GRIGORE T. POPA UNIVERSITY OF MEDICINE AND PHARMACY IASI

HABILITATION THESIS

MULTIDISCIPLINARY APPROACH IN PEDIATRIC HEMATOLOGY AND ONCOLOGY

Miron Ingrith - Crenguța, MD, PhD

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Abbreviations

5-FU - 5 fluorouracil
ABVD - Adriamycin, Bleomycin, Vinblastine, and Dacarbazine
ACC - adrenocortical carcinoma
ACTH - Adrenocorticotropic hormone
ADH - antidiuretic hormone
AFP - *a*-fetoprotein
AKI - acute kidney injury
ALL - acute lymphoblastic leukemia
AML - acute myeloblastic leukemia
ANT - anthracyclines
ARF - acute renal failure
BCNU - Carmustine
BCP-ALL - precursor B cell - acute lymphoblastic leukemia
BEP -bleomycin, etoposide and cisplatin
BFM - Berlin–Frankfurt–Munster protocol
BM - bone marrow
BMI - body mass index
BNP - B- type natriuretic peptide
BP - blood pressure
CBTRUS - Central Brain Tumor Registry of the United States
CCNU - Lomustine
CDDP - Cisplatin
cDNA - complimentary DNA
cHL - classical HL
CID - chemotherapy-induced diarrhea
CIS - cisplatin
CML - chronic myeloid leukemia
CNS - central nervous system
COPP -Cyclophosphamide, Oncovin, Procarbazine, Prednisone
CR - complete remission
CSF - cerebrospinal fluid
CT - computer tomography
cTnI - troponin I
DdLV - diastolic diameters of the left ventricle
ECHO - echocardiography
EF - ejection fraction
EFS - event-free survival
EGIL - European Group for the Immunological Characterization of Leukemia

EKG - electrocardiogram
ELISA - enzyme-linked immunosorbent assay
ESR - erythrocyte sedimentation rate
FcγR - Fcγ receptors
FG - fusion gene
FSGS - focal segmental glomerulosclerosis
HCP - hereditary cancer predisposition
HDL-C - high density lipoprotein cholesterol
HIC - high income country
HL – Hodgkin lymphoma
HOMA - homeostasis model assessment
HR - high-risk
ICAM - intercellular adhesion molecules
ICE - Ifosphamide, Carboplatin, Etoposide regimen
ICT - isovolumic contraction time
IgG - immunoglobulin G
IL-6- interleukin 6
IR - insulin resistance
ITP - immune thrombocytopenia
IVA - ifosfamide, vincristine, actinomycin-D regimen
IVRT - isovolumic relaxation time
IVS - interventricular septum
LD - lymphocyte-depleted
LDH - lactate dehydrogenase
LDL-C - low density lipoprotein cholesterol
LFS - Li-Fraumeni syndrome
LR-LP - lymphocyte-rich lymphocyte-predominant
LVM - left ventricular mass
M-BCR - major breakpoint in BCR
m-BCR - minor breakpoint in BCR
MC - mixed cellularity
methyl-CCNU - Semustine
MIC - middle-income country
MLL - mixed lineage leukemia
MLPA - multiplex ligation probe-dependent amplification
MOPP -Nitrogen Mustard (Mechlorethamine), Vincristine, Procarbazine and Prednisolone
MRD - minimal residual disease
MRSA - methicillin resistant staphylococcus aureus
MTX – methotrexate
NE - neutropenic enterocolitis

NHANES - National Health and Nutrition Examination Survey
NHL – non Hodgkin lymphoma
NK - natural killer
NRSTS - non- rhabdomyosarcoma soft tissue sarcomas
NS - nodular sclerosis
OEPA - Vincristine, Etoposide, Prednisone, Doxorubicin
OS - overall survival
PB - peripheral blood
PCR - polymerase chain reaction
Ph - Philadelphia cromosone
PGR - prednisone good responders
PPR - prednisone poor responders
RMS - rhabdomyosarcoma
RNA -ribonucleic acid
RS - Reed-Sternberg cells
RT-PCR - Real Time polymerase chain reaction
RWT - Relative wall thickness
SEER - Surveillance, Epidemiology, and End Results
SF - shortening fraction
SIADH - syndrome of inappropriate antidiuretic hormone secretion
SIOP - International Society of Pediatric Oncology
STS - Soft tissues sarcomas
T-ALL - T cells acute lymphoblastic leukemia
TC - total cholesterol
TG - triglycerides
TKIs - Tyrosine kinase inhibitors
TMA -thrombotic microangiopathy
VAC - vincristine, actinomycin and cyclophosphamide regimen
VP16 - etoposide
WBC - white blood cells
WC - waist circumference
WHO - World Health Organisation

Abstract

The Habilitation Thesis entitled "Multidisciplinary Approach in Pediatric Hematology and Oncology" outlines the milestones of my postdoctoral professional, academic, and scientific career, as well as the future perspectives for further scientific and professional development. It is a synthesis of my research activity carried out over 35 years of medical practice and 30 years of academic experience. The thesis is structured according to the recommended and approved criteria by the National Council for Attestation of Titles, Diplomas and Certificates (CNATDCU), as summarized below.

Section A entitled "*Professional, Scientific, and Academic Achievements*" is a review of what I have been able to accomplish in the course of my clinical practice, research, and academic pursuits in the field of Pediatric Hematology and Oncology, highlighting the original results obtained and published.

Chapter 1 entitled *"Studies in Pediatric Hematological Malignancies"* includes the results of several studies which illustrate two important directions in pediatric hematology research: acute lymphoblastic leukemia and Hodgkin lymphoma. The studies in which I was directly involved and which assessed the impact of molecular modifications in pediatric acute lymphoblastic leukemia on patient outcomes are presented in the first part of the Chapter. The research grant assembled with the published articles helped our team to expand and to improve the molecular diagnosis technique in these patients. The second part of the Chapter is focused on pediatric Hodgkin Lymphoma and presents the results of researching different treatment regimens used over a 25-year period.

Chapter 2, "*Studies in Pediatric Solid Tumors*" focuses on the research of solid tumors in the pediatric age group. Studies on epidemiological, clinical, pathological features of the patients diagnosed with central nervous system tumors and rhabdomyosarcoma, as well as their treatment response are presented at length.

Chapter 3 entitled "*Chemotherapy – related Toxicities and Late Effects of Childhood Cancer Therapy*" outlines a multidisciplinary approach in dealing with the complications of chemotherapeutic treatment. In this regard, I was involved in conducting several studies in which we evaluated the incidence and the various types of chemotherapeutic complications in pediatric hemathological malignancies and solid tumors (e.g. anthracycline-induced cardiotoxicity, etiological spectrum of systemic infections). Another line of research has been about childhood obesity as a late complication and also as a risk factor for cardiovascular impairment.

Chapter 4, the last in this section is entitled "*Ethical and Social Practices in Pediatric Oncology*". In it, I discuss the ethical dilemmas commonly occurring within the practice of physicians dealing with oncology cases, starting from ethical considerations of obtaining informed consent in pediatric oncology (and notable differences compared to adult oncology) and continuing with end-of-life decisions. Also, this ethical part includes communication about diagnosis and treatment with children suffering from life-threatening illnesses, which presents an additional

psychosocial challenge. The research analyses how adults act either to limit or support patient participation by evaluating the impact of these actions on children.

