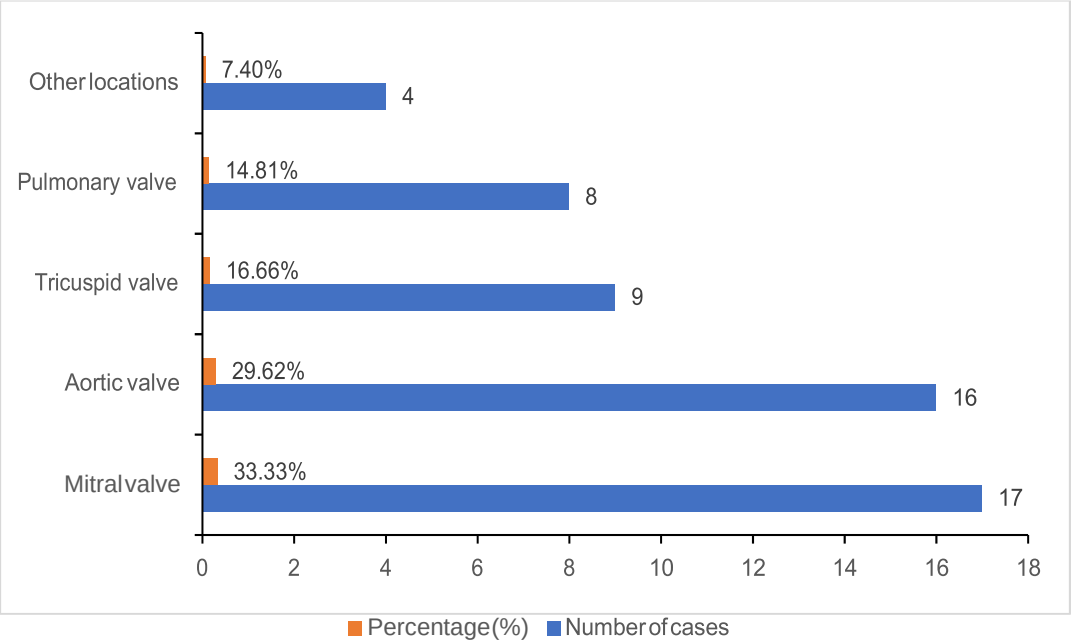


Figure V.2. Infective endocarditis complications illustrated in percentages and the corresponding number of cases

We used blood samples to obtain blood cultures. Blood samples were acquired using BD BACTEC Peds plus/F vials. For newborns, nurslings and children up to 2 years old, 1–3 mL of blood is recommended to be collected, and after this age the amount of collected blood increases dependent on the age of the patient. After blood collection, the vials were placed in BD BACTEC FX40 and incubated at 35.1 ◦C for 5 days. From the positive vials, we Gram-stained smears and examined them with an immersion microscope. Based on the microscope results, we sub-cultivated on adequate solid mediums: Columbia agar with sheep blood, chocolate blood agar, MacConkey agar and Sabouraud agar. We carried out the angiogram and the biochemical identification of the

strain using a MicroScan WalkAway 40 Plus System. The most common pathogen found was *Staphylococcus aureus*, followed by *Viridans Streptococci* and the HACEK group. The blood cultures were negative in nine patients, those that were on antibacterial treatment prior to admission. The therapeutic approach considered the antibiotic sensitivity of the identified microorganisms. For treatment, we used different combinations of antibiotics from the cephalosporin 3rd generation, aminoglycoside, carbapenem and glycopeptide classes. Therefore, the blood cultures became negative during the first week of antibacterial treatment in most patients. However, the therapy was continued until the intracardiac vegetation became sterile, in full agreement with international guidelines. Sterilization was deemed successful in all patients. Only one patient required surgical therapy, with a positive post-surgical evolution.

Patients were also reexamined by cardiological and echocardiographic means to assess therapy effectiveness and dynamic heart function. Due to their early diagnosis and prompt therapy, the evolution of the 48 patients included in the study (88.88%) was positive. Infectious endocarditis was fatal in six of the patients (11.11%), as they all had severe pre-existing heart conditions and one patient was diagnosed with terminal phase oncologic pathology.

V.1.4. Discussions

The clinical picture of IE includes a wide range of symptoms. A new heart murmur is common, especially in patients with IE complicated by heart failure. Splenomegaly is found in about 50% of patients. Neurological complications, such as embolic stroke, brain abscesses, fungal aneurysms and intracerebral hemorrhages, are late manifestations and are usually associated with staphylococcal infections. Myocardial abscesses are generally specific to staphylococcal infections and may cause conduction disorders, blockages or, when the injury occurs in the pericardium, purulent pericarditis. A number of classical peripheral signs are described, which are generally late manifestations: petechiae (most common in the conjunctiva, oral mucosa and extremities), Janeway lesions (erythematous or hemorrhagic maculae located on the palms or soles, sequelae of peripheral septic embolism associated *Staphylococcus aureus* IE), subungual hemorrhages, Osler nodules (painful, firm subcutaneous nodules in the fingers, the result of deposits of immune complexes) and Roth spots (retinal hemorrhage detected in 10–20% of cases)(Kliegman, 2016) some of which were also identified in our patients.

Viridans-type streptococci and *Staphylococcus aureus* remain the leading causative agents for endocarditis in pediatric patients (Kliegman, 2016) .Fungal endocarditis is rare both in children and adults. Although injected drug use was traditionally an important risk factor for Candida IE, care contact has now emerged as the primary risk factor for most patients with this infection. The overall cure rate in cases of fungal IE is poor (Bennet et al., 2020). The poor prognosis may be due to large, bulky vegetations; a tendency for fungal invasion of the myocardium; widespread systemic septic emboli; poor penetration of antifungal agents into the vegetation; low toxic-to-therapeutic ratio of the available antifungal agents and the usual lack of fungicidal activity with these compounds (Bennet et al., 2020). A cure is almost impossible without surgical intervention.

The 2015 European Guide to Infective Endocarditis Management highlights the major role of a multidisciplinary approach, involving a pediatric cardiologist, cardiovascular surgeon, infectious disease specialist, microbiologist and medical imaging specialist. The IE therapy principles are microbial eradication through the use of antibiotics; removal of infected material; drainage of abscesses and repair of valve damage/valve prosthesis (Bennet et al., 2020; Habib et al., 2015).

Extended antimicrobial therapy is the basis of IE treatment, and the treatment of IE on valve prostheses should last longer (at least 6 weeks), while the treatment of IE on native valves

should last 2–6 weeks. In both cases, treatment duration is determined by the first day on which antibiotic therapy is effective and not by the day of surgery. A new treatment plan should be started only if the valve cultures are positive, with the choice of antibiotic being based on the susceptibility of the last isolated bacteria (Habib et al., 2015)In some cases, antibacterial therapy should be supplemented with surgical treatment to eradicate the infection foci. The clinical signs that mark the necessity of surgery are congestive heart failure secondary to valvular dysfunction or prosthetic material dehiscence; growing vegetation and its embolism; perivalvular extension of myocardial abscess and complicated myocardial abscess with atrioventricular block.

A retrospective case study conducted in the USA between 2000 and 2010 on a group of 3840 children diagnosed with IE, with a pediatric population incidence of 0.43/100,000, more than half of whom were aged over 11 years, revealed that 30% of the cases had negative blood cultures. Staphylococcus species were the most common pathogen in the positive cultures, followed by Streptococcus species (Gupta et al., 2017). In the case of our study, only 16.66% of patients had negative blood cultures, but the most common pathogen was indeed Staphylococcus spp followed by Streptococcus spp.

Another large-scale study conducted in Norway at the Clinical Teaching Hospital for Congenital Heart Malformations between 1994 and 2016 showed a much higher incidence among patients with congenital heart disease, i.e., 2.2/10,000/year. Most of these patients (75%) had severe congenital heart diseases and had undergone open heart surgery during the last year prior to IE diagnosis setting (Jortveit et al.2018). In a retrospective study conducted in the USA between 2003 and 2014 in 29 centers on 841 children diagnosed with IE, Bates et al. studied the impact of the antibiotic prophylaxis guidelines suggested by the AHA in 2007. The study concluded that there was no significant decrease in the hospitalization rate of IE children after 2007, with the infection rate decreasing from 0.13 cases/10,000 hospitalizations for 6 months to 0.12 cases/10,000 for 6 months (Bates et al., 2016).