The results of the studies featured in this section were published in national and international journals such as the Romanian Review of Laboratory Medicine (IF=0.171), Romanian Journal Of Bioethics**,** Farmacia (IF=1.057), Medicine (IF=1.804), Journal of the Balkan Union of Oncology (IF=0.741), Journal of Clinical Medicine (IF=4,241), Diagnostics (IF=3.706).

Section B entitled "*Further Academic, Professional, and Scientific Development*" presents my main areas of interest and envisaged opportunities moving forward. The field of Pediatric Hematology and Oncology addresses a wide spectrum of benign and malign blood disorders and solid tumors. My scientific goals in this field are mainly about identifying ways to improve the standards of care for the young patients suffering from these disorders. My career development planning is oriented towards studying new topics such as the identification of molecular modifications in pediatric acute leukemia and the assessment of the TP53 germline mutation in pediatric oncological patients. Moreover, I intend to conduct research aiming to expand the understanding of the implication of genetic polymorphisms of the Fcγ receptor of phagocytes in immune thrombocytopenia and to identify a correlation between the type of polymorphism, the clinical approach and the treatment response.

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

Rezumatul tezei

Teza de abilitare intitulată „Abordarea Multidisciplinară în Hematologia și Oncologia Pediatrică” reflectă principalele repere ale activității profesionale, academice și științifice derulate în perioada postdoctorală, precum și perspectivele pentru dezvoltarea științifică și profesională. Teza este o sinteză a activității mele de cercetare desfășurată în decursul a peste 35 ani de practică medicală și peste 30 ani de experiență academică.

Teza de abilitare este alcătuită conform recomandărilor Consiliului Național de Atestare a Titlurilor, Diplomelor și Certificatelor Universitare (CNATDCU) și este structurată în trei sectiuni.

Secțiunea A a tezei de abilitare prezintă o scurtă trecere în revistă a realizărilor din domeniul academic, profesional și științific și analizează principalele direcții de cercetare urmărite până în prezent, cu menționarea studiilor urmate. Activitatea științifică a abordat mai multe direcții de cercetare din domeniul hematologiei și oncologiei pediatrice. Această secțiune cuprinde 4 capitole.

Capitolul 1, respectiv „Malignitățile hematologice pediatrice” abordează două direcții de cercetare din hematologia pediatrică malignă: leucemia acută limfoblastică și limfom Hodgkin. Aceste linii de cercetare s-au materializat în diferite studii privind impactul modificărilor genetice și moleculare din leucemia limfoblastică acută la copil asupra răspunsului la tratamentul chimioterapic și a supraviețuirii. Ele sunt pe larg prezentate în prima parte a acestui capitol. Grantul de cercetare derulat în această direcție împreună cu articolele publicate ulterior au contribuit la extinderea și îmbunătățirea tehnicii de diagnostic molecular la aceast grup de pacienți. A doua parte a capitolului se concentrează asupra limfomul Hodgkin la copil și sumarizează rezultatele studii utilizate în tratamentul pacientului pe o perioadă de douăzeci și cinci de ani.

C**apitolul 2** este dedicat tumorilor solide la vârsta pediatrică. Sunt integrate rezultatele cercetărilor în acest domeniu, urmărind caracteristicile epidemiologice, clinice, anatomopatologice ale pacienților diagnosticați cu tumori ale sistemului nervos central și cu rabdomiosarcom, precum și răspunsul terapeutic la protocoalele de tratament aplicate.

Capitolul 3, „Toxicitatea chimioterapică și efectele pe termen lung ale terapiei antineoplazice la copil” prezintă o abordare multidisciplinară a complicațiilor tratamentului chimioterapic. În această direcție de cercetare, am dezvoltat mai multe studii care au evaluat incidența și diferitele tipuri de complicații chimioterapeutice (de exemplu, cardiotoxicitatea indusă de antraciclină, spectrul etiologic al infecțiilor sistemice) din tumorile maligne hematologice pediatrice și tumorile solide. O altă direcție de cercetare este legată de una dintre complicațiile pe termen lung postchimioterapie, respectiv obezitatea la vârstă pediatrică și implicarea acesteia ca factor de risc cardiovascular.

Ultimul **capitol** al acestei secțiuni, „Aspecte etice și sociale în oncologia pediatrică”, discută conceptul de dilemă etică ca problemă frecvent întâlnită în practica medicilor care tratează pacienți oncologici, pornind de la considerații etice privind obținerea consimțământului informat

în oncologie pediatrică și a diferențelor acestuia comparativ cu cel din oncologia adultă și continuă cu deciziile etice legate de finalul vieții. De asemenea, partea etică este completată de un aspect social major: comunicarea diagnosticului și tratamentului medical către copiii care suferă de boli oncologice. Cercetarea analizează modul în care adulții acționează fie pentru a limita, fie pentru a susține participarea pacienților la aceste practici evaluând impactul acestor acțiuni asupra copiilor.

Rezultatele studiilor incluse în teza de abiliatare au fost publicate în reviste naționale și internaționale Romanian Review of Laboratory Medicine (IF=0.171), Romanian Journal Of Bioethics**,** Farmacia (IF=1.057), Medicine (IF=1.804), Journal of the Balkan Union of Oncology (IF=0.741), Journal of Clinical Medicine (IF=4,241), Diagnostics (IF=3.706).

Secțiunea B intitulată „Perspective în dezvoltarea academică, profesională și științifică” prezintă principalele proiecte de dezvoltare în domeniul academic, profesional și științific. Hematologia și oncologia pediatrică acoperă un spectru larg de afecțiuni hematologice benigne și maligne și tumori solide. Interesele mele științifice în acest domeniu sunt orientate în principal spre identificarea modalităților de îmbunătățire a standardelor de îngrijire a pacienților care suferă de aceste afecțiuni. Planurile de dezvoltare a carierei academice își propun abordarea a noi direcții de cercetare în acest domeniu, cum ar fi identificarea modificărilor genetice și moleculare cu rol prognostic în leucemia acută pediatrică și evaluarea mutației TP53 la pacienții oncologici pediatrici. De asemenea, intenționez să-mi extind cercetările în înțelegerea implicării polimorfismelor genetice ale receptorului Fcγ în trombocitopenia imună și să identific o corelație între tipul de polimorfism, evoluția clinică și răspunsul la tratament.

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

Section A. Professional, Scientific and Academic Achievements

Professional Achievements

I have been a pediatrician for 35 years, having an uninterrupted practice of this challenging profession since my graduation from 1982; after completing the 6 years study program of Pediatric Section of the Faculty of Medicine, "Grigore T. Popa" University of Medicine and Pharmacy Iași (1976-1982), I entered the internship preparation program through a national contest. I began to work as an intern in the 2nd Pediatric Clinic in Iași and completed a series of internship rotations in pediatrics, pediatric surgery, general surgery, obstetrics-gynecology, infectious diseases, and pediatric neuro-psychiatry fields (1982-1985). In 1985 I was distributed by competition to the Poeni Exterior Unit of the Emergency Clinical Hospital for Children Iași for three years, and acquired experience in the treatment of chronic conditions,(celiac disease, cronic hepatitis, diabetes), severe metabolic and immuno-rheumatological diseases. Then, my top result in the national residency exam enabled me to get the position of pediatrician at the 2nd Pediatric Clinic of the "St. Mary" Emergency Clinical Hospital for Children Iași.

From September 1989, I continued my clinical practice as a resident at the Fundeni Clinical Hospital in București (under the supervision of Prof. Dr. Gheorghe Goldiș) and pursued a secondary specialization by attending courses at the "Prof. Dr. Alfred Rusescu" Institute for the Protection of the Mother and Child (Prof. Dr. M. Geormăneanu and Prof. Dr. E. Ciofu), the "Marie Curie" Children's Hospital (Prof. Dr. M. Maiorescu), the "23 August" Children's Hospital (Prof. Dr. V. Popescu, Prof. Dr. D. Dragomir, and Prof. Dr. C. Arion), the "Grigore Alexandrescu" Emergency Hospital for Children (Prof. Dr. C. Ulmeanu), the "Matei Balș" Institute (Dr. Mariana Mardărescu). I passed specialist exam at the "Prof. Dr. Alfred Rusescu" Institute in Bucharest in September 1991 with the final rating of 9.68/10. My professional development during the residency was facilitated by internship rotation in nephrology, rheumatology – pediatric autoimmune diseases, and hemato-oncology at Fundeni Pediatric Clinic, as well as by internships at the 1st and 2nd Pediatric Clinic in Iași (in intensive care for 12 months, then in allergo-immunology, pneumology, nephrology, general pediatrics for late childhood and for infancy).

In 1991, I got the physician compentency in general pediatrics, working as a doctor in 2nd Pediatric Clinic of the "St. Mary" Emergency Clinical Hospital for Children in Iași, with particular expertise in respiratory, immuno-allergic, rheumatologic and hematologic diseases. From 1994, I continued my work in the Pediatric Oncology Department of the hospital. I received the title of senior specialist/consultant based on the final grade 9.82/10 in the contest and I was employed part time in the 4th Pediatric Clinic. This part time position completed the basic full time position of assistant professor at "Grigore T. Popa" University of Medicine and Pharmacy Iași, aquiered in 1991 . In 1997, I passed the exam attesting my second competence in pediatric oncology. Subsequently, in 2000, I competed for and got the position of Head of the Pediatric Oncology Clinical Department, and in 2007, through a similar process, I became also the Head of the 4th Pediatric Clinic.

In my pursuit of the highest level of professional specialization, I competed successfully for several international mobility scholarships and internships, such as the Rhones-Alpes competition. This enabled me to work in Lyon, France, under the supervision of Prof. Thierry Philip, Head of the Léon Bérard Center, and of Dr. Maud Brunat-Mentigny, Head of the Pediatric Department and President of the French Society of Pediatric Oncology. During this one-year internship, I learned to apply the newest chemotherapy protocols. Furthermore, in September 2001, I participated in a specialist internship at the Pediatric Hospital in Luisville, Kentucky, U.S.A., as part of a team of doctors and nurses from Iași and Tg. Mureș. This experience included lectures, case presentations, patient consultations and monitoring in intensive care, neonatology and pediatric hemato-oncology units. I returned to the U.S. one year later at the "St. Jude's Children Research Hospital "in Memphis, U.S.A., where I was able to expand my knowledge of therapeutic options in the case of hematologic and oncologic diseases resistant to conventional treatments using cytostatic drugs.

Throughout my professional career, I have completed multiple postgraduate, national and international specialization courses in pediatrics and pediatric hemato-oncology, (Freiburg Hemato-Oncological training, mentor professor Charlotte Niemeyer), as well as in other connected medical fields. I also learned about leadership ("The management of programs and projects in healthcare; notions necessary for staff in managerial positions", 40 hours of continued medical education, 2007).

With regard to the professional responsibilities that I have undertaken over the years, I am currently a member in the pediatric oncology sub-committee of the Oncology Committee at the Ministry of Health. Also, I coordinate the national health program for pediatric oncology and the pediatric hematology program for hemophilia and thalassemia. In the hospital, I have been an active member of various committees tasked with the assessment of results following the application of protocols and practice guides, the implementation of hospital accreditation procedures, the international referral of cancer patients, the stockpiling of medication and sanitary materials, the assessment of negative medication side-effects, pharmacovigilance, the analysis of fatalities or the handling of grievances and complaints.

About projects and partnerships with clinical applications, I was part of the specialist team from the "St. Mary" Emergency Clinical Hospital for Children in the 2,5 million dollars international project "Improving Care in the Field of Pediatric Hemato-Oncology in Romania via the Acquisition of Specialized Goods and Services", financed through the Norway Grants 2009-2014 program. The main objective was to facilitate more accurate and timely diagnosis of pediatric hemato-oncology cases by updating the infrastructure and providing specialized training for doctors and nurses involved in hospital care, outpatient care, emergency care, and various medical specialties (gastroenterology, nutrition, nephrology and dialysis).

I have participated in national multi-center studies on the various protocols applicable to hematology, nephrology, oncology or to general pediatric issues. I also received relevant training and two Good Clinical Practice certificates for complex international studies undertaken at the Pediatric Oncology Unit with the approval of the Ethics Committee. Moreover, I am committed

to honoring the hospital's ongoing contracts and partnerships with the Regional Oncology Institute and the Molecular Biology Laboratory in Iași. Such collaborations enable us to address the inherent complexity of pediatric leukemia diagnoses. Last but not least, I have invited specialists in palliative medicine, resuscitation therapists, clinical pharmacologists etc. to deliver detailed lectures and training, such as on the topic of opioid administration in pediatric care, where more advanced understanding is a necessity.

Scientific Achievements

I have always acknowledged the essential role that scientific research plays in medical practice and progress. My early work at Fundeni Hospital made occasioned frequent participation in the reputed "Thursday Medical Sessions". There, I found inspiration and support for pursuing my own scientific interests and for sharing the results with my peers. My university graduation thesis was treating one of the most challenging childhood solid tumors: neuroblastoma.

The doctoral thesis entitled "Clinico-biological and Therapeutic Correlations in Pediatric Histiocytosis X" was my first substantial direction of research, supervised by the reputed Prof. Dr. Gheorghe Goldiș from the "Carol Davila" University of Medecine and Pharmacy, Fundeni hospital, București, and it was completed during 1992-1998. The thesis involved a retrospective study of rare pediatric cases of Langerhans histiocytosis (X), aiming to: 1) review specific epidemiological and clinical features in the Moldavian region of Romania, 2) identify transitional forms between those described in the literature by that time, and 3) identify predictive factors which could yield prognostic significance in terms of response to therapy and the incidence of relapses. For the diagnosis of the studied cases I received support from the Cellular Biology Department at the "Grigore T. Popa" University of Medicine and Pharmacy Iasi and from the immunology laboratories from Bucharest and Lyon. Based on my PhD research of local cases, I was able to propose an algorithm aiming to guide the diagnosis process and the therapeutic attitude, especially in response to transitional forms of the disease. Over the years, I have continued to follow up new cases, to share relevant insights by participating in scientific events, and to inspire professional interest in a younger colleague and collaborator who, 12 years later, completed another PhD thesis in the field. At present, therapy is conducted according to the protocol of the Histiocytosis Society. I participated in several projects and research grants such as:

- COST Action CA 18233 "European Network for Innovatibe Diagnosis and Treatment of Chronic Neutropenias" (19.11.2019 - 18.11.2023) management committee substitute;
- "Perception and attitudes on reproductive health and contraception in obese teenagers" (15.06.2017 -15.09.2019); Project financed by European Society of Contraception and Reproductive Health (ESCRH) contract no 12948/2017, project code P-2016-B-05; UMF "Grigore T Popa" Iasi, project director Conf Dr Laura Trandafir;
- Development of an integrated model for the evaluation of acute leukemia children using a RT-pcr technique for gene fusion. internal research grantof „Grigore T. Popa" University of Medicine and Pharmacy Iasi, no 16842/30.09.2009, grant director Miron Ingrith

- Grant CNCSIS nr. 1219 – Topics: Mutations in Systemic Infections Etiology Panel in Patients with Malignant Hemopathies (Coordinator Prof. Dr. D. Buiuc).
- MEC – CNCSIS - Topics: Research Platform of physiofarmacological and Clinical Research on the mechanisms of nonncological and oncological pain. (Coordinator Prof. Dr. O. C. Mungiu) contract code 68/2006
- Grant of Romanian Academy, Project no. 282/2007; Contract Nr. 148/2007. contract /2008. Title: Study of radiopharmaceutical substances used in oncology; Director: Ştefănescu Cipriana.

Following the thesis, I continued to share my work with the medical community by means of oral presentations in national and internationals conferences and congresses and scientific papers.

My international visibility is reflected by a Hirsch Index of 8 and 133 citations in Clarivate Analytics; 34 international scientific indexing papers (17 as main author, 17 as co-author) and over 60 IDB indexed papers.

Academic Achievements

I began my academic career in October 1991 as a teaching assistant at the "Grigore T. Popa" U.M.Ph. Iași, 2nd Pediatric Clinic, based on my overall grade 9.87/10 in the respective admission exam. In 1992, I started my PhD research at the "Carol Davila" University of Medicine and Pharmacy in București, under the supervision of Prof. Dr. Gheorghe Goldiș, and got the title of Doctor in Medical Science in 1998 upon the successful defense of the thesis entitled "Clinical, Biological and Therapeutic Correlations in Pediatric Histiocytosis X".

The completion of my PhD enabled me to go further on in my academic career by advancing to the position of Lecturer at the 4th Pediatric Clinic in October 1999. This was also facilitated by acquiring certification in didactics following the postgraduate courses organized in 1998-1999 by the Department of Training Didactic Staff at the "Alexandru I. Cuza" University of Iași (certificate no. 99274/21.06.1999, diploma A 1649). I then advanced to the position of Associate Professor in 2003, each time as a result of a competitive selection process.

Throughout these years, I have taught and coordinated courses from the medical and nursing curricula. I held lectures, practical activities, and presentations on all pediatric specialties to students studying in Romanian, English and French languages, and I have supervised graduation theses. I have also been involved in various types of examinations: open-ended questions, MCQ tests, clinical exams etc. I have been a tutor to several student series and I have mentored medical students in the context of extra-curricular student research and congresses. Also, I have helped enrich the medical curriculum by introducing new elective courses and instructional approaches aiming to enhance the knowledge with more detailed insights from pediatric sub-specialties. Given the increasing incidence of hematologic and oncologic conditions in children, such perspectives are purposeful and useful. For instance, in my lectures for 5th year medical students, I explain the normal features of the hematopoietic system throughout the different ages of childhood and adolescence, in contrast to the distinctive features of hematologic pathology depending on age, as

well as the various subtleties of hematologic manifestations in other pediatric diseases.

The bibliography recommended to 5th year medical students includes coursebooks which I helped write or edit, such as the 1995 Neonatology coursebook coordinated by Prof. Dr. I. Tansanu, the 2008 coursebook Practical Diagnostic and Treatment Tools in Pediatric Hematology and Nephrology by Ovidiu Brumariu, myself, and Mihaela Munteanu and the 2016 Pediatrics textbook. Also, in order to facilitate independent study and access to relevant bibliographies, I upload substantial pediatrics course materials in Romanian, English and French on the university's e-learning platform, as well as on the website www.hemoncped.com, which I author. This proves useful information, when objective circumstances render certain didactic activities inappropriate, such as when the patient's condition or prospect is too unfavorable to allow for adequate case presentation and discussion (e.g. during the covid-19 pandemic).

Regarding the education and training of medical graduates, I am the coordinator of the residency program at the Discipline of Pediatrics. As such, I supervise the progress of young pediatricians throughout their residency, but also the modular pediatric internships of residents from other medical specialties (emergency medicine, neonatology, infectious disease, endocrinology, pediatric surgery, family medicine, nephrology). I organize weekly case presentations and lectures tailored to comply with these residents' curricula.

Apart from such regular teaching duties, I have been a lecturer in the Moeciu Summer School for Residents, organized by the Romanian Society of Pediatrics, as well as in annual editions of the international Pediatric Medical School organized in Iași. In 2015, I participated in the project MedVISE, entailing professional counseling in support of successful medical careers. Specifically, I shared my experience as a pediatrician with medical students, helping them understand the professional and personal implications of caring for children suffering from hematologic and oncologic conditions.

Also, I have built additional postgraduate specialization courses in pediatric hematology and oncology for interested doctors, pediatricians and general practitioners from Iași and neighboring counties in the Moldavian region. Last but not least, I have delivered numerous instructional presentations, lectures, and courses in professional and scientific events at our university, at the National an International Conferences (e.g. Italy, China).

In terms of international academic interactions and partnerships, I have so far taken part in several didactic exchanges and study visits. In September 1998, at the Medical University in Freiburg, I had the opportunity to participate in case presentations organized by Prof. Dr. Mathias Brandis and Prof. Charlotte Niemeyer. I could also witness clinical practice focused on immunopathology, genetics, hematology and stem cell transplant. It is thanks to Mrs Niemeyer, currently one of the most knowledgeable and respected specialists in the myelodysplastic syndrome in children, that I was able to gain valuable and detailed understanding of this pathology. Also, in 2008, I took part in the Erasmus mobility program for faculty members, giving lectures on oncohematological topics to French students from Amiens and residents from France. This was also an opportunity to participate in the pediatric clinco-anatomo-radiologic sessions and to establish a professional relationship with the coordinator and President of the French Society of

Hemostasis and Thrombosis, Prof. Dr. Brigitte Pautard.

Regarding my experience in academic management, I am the Coordinator of Didactic Activity of the discipline Pediatrics at the "Grigore T. Popa" U.M.Ph. Iași. This includes: organizing the curriculum to students and residents, managing rotations and didactic contributions of associated teachers, assembling and reporting information requested by the Head of Department and the Dean's Office, leading faculty meetings on various topics, overseeing examination procedures, facilitating career advancement, scientific research, publications, congress participation, etc. I have been a member of numerous committees for the evaluation of interim PhD reports, and an appointed reviewer of PhD theses.