Life-threatening complications include stroke and intracerebral and subarachnoid hemorrhage. Thus, in a 13-year study conducted in China between January 2002 and December 2015 on 60 children with IE, the neurology department of Beijing Anzhen Hospital found a higher incidence of stroke in children with IE of the left half of the heart than in those with IE in the right half of the heart (32% compared to 2.8%). The most common manifestation of stroke was hemiparesis (55.5%), and the mortality rate was significantly higher in patients with stroke than in those who did not suffer any stroke (22.2% compared to 3.9%) (Cao & Bi, 2019).

The Italian Society for Pediatric Infectious Diseases conducted a retrospective study on 47 IE patients aged 2 to 17 years between 2000 and 2015 and concluded that the most common pathogen was *Streptococcus viridians* in patients with pre-existing heart disease, and *Staphylococcus aureus* in those without pre-existing heart disease (37.9% compared to 5.5%, and 6.9% compared to 27.8%), while 85.7% were methicillin-resistant S. aureus (Habib et al., 2015; Esposito et al., 2016).

Dixon and Chistov concluded that despite all the advances in its management, pediatric infective endocarditis still poses clinical problems. Improved and more sophisticated laboratory techniques, wider use of echocardiography and the use of new medical imaging methods allow for better diagnosis. However, the increasing number of intracardiac implants (the incidence of pulmonary valves of the bovine jugular vein implanted with a transcatheter is relatively high) leads to a change in predisposing factors and brings about new diagnosis and treatment challenges. The impact of changes in prophylaxis guidelines remains uncertain and there is still no evidence to support their effectiveness (Dixon & Chistov, 2017).

A retrospective study conducted at the Rawalpindi Institute of Cardiology between 1 January and 15 October 2017 on 120 pediatric patients with suspicions of IE showed that the incidence in male patients was higher than that in female patients (70% compared to 30%), while the mean age of the patients was 5.5 (1.7) years. Additionally, the most common complaints of patients were fever, shortness of breath, chest discomfort and cyanotic episodes. The most common underlying disease associated with endocarditis was CHD (52%) followed by rheumatic heart disease (32%). Blood cultures were positive in 32% of the patients, while in 68% of them blood cultures were negative.

Coagulase-negative Staphylococcus was the most common isolated organism (31.2%) in the research patients, followed by *Streptococcus viridians* (Xu et al., 2016). Our findings are in agreement with these observations, except the number of positive blood cultures (45 in our case) and the most common pathogen being coagulase-positive S. aureus. Another retrospective study conducted in Belgium at the Department of Pediatric Cardiology, KU Leuven, between 2000 and 2017, showed that the incidence of patients who needed heart surgery was 36%, with the mortality rate being 13% and 87% of the patients with congenital heart defects. The pathogenic factor was detected in 92% of the cases: Streptococcus viridians (32%), S. aureus (25%), coagulase-negative Staphylococci (20%) (Kelchtermans et al., 2019).

As far as medication is concerned, in 2016, Nichols et al. studied the recommendations of the updated IE guidelines published by the AHA in 2015 regarding certain antibiotics, such as: vancomycin, gentamicin and tobramycin, cefepime, piperacillin/tazobactam, nafcillin and penicillin G. Thus, they concluded that the doses listed here respected the vancomycin, aminoglycoside and β-lactam dosing and follow-up recommendations, and they were based primarily on experts' opinions and failed to take into account the available dose-optimization evidence based on pharmacokinetic and pharmacodynamic principles in children and adolescents. These findings are disconcerting, since, in a clinical setting, some practitioners may be reluctant to deviate from the doses recommended by the guidelines (Nichols et al., 2016).

Despite the positive results of our cases, our study is limited by a small sample size (54 cases), and imbalance between the numbers of each gender, but we believe that our wider age distribution (with the highest prevalence of IE in the 11–17-year-old patients) provides a good insight regarding treatment efficacy in different age groups and different backgrounds. However, the retrospective nature of our paper brings several limitations to our study, as this type of work is more prone to biases and missing information since the cases were first registered for clinical purposes and not research.

V.1.5. Conclusions

Infectious endocarditis is a severe condition that unfortunately also affects pediatric patients, in some cases even being fatal. The presence of pre-existing heart conditions such as congenital heart defects and valvular injuries is a major predisposing factor to infective endocarditis in children. Medical imaging diagnosis and blood cultures are particularly important for fast and efficient intervention, for successful therapy and for a positive outcome. Our study illustrated the importance of early diagnosis and therapy, with a successful recovery rate of 88.88% in our case, with only one patient needing surgery, and the most common causes were S. aureus, S. viridans and the HACEK group for IE. The lethality cause was severe congenital heart defects, which were observed in six of our initial patients. Future studies should focus more on establishing the optimum dosages for the antibacterial therapy as well as developing more personalized therapies based on the observed symptoms and complications of every patient in particular, as it might increase the chance of a favorable outcome.

V.2. Prevention of infectious cardiac diseases- age of antibiotics

V.2.1. Background

Valvular and non-valvular infections of the heart pose serious threats due to the high degree of mortality and morbidity they associate. In the antibiotic era, the prevalence of exitus is significantly lower than before, however, the morbidity, financial, social and personal burden caused by prolonged, invasive treatment and sometimes sequels, remain at high levels. Heart infections are more often caused by hematogenous or direct spread of infection from another site, although in patients with congenital heart defects, turbulent blood flow and damaged endocardium offer the propitious context for local colonization.

The most frequent culprits involved in bacterial cardiac infections are detailed in the chapter V.1. It is important to consider that hematogenous spread of a bacterial infection accounts for a significant number of cases, all of which could be prevent by appropriate antibiotic therapy and sterilization of the primary site of infection.

The personal contribution in understanding mechanisms of microbial resistance and finding new ways of delivering targeted antibiotic treatment is highlighted in the articles:

1. Ichim, D.L.; Duceac, L.D.; Marcu, C. ; Iordache, A.C.; Ciomaga, I.M.; **Luca, A.C.**; Goroftei, ERB; Mitrea, G; Damir, D.; Stafie, L. Synthesis and Characterization of Colistin Loaded Nanoparticles Used to Combat Multi-drug Resistant Microorganisms, *Journal of Chemistry*, 2019; 70(10), pp.3734-3737**; IF 1.755**
2. Duceac, L.D.; Marcu, C.; Ichim, D.L.; Ciomaga, I.M.; Tarca, E.; Iordache, A.C.; Ciuhodaru, M.I.; Florescu, L.; Tutunaru, D.; **Luca, A.C.;** Stafie, L. Antibiotic Molecules Involved in Increasing Microbial Resistance, *Journal of Chemistry*, 2019; 70(7), pp. 2622-2626 ; **IF 1.755**
3. Duceac, L.D.; Mitrea, G.; Banu, E.A.; Ciuhodaru, M.I.; Ciomaga, I.M.; Ichim, D.L.; Constantin, M ; **Luca, A.C.** Synthesis and Characterization of Carbapenem Based Nanohybrids as Antimicrobial Agents for Multidrug Resistant Bacteria, *Journal of Plastic Materials*, 2019; 56(2), pp. 388-391, **IF 1.517**
4. Duceac, L.D.; Banu, E.A.; Baciu, G.; Lupu, V.V.; Ciomaga, I.M.; Tarca, E.; Mitrea, G.; Ichim, D.L.; Damir, D.; Constantin, M.; **Luca, A.C.** Assessment of Bacteria Resistance According to Antibiotic Chemical Structure, *Journal of Chemistry*, 2019; 70(3), pp.906-908; **IF 1.755**
5. Duceac, L.D.; Tarca, E.; Ciuhodaru, M.I.; Tantu, M.M.; Goroftei, REB.; Banu, E.A.; Damir, D.; Glod, M.; **Luca, A.C.** Study on the Mechanism of Antibiotic Resistance, *Journal of Chemistry*, 2019; 70(1), pp 199-201; **IF 1.755**

V.2.2. Materials and methods

Mechanisms of microbial resistance: The major aim of this study was to investigate the connection between antibiotic chemical structure and the bacteria resistance to those types of drugs.