Chapter 1. Studies In Pediatric Hematological Malignancies

1.1. Background

Childhood malignancy, although a rare phenomenon, is still the leading cause of mortality in the pediatric population. The major hematologic malignancies affecting this population are acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), Hodgkin lymphoma (HL) and non-Hodgkin lymphoma (NHL). Progress in treatment for pediatric hematological malignancies over the last decades has resulted in remarkable survival for these children, with an estimated cure up to 70%. Five year survival exceeds 85% in children diagnosed with ALL, Hodgkin lymphoma or non-Hodgkin lymphoma and has reached 60% for children diagnosed with AML (Jemal A et al., 2010)

The most common type of cancer in children is acute lymphoblastic leukemia. Located in the bone marrow, this malignant disease features blocked lymphoid differentiation and fast clonal expansion of immature immune cells (T- or B-cells) (Cooper et al., 2015, Huang et al., 2020, Pui et al., 2012). Certain chromosomal translocations and inversions are involved in the clinical presentation and response to treatment of pediatric ALL. These have been thoroughly researched and classified, such as by World Health Organisation (WHO) in 2008, and the knowledge gained has been used to develop a range of effective treatments adapted to each subtype and risk group. Concurrently, supportive care has also improved, although not universally (Pui et al., 2012; Biondi and Rambaldi, 1996; Rubnitz and Pui, 1997; McLean et al., 1996).

Decades of clinical and therapeutical experience have converged towards the conclusion that precise identification of different types and subtypes of leukemia is absolutely necessary and depends on genetics. Understanding the genetic variants and clinical features combined with the treatment response may improve the prognostis for ALL patients and may help for selection of adapted therapies. In a nutshell, fusion genes result from the breakage of 2 separate genes, whose subsequent fusion produces a new gene; this, in turn determines intensive proliferation of a specific clone, leading to neoplasia (Good et al., 2018). Studies revealed that translocations in ALL and in acute myeloblastic leukemia may be used to classify patients in different risk groups. The 2008 WHO classification hematologic malignancies include several genetic abnormalities such t(9;22) BCR-ABL1, mixed lineage leukemia (MLL) rearrangement, t(12;21) ETV6-RUNX1, t(5,14) IL3-IGH, t(1,19) TCF3-PBX1 and hypo-, hyperdiploidy. Also mature B-ALL was included (Pui et al., 2012). The most important translocations with a distinctive immunophenotypic characteristics and outcomes are represented by TCF3-PBX1, MLL-AFF1, BCR-ABL1, ETV6-RUNX1 (De Braekeleer et al., 2012).

Regarding T-cell acute lymphoblastic leukemia (T-ALL) genetic alterations a dysregulation in the expression of normal transcription factor proteins is produced. Chromosomal abnormalities affect a subset of oncogenes such as TAL1, LCK, MYC, TAL2, TAN1/NOTCH1.

Also, certain rearrangement like translocations are implied in the formation of fusion genes met in specific T-ALL subgroups (CALM-AF10, MLL-t or ABL1-fusions) (Gorello R, et al., 2010).

The development of the genetic analysis in pediatric cancers, mostly in acute leukemia, has provided critical information about genetic changes accompanying this disease. The understanding of cytogenetics and molecular modifications in acute leukemia could provide new directions in the management and tailored targeted therapy for ALL.

Hodgkin lymphoma in children and adolescents is one of the most curable malignancies in pediatric oncology with a cure rate over 90%. The incidence of HL represents around 10% of all lymphoma types and approximately 0.6% of malignant diseases in Western European countries (Siegel et al., 2020). The disease occurrence is bimodal: the first is observed in young adulthood (age ranged from 15 -30 years old) and the second in group over 55 years old (King et al., 2014).

According to the data from United States Surveillance, Epidemiology, and End Results (SEER), Hodgkin's lymphoma constitutes 4% of annual cancer cases in the 0-14 years age group and 16.2% in the 15-19 years age group, making it the most common type of cancer in the older group (Amer et al., 2019). In developing countries, an inverse relationship in pediatric age between the incidence of HL and socio-economic is noted and it highlight a occurrence pattern of this disease (Mauz-Körholz et al., 2015, Gupta et al., 2020).

Thus, 20-30% of HL is diagnosed before 5 years of age compared to 5% in developed countries. The etiology seems to be multifactorial and may include several factors suc as infectious agents (epstein barr virus), genetic predisposition, immune dysregulation(human immunodeficiency virus infection, congenital immunodeficiency syndromes), environmental factors (King et al., 2014).

The treatment of HL at pediatric age requires a balance between the necessary therapy to eradicate the disease and the excessive treatment's related late toxicities. Late toxicity among survivors may affect the quality of life, even limit long term survival. A combined modality treatment approach – chemotherapy and radiotherapy – has superior event free survival rates and less late toxicity compare to chemotherapy alone. Pediatric chemotherapy for HL focused on two main strategies: avoidance of late effects and minimization of infertility using gender adapted chemotherapy (Tebbi et al., 2006; Mauz-Körholz et al., 2015). Thus, chemotherapic protocols such as ABVD (Adriamycin, Bleomycin, Vinblastine, and Dacarbazine) had demonstrated a superior efficacy but with cardiac and pulmonary toxicity due to anthracycline and bleomycin. Less toxic regimens that use lower total doses of alkylators, doxorubicin or bleomycin are indicated in favorable risk HL. In order to maintain a treatment efficacy and to preserv the male fertility, etoposide has been substituted for procarbazine in several protocols (Mauz-Korholz et al., 2010).

The relevant papers and a research grant are listed and revisited below.

Research grant:
Internal grant Depvelopment of an integrated model for the evaluation of acute lymphoblastic leukemia in children using a RT-PCR technique for gene fusion. UMF GR.T.POPA, no. 16842/30.09.2009, Iasi

Published papers:

1. Alecsa, M.-S.; Moscalu, M.; Trandafir, L.-M.; Ivanov, A.-V.; Rusu, C.; **Miron, I.-C.** Outcomes in Pediatric Acute Lymphoblastic Leukemia—A Single-Center Romanian Experience. *Journal of Clinical Medicine* 2020, 9, 4052. **IF=4,241**
2. Ivanov IC, Jitam D, Grigore GE, Zlei M, Ivanov AV, Dumitraş S, Carasevici E, **Miron IC.** Infant acute leukemia with lineage switch at relapse expressing a novel t (4; 11)(q21; q23) MLL-AF4 fusion transcript. *Romanian Review of Laboratory Medicine*. 2013 Mar 1;21(1):47-58. **IF=0,171**
3. **Miron I**, Ivanov A, Ivanov I, Dumitraş S. Detection of fusion gene--integral part of the assessment of children with acute leukemia. *Medical Surgical Journal*. 2011;115(3):731-5.
4. Efrosă I, Miron I, Tansanu I. Statistical evaluation of clinical characteristics and therapeutic management of Hodgkin disease in children over a 10 year period. *Medical Surgical Journal*. 2010;114(1):111-4.
5. Efrosă I, Miron I, Tansanu I. Clinical features and therapeutics in Hodgkin disease of children over a 25 year period. *Medical Surgical Journal.* 2009;113(1):93-6.

1.2. Prognostic Signifiance of Molecular Modification in Pediatric Acute Lymphoblastic Leukemia

1.2.1. Introduction

Acute lymphoblastic leukemia is the most common pediatric cancer in both high-income countries and low-income countries, with survival rates close to 90% in most developed countries, with low treatment-related mortality. Fortunately, this illness is also one of the better understood and effective treatments are now available, tailored to different risk groups and enhanced by upgrades in supportive care (Cooper et al., 2015).