I. Purchased E.Coli bacterial suspension was prepared inoculating 500 µL of a glycerol stock in a total volume of 200µL of inoculation medium. Culture medium consisted of 5.5 g/L glucose, 2.5 g/L peptone and 1.25 g/L yeast extract in phosphate buffer was grown on a 1 L shake-flask incubated at 37°C under agitation. Cell were then harvested by centrifugation and suspended in broth for removing of all traces of medium and again harvested by centrifugation and suspended in broth for obtaining an inoculum containing about 1x107 cell/mL. Glass and silicone surfaces were prepared by washing and drying them for further determination. A β-lactam antibiotic,

ampicillin, was used in this study which acts by blocking a specific cross-linking step in the cell wall synthesis, this process creating weak bacterial cell walls inducing thus cell lysis.

II. We conducted a descriptive and retrospective study in the period 2012-2017, on a batch of 35 patients, admitted to the Saint Mary Emergency Clinical Hospital for Children of Iasi, from whom various pathological products were collected to highlight the *Enterobacter spp* strains involved in the production of infections associated with the inpatient medical care. The data were collected from the patients' observation sheets and from the statistical reports, and the results of the antibiograms performed from the existing reports, according to the hospital's microbiology laboratory. The antimicrobial sensitivity of each strain was determined by diffusimetric method, and the interpretation criteria were considered those of the laboratory standards. The data were statistically processed using the computer software SPSS v.22.0.

Targeted antibiotic treatment- The major goal of this work was to design antimicrobial nanohybrids by incorporation of antibiotics into LDHs nanomaterials thus enhancing antibacterial activity of these formulations.

I. Coprecipitation method was used to synthesize MgAlLDHs, where metal salts solutions were mixed together under vigorous stirring. The pH of the mixed solution was adjusted at the desired value of 9.5 by adding a soda solution to support simultaneous precipitation of cation hydroxides. The obtained solution was aged for 24 hours then filtered and dried at 60°C to obtain nanostructures with high crystallinity. Synthesis of drug delivery nanocomposites involves anion exchange and reconstruction method. For anion exchange route, the initial layered double hydroxides containing NO_3- as interlayer anions is added to a solution containing 1g amoxicillin and 0.2g clavulanic acid. Next step consist in maintaining the obtained solution under stirring for 20h at 50°C followed by filtration and drying at 55°C of final product. Reconstruction method refers to the memory effect of some LDHs nanomaterials when heated at 550°C form metal oxides that are able to reconstruct the pristine structure by exposing to an aqueous solution of amoxicillin and clavulanic acid. Following steps are same as described for the other mentioned method.

II. Imipenem loaded nanocapsules were obtained using ionic gelation method with minor improvements. Chitosan was dissolved in 1% acetic acid solution to an adjusted pH of 5.5 using a 6M NaOH solution subjected to sonication for 30 min. Tripolyphosphate (TPP) was dissolved in distilled water to an adjusted pH of 5.5 using 0.5M HCl, and then both solutions were filtered using a 0.22µL syringe filter before use. Antibiotic was incorporated into an aqueous solution containing TPP and over mixing different volumes of chitosan and imipenem-containing TPP solutions, nanoparticles were spontaneously formed at varied chitosan-TPP weight ratios of 9:1; 8:1; 7:1; 6:1; 5:1;4:1; and 3;1.

III. Ceftriaxone drug-based nanocomposite was prepared by intercalation of antibiotic into the interlayer gallery of layered double hydroxide by co-precipitation. Ceftriaxone, Mg(NO3)2•6H2O and Al(NO3)3•9H2O compounds in a molar ratio of 0,2:2:1 were dissolved in double distilled water. The pH of mixed solution was adjusted at 9.0 by adding a NaOH/Na2CO3 aqueous solution. Then, the solution was vigorously stirred for 20h and the obtained precipitate of Ceftriaxone/LDH nanohybrid was washed with double distilled water, filtered and dried for further structural and morphological analysis.

V.2.3. Results and Discussions

Mechanisms of microbial resistance

I. For quantification of *E.Coli* biofilm formation and antibiotic sensibility on glass and silicone surfaces was used epifluorescence microscopy. After 24 h of biofilm development silicone surfaces exhibits enhance biofilm formation compared to glass. Viable cells number remained constant

during the first 3 hours for both materials (Fig.V.3.) and then the viability of biofilms formed on glass markedly decreased and a 7-log reduction was achieved after 7.5 h of treatment.

E.Coli biofilm formed on silicone was more resistant to antibiotic than those formed on glass and had a reduction of 1-log in the amount of viable bacteria.

SEM images analyzed the morphological changes on the senssile cells exposed to antibiotic. The micrographs showed that the size of protrusions varies with features up to 10 µm and yet most of them exceed the *E.Coli* cells size (Fig.V.4.). Bacteria cells adhered to silicone appeared to be extracellular polymeric substances compared to the cells observed on the glass surfaces (Fig.V.4.A) which are distributed in aggregates or as individualized cells without adhesive material in their vicinity. After 6 h ampicillin exposure, the amount of *E.Coli* biofilm cells adhered to silicone and glass decreased (Fig.V.4.B and Fig.V.4.D respectively). The antibiotic-treated cells are more elongated on both materials compared to untreated cells and in the case of *E.Coli* biofilm developed on glass (Fig.V.4.B), the treated cells are longer than on silicone (Fig.V.4.D).In both tested materials, the cell wall of sessile cells showed no severe damage after 6 h of ampicillin treatment (Fig.V.4.B and Fig.V.4.D). Cell length determined from SEM micrographs consisted of histograms revealing the size distribution of biofilms cells exposed (Fig. V.5.B) and not exposed (Fig. V.5.A) to ampicillin.Treated cells present on glass were more elongated than those present on silicone surfaces (Fig. V.5.B).

Moreover, after antibiotic treatment, cells adhered to silicone measured between 1.2 and 6.5 µm while cell lengths between 3.5 and 9.2µm were determined for glass. Additionally, a narrower size distribution was found for the untreated cells for both materials tested.

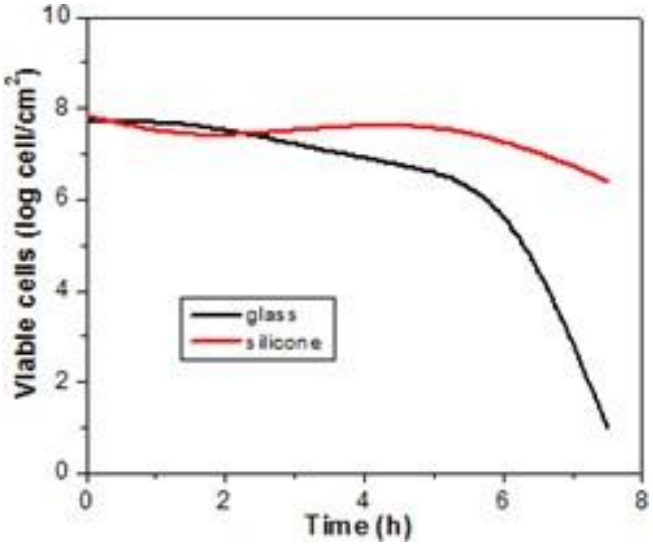


Figure V.3. Evolution of the amount of viable cells within 24 h biofilmsformed on glass and silicone during exposure to ampicillin

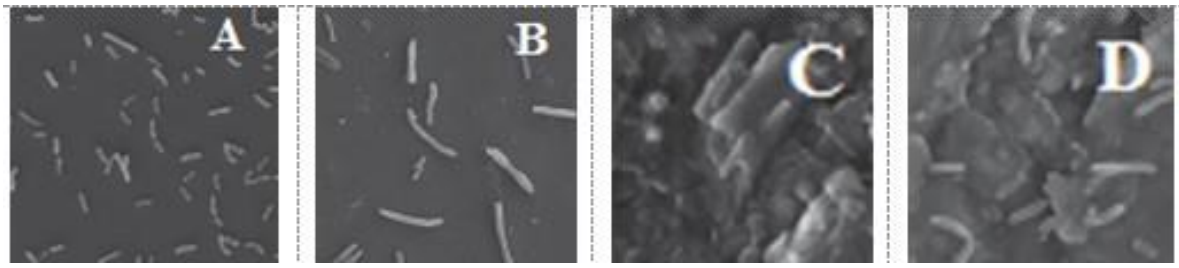


Figure V.4.. SEM micrographs of 24 h biofilms not exposed to ampicillin formed on (A) glass and (C) silicone and after 6 h of exposure to ampicillin (B) glass and (D) silicone

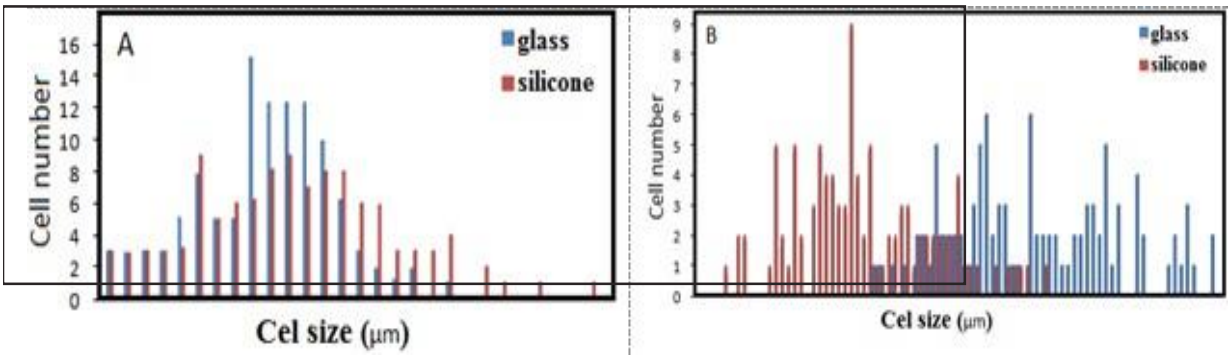


Figure V.5. Cell length distribution of 24-hour biofilms not exposed to ampicillin (A) and after 6 h of exposure to ampicillin (B).

II. Over the past two decades, antibiotic resistance, especially for Gram-negative bacteria, has increased at an alarming rate, requiring constant concern for resolving and controlling this extremely important therapeutic aspect in any medical department, but in particular, in Anaesthesia and Intensive Care Unit, in units of neonatology, paediatrics, neurosurgery, burns and immunosuppressed. Specialists note a particular concern for the resistance of *Enterobacteriaceae spp* to third-generation cephalosporins and aztreonam, with a resistance profile frequently associated with the expression of extended-spectrum β-lactamases (ESBL). This family of enzymes was identified in the 1980s and confers resistance to almost all β-lactam antibiotics, except for carbapenems and cephamycins. Genes encoding ESBL are found on plasmids that also carry other antibiotic resistance genes, which make the ESBL- producing strains to be multidrug-resistant. In addition to ESBLs, the broad-spectrum β-lactam resistance of *Enterobacteriaceae spp* is increasingly provided by other chromosomal-encoded plasmid enzymes, such as carbapenemases and cephalosporinases AmpC. (Bush & Jacoby, 2010; Logan et al., 2014). Such mobile genetic and molecular equipment of resistant bacteria has further complicated the detection of ESBL- producing species. Due to the limitations and variability of the tests and the different reporting, third generation cephalosporin resistance is used as a more reliable indicator for the actual reporting of the prevalence of ESBL strains (Gazin et al., 2012; ECDC, 2015; Logan et al., 2014).

Potentially pathogenic enterobacteria have developed multiple antibiotic resistance, chromosomal or plasmid- mediated resistance, becoming feared enemies in infections associated with inpatient healthcare. Enterobacter genus comprises 14 species, two are of medical interest, *Enterobacter aerogenes* and *E. cloacae*, which are involved in the induction of healthcare

associated infections such as urinary infections, pneumonias associated with mechanical ventilation, bacteremia, septicemia, etc. Infections caused by potential or occasionally pathogenic enterobacteria occur due to the alteration of the natural defense mechanisms, of the terrain on which the bacteria are implanted and due to the extreme age of the patients. Although receptivity is general, it is increased in long- term hospitalized individuals for chronic conditions and in immunosuppressed patients. The prevention of these health care associated infections includes measures similar to those instituted for such pathologies, considering that these isolates are frequently MDR (Ivan, 2002; Duceac et al., 2019). The aim of the study was to highlight the antibiotic molecules in which case the microbial resistance of some circulating strains of enterobacteria was detected. The distribution by years of study shows that most cases were reported in 2017 (11 cases - 31.42%). The quarterly distribution of patients showed that in the first and third quarters most cases were reported (respectively 12 patients - 34.28%), in the fourth quarter 9 cases (25.71%) were reported, and in the second quarter, the least (2 cases - 5.71%) ($p < 0.01$).

The age distribution of patients with infections in whom strains of *Enterobacter spp* were isolated showed an average age of approximaely 2.6 years. Most cases were registered in newborns, up to 1 month (15 cases - 42.85%), then in infants up to 12 months (9 cases - 25.71%), then in children between 2 -5 years old (7 cases - 20.00%) and in children between 12-18 years old (4 cases - 11.43%).

Gender distribution showed that there were more cases in boys than in girls, and the ratio M: F = 24/9 (2.67).

The most commonly diagnosed types of infections were wound infection (8 isolates - 22.85%), skin infection (7 isolates - 20.00%), urinary tract infection (5 cases - 14.28%) sepsis (5 cases -14.28%), pneumonia associated with mechanical ventilation (4 cases - 11.42%), catheter infection (3 isolates - 8.57%), digestive infection (2 cases - 5.71%) and respiratory infection (1 isolate - 2.85%). Fig.V.6. exemplifies sites and types of pathological samples used in our study.

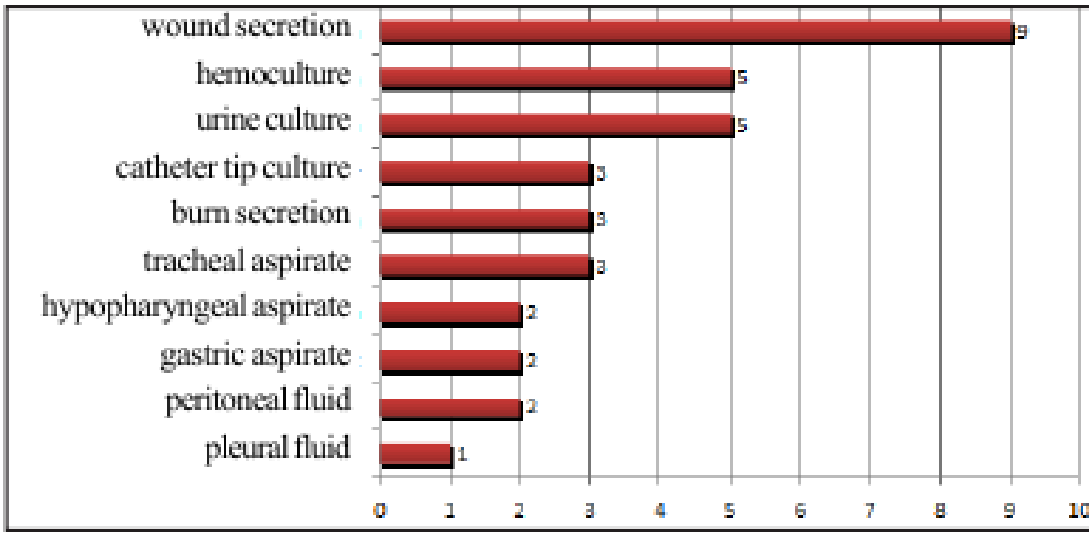


Figure V.6. Types of pathological products taken as samples

Microbial antibacterial resistance of *Enterobacter spp* isolates showed that all isolates exhibited resistance to ampicillin, amoxicillin and clavulanic acid (100%), 17 isolates (48.57%) were resistant to cefuroxime (CXM), 15 isolates (42.85%) resistant to ceftazidime (CAZ) and