The treatment can be adapted to the genetic profile of each patient, toxicity can be reduced and compliance maintained, resulting in optimal outcomes and remission. Cytogenetics and molecular cytogenetics completed by molecular genetics have been commonly used to detect chromosomal and genetic changes in childhood ALL. Molecular genetics, in particular, has contributed substantially to the assessment of risks and prognoses depending on the course of action, thus enhancing customized care, maximized benefits, and lower relapse rates in pediatric ALL (Friedrich et al., 2016).

Three fusion genes (FG) are known to be very important for risk classification and adequate therapy decisions: t(9:22)p190, t(4:11) and t(12.21). More specifically, t(9:22) and t(4:11) are associated with unfavourable prognosis , whereas t(12.21) in ALL, as well as t(8:21), t(15:17) and inv(16) in AML are associated with favourable prognosis (Pui and Evans, 1998; Biondi and Rambaldi, 1996; McLean et al., 1996; Borkhardt et al., 1997; Rubnitz et al., 1997).

Molecular studies have shown that translocations employing mixed lineage leukemia gene on 11q23 occur frequently in various types leukemia (De Braekeleer et al, 2012; Super et al., 1997). The previously mentioned translocation t(4;11) (q21;q23), involving the AF4 gene partner on chromosome 4, is found in 50-70% of ALL in young children and in approximately 5% of ALL in older children and adults (Foroni et al., 1997; Pui et al, 2003; De Braekeleer et al, 2005; Pui et al., 1998; van Dongen et al., 1999). At least 10 breakage points have been identified inside MLL-AF4, but the correlation between different types of FG MLL-AF4 and the patients' survival rate is yet to be established. There is limited data regarding minimal residual disease (MRD) monitoring using MLL-AF4, and some suggest that MLL-AF4 disappears in patients with total remission (Cimino et al., 1996).

Also, the Philadelphia chromosome (Ph), the result of t(9:22) (BCR-ABL fusion genes) and a marker for chronic myeloid leukemia (CML) is found in 5% of pediatric ALL and in 20-50% of adult ALL (incidence increases with age) (Maurer et al., 1991). In CML, the synthesis of a 210kDa hybrid protein named p210 occurs in the gene breakage area known as the *„major breakpoint cluster region"* (M-BCR) (Shtivelman et al., 1985). The second breakage region in BCR (m-BCR) is found almost exclusively in Philadelphia chromosome positive ALL and produces a 190kDa protein named p190 (Clark et al., 1987). In fact, 40% of Ph positive ALL have M-BCR like in CML and 60% of Ph positive ALL have m-BCR. t(12:21)(p13;q22).

The most frequent anomaly in ALL in children is ETV6-RUNX1 (Borkhardt et al., 1997). It occurs in 25% of patients, most of whom are between 1-12 years old (with a peak incidence at the age of 2-5). Until now, this translocation has not been found in T-cell acute lymphoblastic leukemia and AML. Some studies reported favourable prognosis for patients with ETV6-RUNX1, although investigations of ALL relapses showed that the frequency of these translocations was similar to that found upon initial diagnosis.

Three different techniques are used to detect minimal residual disease: flow cytometry, polymerase chain reaction (PCR) for Ig or TCR, and PCR for fusion genes produced by chromosomal translocations/inversions with breakage inside genes (Campana and Pui, 1995).

Whenever treatment can be promptly initiated and, importantly, adapted to the genetic profile of each patient, toxicity can be reduced and compliance maintained, resulting in optimal outcomes and remission (Erdmann et al., 2019) (Friedrich et al., 2016). Molecular genetics, in particular, has contributed substantially to the assessment of risks and prognoses depending on the course of action, thus enhancing customized care, maximized benefits, and lower relapse rates in pediatric ALL (Kato and Manabe, 2018; Goto, 2015; Halalsheh et al., 2011; Stary et al., 2014). The monitoring of acute leukemia patients before, during and after treatment in order to detect any presence or persistence leukemia cells was confirmed as a key factor for therapy efficiency (Foroni et al, 1997). However, universal accessibility is still a challenge and survival rates seem to echo economic and social disparities to a certain degree. In the more developed, high-income countries, survival rates have gone up to almost 90%, while in less prosperous countries, 30% of children are lost to the disease (Rivera and Ribeiro, 2014; Arora and Arora, 2016).

1.2.2. Aim

The international literature lists few studies from countries such as Romania, where pediatric patient families and oncologists must navigate less favorable social and economic conditions. The research we have been conducting and publishing aims to appraise the clinical, hematological, and the molecular modifications (MLL-AF4 t(4:11), ETV6-RUNX1 t(12:21), BCR-ABLp190 t(9:22)) associated with ALL in our Romanian patients. In doing so, we have also considered the socio-economic context and its implications for the quality and effectiveness of care.

1.2.3. Material and methods

Patients and Clinical Data

- ***Detection of fusion gene--integral part of the assessment of children with acute leukemia***

Patient cohort: The clinical prospective study from 2011 was carried out on a series of 30 cases referred to the Hematology and Oncology Department of "Sf. Maria" Children's Hospital Iasi and diagnosed with ALL and AML over the course of two years. Based on age, the patients were grouped as follows: under 2 years of age (4 patients), between 2 and 10 years old (12 patients) and over 10 years of age (11 patients) (Fig.1.1). One case presented scientific interest justifying in-depth analysis and publication subsequently in 2013.

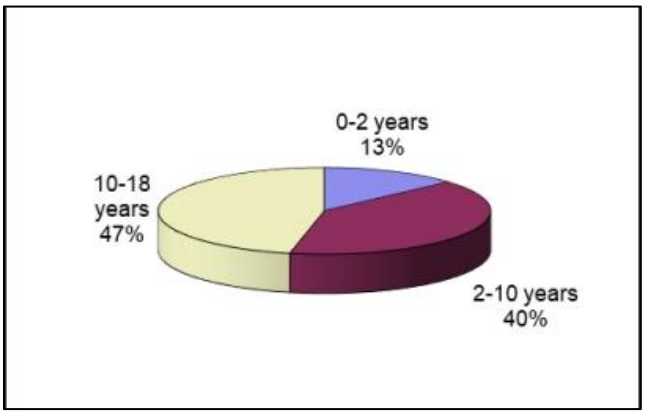

Figure 1.1. Patients number according to age groups: 0-2 years old, 2-10 years old, 10-18 years old.

- ***Outcomes in Pediatric Acute Lymphoblastic Leukemia—A Single-Center Romanian Experience***

Patient cohort: More recently, we were able to expand our research to include patient data collected over a period of 6 years and with an average follow up of 60 months. Our retrospective observational study from 2020 enrolled 132 consecutive patients newly diagnosed with ALL and admitted at the Oncology Department of the Sf. Maria Clinical Emergency Hospital for Children Iasi between 1.01.2010 and 31.12.2016. Fourteen patients were excluded after quitting treatment, leaving a total of 125 cases eligible for analysis.

Diagnosis

In all three aforementioned studies, blood samples, bone marrow (BM) aspirates, and lumbar punctures were collected at the time of diagnostic procedures and prior to any treatment. The ALL diagnosis was based primarily on the cytological examination of peripheral blood (PB) and bone marrow infiltration ≥25% blast cells (morphological and cytochemical evaluation of BM smears), and it was also confirmed through immunophenotypic analysis.

Cerebrospinal fluid (CSF) was analyzed to determine the involvement of the central nervous system (CNS). Clinical and paraclinical variables such as age; gender; clinical status at admission; white blood cells (WBC) count; immunophenotyped; molecular biology; involvement of central nervous system; response to chemotherapy; relapse; last recorded follow-up; and, where applicable, death and cause of death.