ceftriaxone (CRO), 11 isolates (31.42%) resistant to trimethoprim-sulfamethoxazole (SXT), 9 isolates (25.71%) resistant to gentamicin, 7 (20.00%) resistant to tazobactam-piperacillin (TZP), 6 isolates (17.14%) resistant to cefoperazone (CFP) and tobramycin, 5 (14.28%) to ciprofloxacin, 4 (11.42%) to ertapenem, 2 (5.71%) in meronem (MEM). The types of strains were *Enterobacter spp* - 20 isolates (57.14%), *E. cloacae* - 11 isolates (31.42%) and *E. aerogenes*- 4 isolates (11.42%). Of these, 4 isolates were found to produce beta-lactamases ESBL (11.42%) and one CRE (Carbapenem-resistant Enterobacteriaceae) (2.85%).

The time interval from hospitalization to diagnosis of the healthcare associated infection was, on average, 27.6 days, with a minimum of 3 days and a maximum of 94 days. Most cases were diagnosed 15-30 days after admission (10 cases - 28.57%), followed by an interval of 31-90 days (9 cases - 25.71%), between 8-14 days – 8 patients (22.85%) and between 0-7 days - 6 cases (17.14%),and over 90 days - 2 cases (8.57%).

Targeted antibiotic treatment

I. The obtained products subjected to analysis are denoted as Amox/CA-LDHs(c), for the one obtained by reconstruction method using calcined LDHs and Amox/ CA-LDHs for the nanohybrid obtained by anion exchange way. Their structural and morphological characterizations were performed using Fourier-transform infrared spectroscopy (FTIR) and Scaning electron microscopy (SEM) techniques.

Figure V.7. displays the IR spectra of Amox/CA-LDHs and amox/CA-LDHs(c), respectively. Both spectra show an intense and broad peak at nearly 3450 cm-1 and 3000 cm-1, corresponding to the stretching vibration of –OH groups of hydrotalcite layers and interlayer water molecules. The band ascribed to an asymmetric stretching vibration of carbonate anions can be observed at 1350 cm-1. IR bands at 1507 cm-1 and 1595 cm-1 are attributed to characteristic stretching vibration of carboxyl groups. A strong absorption band at around 1360 cm-1 corresponds to nitrate anions. The other bands below 1000 cm-1 refers to vibration of metal oxides bonds. SEM images (Fig.V.8.) reveal crystal morphologies of amoxicillin and clavulanic acid intercalated layered double hydroxides. The micrographs present a relatively narrow particle size distribution between 100 and 250 nm and aggregates of non-porous and compact plate-like structures which are typical for the LDHs nanoparticles and intercalated ones .These morphology characteristics of hydrotalcite based nanocomposites indicate that drug molecules were securely intercalated into LDHs structure. Moreover, drug anions are protected by inorganic sheets and released in a controlled way being excellent reservoir of antimicrobial compounds.

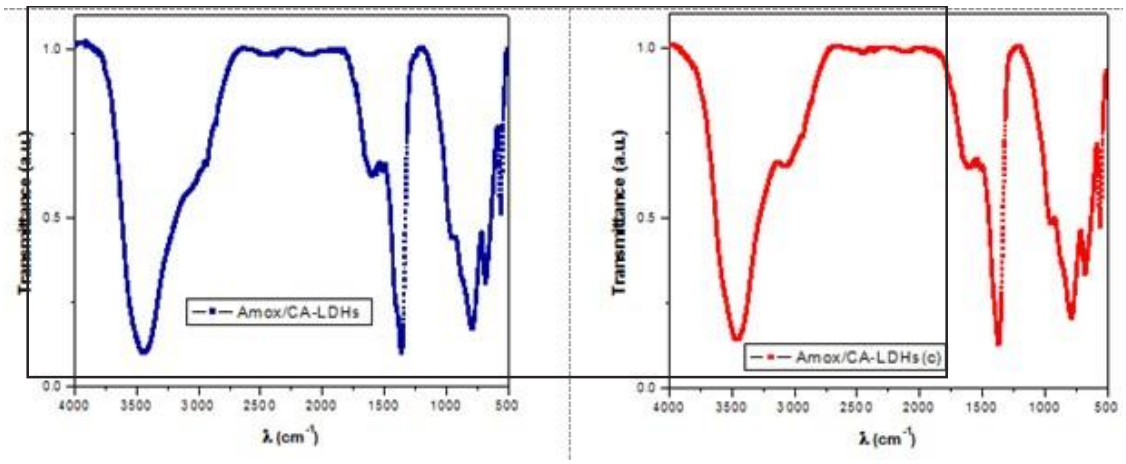


Figure V.7. FTIR spectra of nanohybrid samples: Amox/CA-LDHs and Amox/CA-LDHs(c)

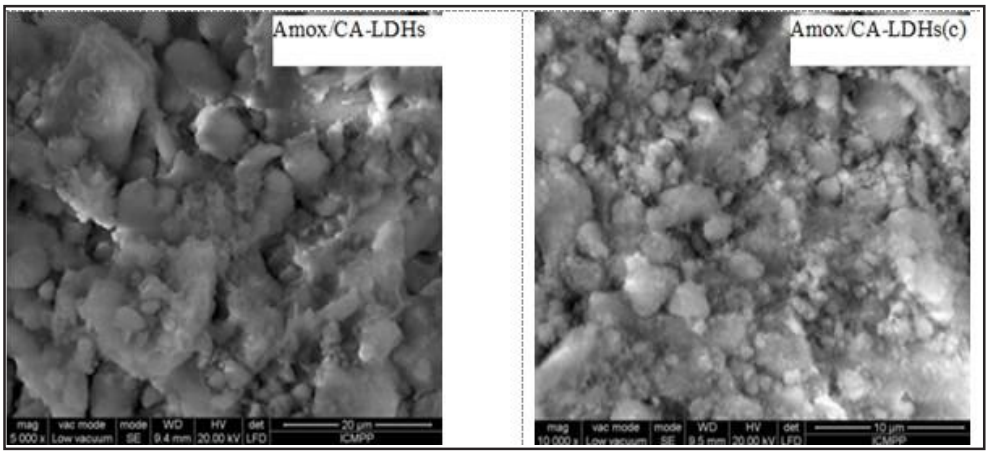


Figure V.8. SEM images of Amox/CA- LDHs and Amox/CA-LDHs(c)

II. Zetasizer Nano ZS instrument was used for measure mean particle size and polydispersity index (PDI) before and after separation of free imipenem. Laser Doppler anemometry was used for determination of zeta-potential values. High yields (70-75%) of imipenem-loaded chitosan were obtained at different chitosan-TPP ratios. The average particle size diameter for imipenem-loaded nanoparticles ranged from 245 to 445 nm before separation and 245-375 after separation of free antibiotic (Fig.V.9a).

Chitosan-TPP ratio played a significant role in the formation of nanoparticles and in setting their physicochemical features. Nanoparticles size decreased when increased the chitosan-TPP ratio from 3:1 to 4:1. From a chitosan-TPP ratio higher than 5:1, before and after separation of free drug, nanoparticles had PDI values larger than 0.5 due to the formation of aggregates.

Analyses of number-, intensity- and volume- size distribution histograms (Fig.V.9b-d) of the various formulations revealed the homogeneity of the nanoparticles prepared at chitosan-TPP ratio of 5:1, this formulation being selected for further analyses.

FTIR spectra of imipenem, chitosan, TPP, and imipenem loaded chitosan nanoparticles analysed chemical structure and complexes formation as shown in Fig.V.9. The main peaks corresponding to imipenem are at 3565 cm-1 for - OH stretching vibration, 3402 cm-1 for –NH stretching, 3104 cm-1, 2977 cm-1, 2998 cm-1 for -OH stretching in COOH, 1750 cm-1 for COO stretching in COOH group and 670 cm- 1 for O-H bending in COOH.

Characteristic peaks of chitosan can be observed at 3370 cm-1 for -OH and -NH stretching, at 2870 cm-1 for -CH stretching vibrations, for 12 653 cm-1 is attributed to C=O stretching, 1595 cm-1 for N-H bending, 1380 cm-1 for -CH symetrical deformation, for 1157 cm-1 for C-O-C anti-symetric stretching and C-N stretching, 1076 cm-1 is assigned to skeletal vibration of C-O stretching.

For antibiotic-loaded chitosan nanoparticles, peaks of amide I and amide II of chitosan were shifted to 1640 cm-1 and 1560 cm-1 respectively and might be attributed to the electrostatic interactions between amino groups of chitosan and phosphoric groups of TP. XRD pattern of chitosan, TPP, imipenem and imipenem- loaded chitosan nanoparticles revealed the crystallinity of these compounds.

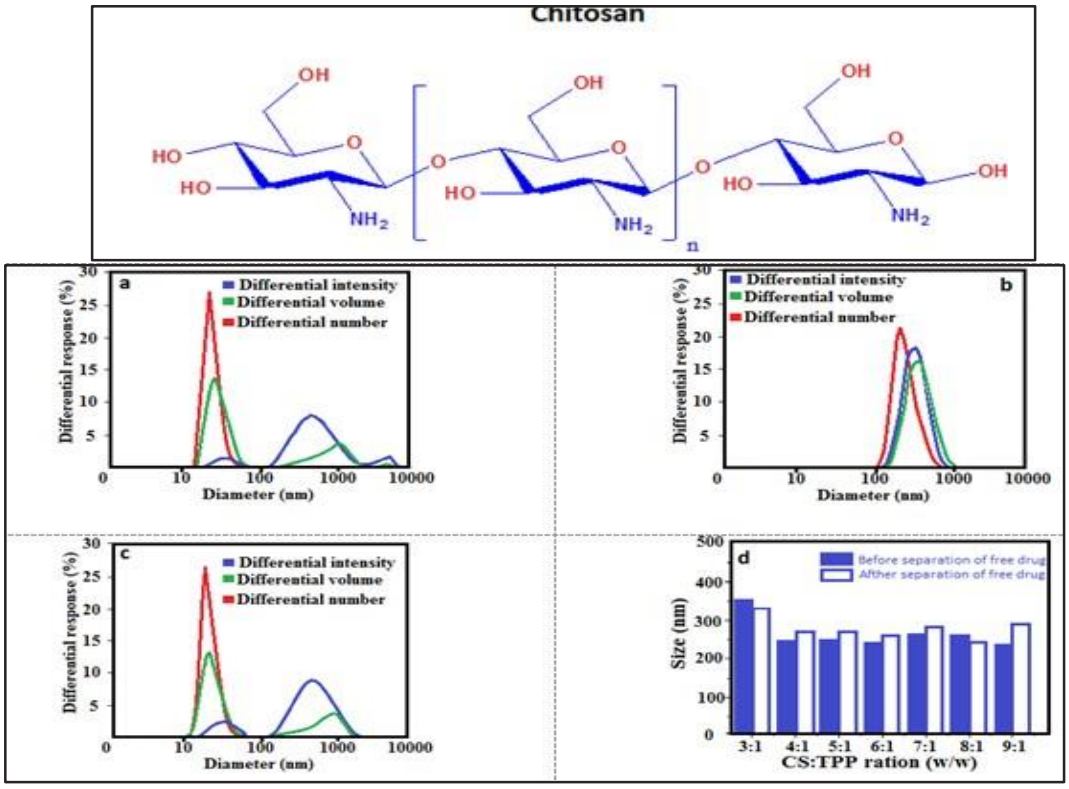
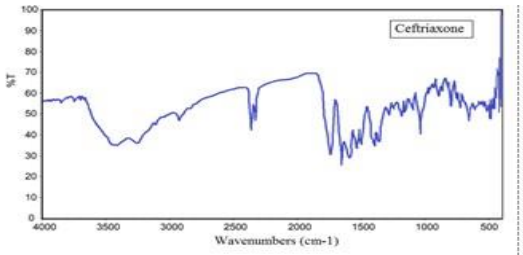
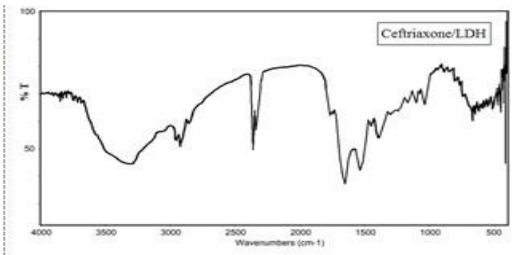


Figure V.9. Intensity-, volume- and number-averaged hydrodynamic diameter histograms of imipenem-loaded nanoparticles prepared at chitosan:TPP weight ratio of 3:1 (a), 5:1 (b) and 9:1 (c). The effect of chitosan:TPP weight ratio (9:1–3:1) on the size of imipenem-loaded nanoparticles before and after separation of the free antibiotic (d)

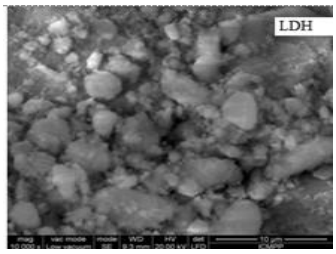
III. FTIR characterization is an important analysis for identification of intercalated ceftriaxone molecules into LDH structure (Fig.V.10.). IR spectra for ceftriaxone present characteristic peaks at nearly 3430 cm-1 asigned to N-H stretching vibration of H-bonded amide group, at 1740 cm-1 attributed to C=O stretching vibration and 1590 cm-1 to C=N stretching vibration. For ceftriaxone/LDH nanocomposite the peaks noticed at 3380 cm-1 corresponds to the -OH groups stretching vibration, at around 2450 cm-1 to C-H stretching vibration. Band around 1640 cm-1 and 1290 cm-1 are attributed to C=O groups and for C-C and C-N stretching vibration. Elemental analysis was determined by Energy Dispersive X-Ray (EDX) technique. SEM micrographs of LDH and Ceftriaxone/LDH samples are shown in Fig.V.10. Antibiotic loaded hydrotalcite suggest the presence of aggregates specific for layered double hydroxides nanoparticles.

The sheet-like morphology is characterized by a particle size about 150 nm and thickness that suggest the small number of layers. They are larger than LDH sample but in the same area and aggregation degree.





Sample	Elements					
	Al	Mg	C	O	N	S
LDH	36.17	8.64	4.74	50.49	0	0
Ceftriaxone/LDH	33.36	6.5	10.58	46.7	3.64	1.35



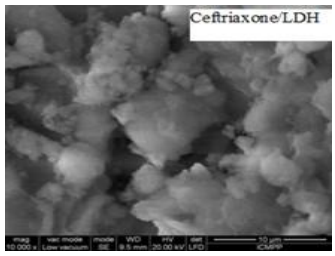


Figure V.10.. IR spectra for Ceftriaxone and Ceftriaxone/LDH; Elements of LDH and Ceftriaxone/LDH samples; SEM images of LDH and Ceftriaxone/LDH nanoparticles

V.2.4. Conclusions

In last few years, bacteria have developed resistance to all classes of antimicrobial agents and the novel mechanism of multi-drug resistance caused considerable problems in the treatment of infections caused by some pathogenic bacteria. Researchers planned new strategies to combat antibiotic resistance that induced the altering of antibiotics already in use concomitantly with the administration of nonantibiotic drugs that inhibit antibiotic biodegradation mediated by bacterial enzymes. Despite these discoveries, antibiotic resistance continues to be a major clinical issue.