Immunophenotyping and Molecular Genetic Analysis

Immunophenotyping was carried out using a FacsCanto II Flow Cytometer and the classification criteria issued by the European Group for the Immunological Characterization of Leukemia (EGIL) (Bene et al., 1995).

Molecular tests were performed at diagnosis for the detection of the most common translocations in precursor B-cell acute lymphoblastic leukemia: MLL-AF4, ETV6-RUNX1, E2A-PBX-1, BCR-ABL-p190 and STIL-TAL1 translocation for T-cell acute lymphoblastic leukemia.

In our earlier studies, the presence of an additional set of transcripts (for AML) was investigated: PML-RARA, AML-ETO, and CBFB-MYH11.

Ribonucleic acid (RNA) extraction was made from $2x10^7$ WBC. The cells were suspended in 0.5 mL of Guanidine Thiocyanate reagent and stored at −80 °C until use. Total RNA was later isolated from the thawed cells according to the manufacturer's instructions.

For reverse transcription, 4 µl of total RNA (with concentration of 500 ng/µl) was processed with the GoScript™ Reverse Transcription Kit.

The complimentary DNA (cDNA)was diluted to a final volume of 100 µl, and 5 µl was amplified by polymerase chain reaction for the detection of fusion gene transcripts. RNA integrity was confirmed by PCR amplification of the mRNA of ABL gene, which is expressed ubiquitously in human hematopoietic cells.

RNA integrity was confirmed by PCR amplification of the mRNA of ABL gene, which is expressed ubiquitously in human hematopoietic cells.

For the amplifications, the GoTaq Green Master Mix and 10 pmol of forward and reverse primers were used (Table 1.1.) under strictly controlled conditions: 3 min at 95 °C, then 30 s at 95 °C, followed by 35 cycles of 30 s at the annealing temperature specific for each primer sets according to Table 1.1., 30 s at 72 °C, followed by 7 min at 72 °C.

Cell lines were used as positive controls for the investigated fusion genes obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen DSMZ-German Collection of Microorganisms and Cell Cultures, Department of Human and Animal Cell Cultures, Braunschweig, Germany and maintained in culture according to the recommendations from the DSMZ.

The cell line used as positive control for STIL-TAL1 fusion gene was RPMI-8402 (DSMZ ACC-290). The PCR products were migrated in a 2% agarose gel, and stained with ethidium bromide at 5 V/cm. The gel documentation was obtained with the UVP BioDoc-It System.

Table 1.1. Sequences and annealing temperatures for primer sets used for polymerase chain reaction (PCR) amplifications, for fusion genes and the reference gene (van Dongen et al., 1999)

Fusion Gene	Forward Primer	Reverse Primer	Annealing temperature
MLL-AF4	5′-CCGCCTCAGCCACCTAC-3′	5′-TGTCACTGAGCTGAAGGTCG-3′	65
ETV6-RUNX1	5′-TGCACCCTCTGATCCTGAAC-3′	5′-AACGCCTCGCTCATCTTGC-3′	
E2A-PBX1	5′-CACCAGCCTCATGCACAAC-3′	5′-TCGCAGGAGATTCATCACG-3′	
BCR-ABLp190	5′-GACTGCAGCTCCAATGAGAAC-3′	5′-GTTTGGGCTTCACACCATTCC-3′	
STIL-TAL1	5′-TCCCGCTCCTACCCTGCAA-3′	5′-CGCGCCCAGTTCGATGAC-3′	

- ***Infant acute leukemia with lineage switch at relapse expressing a novel t (4; 11)(q21; q23) MLL-AF4 fusion transcript***

In the 2013 case study, to confirm the presence of the less common MLL-AF4, additional primer pairs and standards were used (MLL-AF4 e11-e5, e10-e4, e9-e5 from IPSOGEN, Luminy Biotech Enterprises, Marseille, France) (Table 1.2.). The amplification parameters were: initial denaturation (95°C/ 2 min), followed by 35 cycles of denaturation (94°C/ 30 sec), annealing (60°C/ 60 sec), and extension (72°C/ 1 min), with an extension (72°C/ 10 min), using PalmCycler™ (Corbett, LifeSciences/ Qiagen, Germantown, MD, USA). Following electrophoresis, gels were visualized under UV in a G:BOX Chemi TM Gel Documentation System (Syngene, Cambridge, UK) and interpreted with GeneSnapTM and GeneTools TM (Fig. 1.2.).

Table 1.2. The expected dimensions of different primer pairs used as standards

	Expected dimensions for MLL1 and AF4 primer pairs	**Expected dimensions for MLL2 and AF4 primer pairs**
STD e11e5	361 bp	225 bp
STD e10e4	292 bp	156 bp
STD e9e5	115 bp	Negative
HR 339	406 bp	270 bp
bp = base pairs; STD = standard; HR = patient code.		

Sequence analysis was used for the case investigated in more detail in 2013. The MLL-AF4 fragment was purified from gel, with Wizard® SV Gel and PCR Clean-up System Promega

Inc, Madison, WI, USA (according to the manufacturer's instructions) and then analyzed by Sanger sequencing. The product was sequenced in forward and reverse reactions, using a Beckman Coulter kit (Dye Terminator Cycle Sequencing - DTCS, Quick Start Kit), a Beckman Coulter analysis software (CEQ8000 Investigator), primers previously described (Gabert et al., 2003), and the following sequencing parameters: 30 cycles at 96ºC for 20 sec, at 50ºC for 20 sec, and at 60ºC for 4 min. When the molecular response to treatment was investigated, Real Time PCR testing (RT-PCR) was performed using the same primers, a TaqMan probe (6-Fam/Tamra labeled) with the following sequence: 5'-CATGGCCGCCTCCTTTGACAGC-3' (Gabert et al., 2003) and the same standards described above as positive controls (Ipsogen, Luminy Biotech Enterprises, Marseille, France) (Table 1.2.).

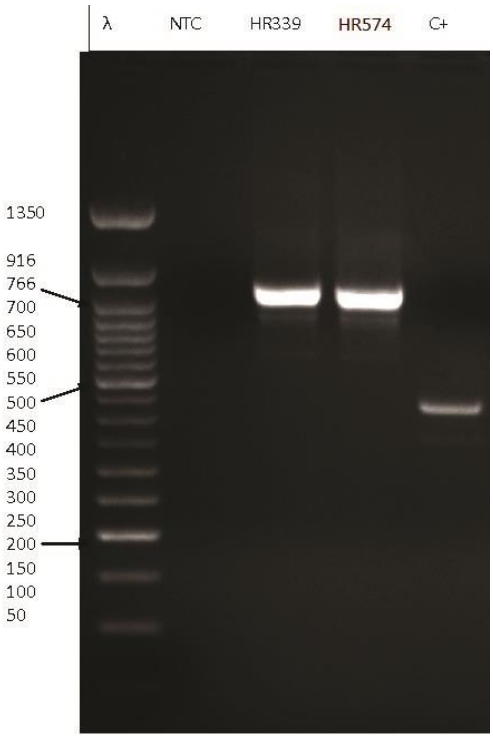

Figure 1.2. The first PCR amplification of the MLL-AF4 fusion transcript. = 50 nucleotide ladder; HR = patient codes (HR339 –at diagnosis, HR574-at relapse); NTC = no template control; C+ = MLL-AF4 e9e4 positive control.