Some methods were developed to lower the risk of infections with antibiotic-resistant bacteria including the choice of an antibiotic with a narrow spectrum when the pathogen is known, shortening the duration of antibiotic prophylaxis and restricting topical and oral therapy with drugs of parenterally use. Although the use of antibiotics increases the occurrence of drug-resistant pathogen agents, these drugs need to be used carefully in order to prolong their efficacy.

SECTION B. PERSPECTIVES IN THE PROFESSIONAL, ACADEMIC AND SCIENTIFIC FIELD

Self-improvement, based on an individual plan of action and continuous didactic and scientific upgrade are the main trajectories for my future. Obtaining the habilitation certificate, as a basis for becoming a PhD supervisor would grant me the opportunity to enhance my level of professionalism and scientific visibility by participating in national and international research projects as well as granting me the possibilities for shaping students' personalities by instilling a passion for learning-oriented studying based on self-discovery that ensures the ability to use information. I plan to deploy all my intellectual resources and skills to achieve my goals and performances in my field.

ACADEMIC AND PROFESSIONAL PERSPECTIVES

My teaching activity aims at the dual role of mentor for students and residents. This duality is also reflected in my goals. In this regard, I would like to initiate a pediatric cardiac emergency course for both students and residents, a course with a cardiopulmonary resuscitation section.

Also in the teaching activity, I want to continuously improve my courses and supports for practical work and internships, to carry out learning software and to continue conducting grid-type tests to help students and residents.

Regarding the extracurricular activity, I want to coordinate topics within the Student Scientific Circles and to organize workshops for students and workshops on various topics in the field of pediatrics, pediatric cardiology and pediatric intensive care.

The student stage is significant for establishing a *modus operandi* throughout the medical career, which consists in the perpetual accumulation of new knowledge, the ability to make connections between multiple related fields, and the capacity of rapid retrieval of information under pressure. Therefore, I consider that my job as a teacher is not just to pass on information and encourage practical skills, but to turn students into partners in their own training process. I intend to stimulate the students' responsibility by increasing the level of exigency corroborated with the maintenance of a collaborative relationship and the use of focused and individualized evaluation methods according to the notions that must be assimilated. To this end, I will develop educational materials with rich imaging, thematic modules (lecture sets, learning aids), and revised versions of university courses. Also, I intend to keep the communication channel with my students opened through UMF's e-learning platform and websites such as www.doctor-pediatru .ro (RO); www.pediatru-iasi.ro, (EN); www.cardiologie pediatrica.ro (profile courses). Another important point on my didactic development agenda, which is student counseling, will focus on 3 axes: individualized guidance; organization of student scientific circles; carrying out tutoring activities.

In fulfilling my vocation as a mentor, I intend to develop collaborative relationships with faculty members in the discipline and department. Participation as a guest / associate professor at foreign universities is a goal that I wish to achieve in the near future, considering that the training of medical students in other countries has certain features that can improve our own teaching process.

Regarding the training of residents, I consider that encouraging their accountability to patients, doubled by the development and printing of revised versions of university courses and a joint course in Pediatric Cardiology redacted with the contributions of my colleagues from other Pediatric Cardiology centers would be the best way to start. Identifying and stimulating young

people who want to pursue a career in the field of Pediatric Cardiology is part of my objectives regarding the development of my young colleagues.

As part of the faculty members group, I also consider it my duty to get involved in the academic and administrative life of the department, the faculty and the university, bring my contributions to the elaboration and implementation of policies and strategies for the upgrade of the faculty and the university and increase involvement in professional-administrative activities: admission, bachelor's, residency, specialty and senior exams. I wish to promote the image and professional expertise of the institution, both in academia and in the media while developing inter-institutional partnership relations.

SCIENTIFIC PERSPECTIVES

The conditions which are the scope of pediatric cardiology are major public health problems. Many of them with a genetic basis are the beneficiaries of intense research programs aimed at finding both the cause and the targeted treatment, through personalized medication or even gene therapy, favored by recent successes in the field of gene editing through the CRISPR technique.

In the hope of obtaining concrete scientific results, I propose to communicate them in both national and international scientific events in the field, as well as publication in ISI-listed journals, ISI-indexed proceedings, journals included in BDI, and national and international appreciation by citations in high impact magazines.

The continuous improvement of my scientific activity will be based on organizing national and international scientific organizations, learning new genetic diagnosis techniques, development of protocols for evaluation and investigations. Also, by actively participating in committees of the Ministry of Health, establishing and cementing existing collaboration with specialists from the country or abroad and developing leaflets / brochures for public information, I aim to popularize the results of my research, so that the objectives of health education of the population as a partner with equal rights in the process of prevention and treatment of the disease are achieved.

Biomarkers for early detection of cardiac injury

The problem of progression of heart damage in children in various clinical scenarios is even more acute as there are few identification markers. Echocardiography can reveal heart dysfunction once it occurs, when drug therapy is often needed. We are not talking about cases of myocarditis or postviral pericarditis, which have a favorable prognosis under symptomatic and sometimes etiological treatment and which, in a child without other heart conditions, does not leave sequelae. The problem relays with cases of congenital heart defects, cardiomyopathies, arrhythmias or systemic diseases with an impact on the cardiovascular system, in which detecting the onset of changes in the heart before they reach the point of being diagnosed paraclinically, can make the difference between a positive and negative prognosis.

Another problem is the risk stratification after surgery to correct congenital heart defects. A number of conditions can be seen in the context of the postoperative period, from coagulation disorders to liver or kidney failure. L-FABP and NGAL are markers of renal failure whose predictive role in the onset of renal failure in children undergoing heart surgery is under ongoing investigation (Yoneyama et al., 2020).

Similarly, Galectin-3 has been proposed as a promising biomarker for the early detection of HF in children with CHD. It has a positive correlation with the ROSS classification and a negative correlation with the ventricular ejection fraction. The preliminary results seem to indicate a good prognostic value for the onset of heart failure, but no or little value in the assessment of mortality risk (Saleh et al., 2020).

Copeptin is another novel biomarker which showed promising results in heart failure stratification risk, prognosis and treatment choice in adults (Karki at al., 2019). Several studies have sought to establish if a similar utility profile exists in children, notably those with cardiomyopathies. The research is currently ongoing.

Weber et al, studied miRNA profile in human body fluids- plasma, serum, pleural, amniotic and cerebrospinal fluid, saliva, urine, colostrum, breast milk and seminal fluid- concluding that there are significant differences in the type and concentration of various miRNAs, but, nonetheless, that their presence can be used as a distinctive disease marker (Weber et al., 2010). miR-204 can be useful in detecting the risk for pulmonary hypertension in children with CHD (Li et al., 2019) hsa-miR-16-5p, hsa-miR-223-3p and hsa-miR-92a-3p have been investigated as possible early biomarkers for rheumatic carditis (Gumus et al. 2018) and miR-29b showed promising results when used as an indicator of progression to heart failure (Yang et al., 2020).

The main purpose of my scientific research will be to asses if molecules such as those mentioned above can be reliable as predictive factors for heart failure and other cardiac complications, considering that they can be influenced not only by the type of disease, but also by other individual aspects, such as smoking habits, alcohol consumption, eating habits, chronic medication, other concurrent diseases, playing sports, etc…

Study groups will be represented by:

1. Patients with diagnosed CHD and with no signs of pulmonary hypertension or heart failure

2. Patients with systemic autoimmune disorders and no cardiac involvement at the time of diagnostic

3. Patients with cardiomyopathies and no signs of heart failure

Besides evaluating the levels of biomarkers prior to the development of any signs of cardiac injury, I will aim to establish a correlation pattern between treatment response and any modification in the levels of said biomarkers.

Chaperone molecules- targeted treatment in metabolic diseases

Lysosomal storage diseases is a category comprising over 70 diseases caused by enzyme deficiencies in lysosomes, leading to substrate accumulation. Pompe , Gaucher and Fabry diseases , and mucopolysaccharidoses are just a few examples.

Pompe disease or type 2 glycogen storage disease is a rare neuromuscular disorder caused by a deficiency in acid alpha-glucosidase (GAA), an enzyme responsible for the glycogen degradation in muscles. As exemplified in chapter I.2.2. of this thesis, Pompe disease can cause serious harm to the cardiac system in the absence of appropriate treatment, which is currently widely done through enzyme replacement therapy, meaning that compounds such as Myozyme®, that contains Alglucosidase alfa are administered intravenously.

Pharmacological chaperone therapy uses molecules capable of restoring a protein's normal conformation by binding themselves to the target. The main advantages of PC consist in oral administration, the ability to cross the blood-brain barrier , enhanced bioavailability and the

capacity to restore protein function up to 20% , enough to suppress disease manifestations (Borie-Guichot et al., 2021).

Galafold™ , received accelerated approval from FDA in 2018 for the treatment of adults with Fabry disease. 123 mg of Migalastat, the active compound is administered every other day to patients with an amenable GLA variant, and it binds to alpha-galactosidase A, allowing it to function normally. The phase 3 ATTRACT and FACETS trials showed a significant reduction of the cardiac mass in patients that received Galafold, compared to those who underwent ERT, which suggests a superior degree of cardiac penetrability of this compound, not to mention higher patient compliance to the oral treatment and the possibility for early intervention when compared to the iv option offered by enzyme replacement (Borie-Guichot et al., 2021; Chimenti et al., 2020).

However, as I mentioned, the effectiveness of PC is dictated by the type of mutation encountered in the genes encoding enzyme proteins. Only missense mutations are candidate for chaperone therapy. The vast majority of mutations associated with Pompe disease are missense, making this disease into a promising candidate for PC treatment.

The first molecules studied as possible options for PC where DNJ and its derivatives, one of which was recently submitted to FDA for approval as treatment in Gaucher disease. Seeing as there is a similarity between GAA and GBA, it could be a possibility for this treatment regime to apply to Pompe disease as well. Other molecules, such as Ambroxol and N-acetyl-cysteine showed promising enhancement proprieties of the GAA activity (Borie-Guichot et al., 2021).

Moreover, a combination between a mRNA-based ERT and PC is under development, pending patent approval.

Pompe disease has a lower prevalence when compared with Fabry or Gaucher disease, which makes it harder to study as a candidate for PC. However, in the past 15 years, important breakthrough has been made.

In Romania, the possibilities to develop new molecules for PC therapy studies are reduced if not inexistent. However, I aim to increase the chances of my patients diagnosed with Pompe disease to receive state-of-the-art treatments in order to remit the disease and improve their quality of life. To this end, I intend to identify patients who are candidates for PC therapy by increasing addressability to genetic centers that can perform GAA gene sequencing. In my activity so far, I have managed to create partnerships with the Regional Genetics Center in Iasi, but also with other regional centers in the country and centers abroad. This collaborative network can and should be used to identify the target group for PC therapy among the patients I have under observation. The next step will be to include them in ongoing trials and to monitor the progress of treatment, while identifying variables, other than the genotype that may influence the end results of PC therapy.

Also, I wish to further investigate genotype-phenotype correlations in patients with Pompe disease. In 2021, 4 new variants of the GAA gene were discovered. The missense mutations c.1328A>T, c.1831G>A, c.2819C>A, c.1889-1G>A were not previously reported and the authors of the study considered them as likely pathogenic. The patients exhibited muscle weakness and/or high CPK in serum, respiratory distress, with only one of them displaying mild left ventricular hypertrophy. Pompe disease was not the first suspicion in neither of these patients (Hermandez-Arevalo et al. 2021).

In light of this information, it is self-evident why further studies on genotype-phenotype correlations are important, especially with the advent of new PC therapies.

To this end, I will evaluate the possibilities for genetic sequencing of the GAA gene and publish the research results. Besides establishing the impact individual mutations have over the clinical presentation, I will also evaluate the response to ERT treatment and the occurrence of extracardiac involvement , in a genotype-dependent manner.

Antioxidant agents for neurological and cardiac protection

It has long been thought that abnormalities in the stage of neuronal development have irreparable effects, but recent studies indicate the possibility of reversibility of some electrophysiological, molecular or behavioral deficits by pharmacological therapy. Because neurological damage allows survival, but significantly decreases the quality of life, optimizing the evaluation and therapy of this clinical category is particularly important**.**

As I have shown in my research, presented in chapter IV.2, neurological disabilities have a significant impact on cardiac function, either through the behavioral and eating disorders they associate, or through motor deficit.

Statins are a class of molecules that inhibit HMG-CoA reductase and with multiple pleiotropic effects caused by inhibition of free radical release, decreased levels of inflammation markers (C-reactive protein), decreased T cells activity, followed by decreased cytokine secretion, reduced production of metalloproteinases, increased nitric oxide synthesis, reduced β-amyloid production and serum ApoE levels (Liao & Laufs, 2005).

Clinical trials have shown the neuroprotective effect of statins in the context of ischemia and Alzheimer's dementia (Wang et al., 2011) as well as the ameliorating effect on the motor deficit associated with Parkinson's disease and Duchenne (Whitehead et al., 2012) muscular dystrophy, turning this class of drugs into potential therapeutic variants in neurodegenerative diseases with genetic substrate.

Multiple clinical trails have established a good safety profile for statin administration (Lovastatin) in patients with RASopathies aged 10-17 years, highlighting absent or moderate side effects. The main positive effects of statin therapy in these patients were in the area of cognitive development, the effects on motor disorders not being sufficiently investigated (Buchovecky et al 2013; Acosta et al., 2011).

Another important aspect to consider is the structural brain damage associated with HF. Decreased cerebral perfusion, vascular remodeling, systemic inflammation and endothelial dysfunction are just some possible mechanisms in the heart-brain interaction in patients with cardiac structural and functional anomalies. To add insult to injury, most children with cardiac disorders, such as cardiomyopathies or rhythm disturbances, associate a variable degree of HF early on, meaning that the impact on the developmental brain can be serious and with life-long consequences. The prevention of cognitive impairment in these patients is still understudied. While it can be difficult to ensure an adequate brain perfusion by medication alone, some cardiac drugs may help reduce the other precipitating factors of cerebral structural damage, such as oxidative stress and endothelial dysfunction. ACE inhibitors have proven useful in the reduction of ROS production (Mikrut et al., 2016) as have mineralocorticoid receptor blockade, AT1-receptor blockade, and direct renin inhibitor therapy. All this therapeutic regimes are currently used for the treatment of hypertension and heart failure in children (Bahbah et al, 2021).

However, recent research found a plethora of synthetic and natural compounds that mimic the effects of cardiovascular medicine mentioned above, but whose antioxidant effects outweigh the cardiovascular ones. Of these, nutraceuticals are a promising new class of natural or synthetic molecules at the border between nutrient and drug, used as therapeutic adjuvants. For instance, astaxanthin has proven to be a valuable anti-inflammatory and antioxidant agent, with both cardioprotective and neuroprotective effects, approved as a nutraceutical by the FDA for use in adults. Polyphenols also exert antioxidative effects and promote DNA repair, as well as improve mitochondrial function. Studies have shown significant neurological improvement in children with autism spectrum disorders following treatment with polyphenol**s** (Pangrazzi et al, 2020).

On one hand, my aim is to investigate nutraceuticals as therapeutic adjuvants in children with mild to moderate cardiac diseases. On the other hand, I am interested in the evaluation of patients with specific genetic syndromes, such as RASopathies, with mild to moderate heart damage and establishing a safety profile for the administration of cardioactive medication. I will use standardized tests for cognitive and motor functions as well as cardiac evaluation instruments to establish the impact of cardiac medication on both neurological improvement and the cardiac function.

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