The ABL kit from IPSOGEN was used as reference (Luminy Biotech Enterprises, Marseille, France).

Risk Stratification and Treatment Protocol

The patients received intensive chemotherapy treatment according to the Berlin–Frankfurt–Munster (BFM) ALL 2002 protocol. The protocol entailed a 4-week course of chemotherapy with three specialized drugs (vincristine, anthracycline, and asparaginase) and a corticosteroid for the induction phase, followed by consolidation and reinduction therapy adapted to risk groups, and then maintenance therapy. Tyrosine kinase inhibitors (TKIs) were added for BCR-ABL positive patients. For ALL patients under the age of 1, the treatment was established according to the INTERFANT 99 protocol. For AML patients, the AIOEP-AML protocol was used.

Treatment response in ALL patients was evaluated based on absolute blast count in the peripheral blood on the eighth day of induction therapy (prednisone good responders / PGR < 1000 blasts/μL and prednisone poor responders / PPR ≥ 1000 blasts/μL) and based on bone marrow status on day 33 of induction treatment. Bone marrow punctures and evidence of extramedullary leukemia were assessed on day 33 of induction treatment or at the end of induction phase in AML patients. Patients who had <5% leukemic blast cells in their bone marrow and no signs of extramedullary disease on day 33 were considered to be in complete remission (CR). For BCP and T-ALL, the risk stratification of patients was done based on BM evaluation on day 33 of induction therapy, absolute blast count in PB on day 8, and the presence of BCR-ABL or MLL-AF4. Patients meeting at least one of the relevant criteria for high-risk (HR) were assigned to the high-risk group and were treated with the corresponding HR therapy branch.

Statistical Analyses

- ***Outcomes in Pediatric Acute Lymphoblastic Leukemia—A Single-Center Romanian Experience***

The data were collected and analyzed using different versions of the well-known software SPSS, e.g. most recently we used SPSS v.25 (IMB Corporation, Armonk, NY, USA)**.** For continuous variables, we assessed the averages and standard deviation or the medians, depending on the normal distribution of the values. The comparisons between the statistical groups were done with the Mann–Whitney U test or the Kruskal Wallis test for continuous variables. The Levene test was used to assess the homogeneity of variances. For qualitative variables, we analyzed frequencies (absolute and relative %) and performed comparisons between groups based on the results of non-parametric tests (Yates and Chi-square). The Kaplan–Meier method was used to evaluate event-free survival (EFS) and overall survival (OS), and the log-rank test to make comparisons. The threshold for statistical significance (p) was set at $p < 0.50$. Event-free survival was defined as the time from diagnosis to the date of the last follow-up indicating complete remission or the first significant event such as signs of resistance to chemotherapy (nonresponse), abandonment of treatment, relapse, or death from any cause. Induction failure was defined as either morphological persistence of leukemic blasts in BM or extramedullary site(s) after the completion of the induction therapy. Relapse was defined as the re-infiltration of bone marrow with more than 25% blast cells or the presence of blast cells in any other extramedullary site. The period of time between diagnosis and a first significant event was considered as the first remission time. For the analysis of overall survival rates, death regardless of cause was the endpoint.

1.2.4. Results

Clinical, Paraclinical and Molecular Characteristics of Acute Leukemia Patients

- ***Detection of fusion gene--integral part of the assessment of children with acute leukemia***

In our 2011 study, 27 patients were diagnosed with BCP - acute lymphoblastic leukemia and 3 patients were diagnosed with acute myeloid leukemia. Two of the patients were diagnosed with Down Syndrome.

The most frequent clinical manifestations were: physical fatigue, loss of appetite, bone pain. Also, 19 patients presented with adenopathies, 23 with splenomegaly, 25 with paleness, while weight loss was observed in most of the patients (22 patients).

For reverse transcription, 42 ARN samples were extracted from 30 patients suffering from acute leukemia (27 patients with ALL and 3 patients with AML). The presence of fusion genes tested by RT-PCR was evaluated at diagnosis (for all patients), on the 33rd day of treatment (for 10 patients) and on the 56th day of treatment (for 2 patients).

Two patients were discharged immediately after diagnosis, while the others were discharged at the end of the induction treatment, either in order to be treated abroad or because of treatment refusal. Seven patients were still undergoing the induction treatment at the moment of publishing the paper.

Seven patients were included in the high-risk group, 20 patients were included in standard risk group, while 3 patients were diagnosed with AML.

Minor-BCR was identified in 2 patients. One of the patients presented a hypercellular form of ALL (WBC=250.000/mmc) and unfortunately died 3 days after being diagnosed.

The other patient displayed a rare type of ALL which started with bone swelling, the diagnosis being established by bone biopsy. Response to prednisone was poor on the 8th day of treatment and induction failure was noted on the 33rd day of treatment, leading to guarded prognosis.

In the 2011 cohort, the ETV6-RUNX1 translocation was found in only one, 4-year-old patient. Similarly, the MLL-AF4 gene was identified in just one newborn diagnosed with ALL 27 days after birth (Fig. 1.3.).

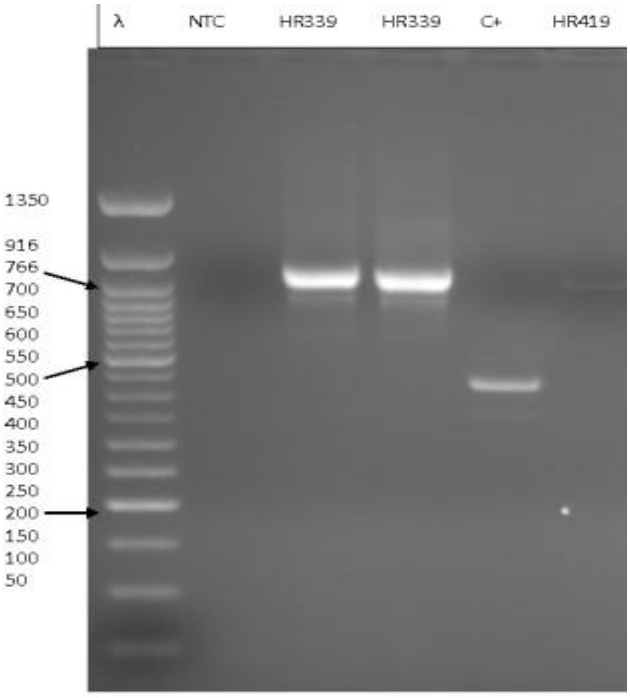

Figure 1.3. The first PCR amplification of the MLL-AF4 fusion transcript.

- ***Infant acute leukemia with lineage switch at relapse expressing a novel t (4; 11)(q21; q23) MLL-AF4 fusion transcript***

The case of the newborn, in particular, presented unique features which justified additional scientific interest, further complex molecular tests, and subsequent publication of results in 2013. The patient was a 27-day-old baby boy admitted for pallor, adenopathies, hepatosplenomegaly, and refusal to breastfeed. An abrupt increase in WBC (from 20000/mmc to 40000/mmc in only 24 hours), associated with severe thrombocytopenia (19000/ mmc) and a substantial presence of blasts with lymphoid morphology both in the bone marrow (92%) and peripheral blood (52%) were observed.

At diagnosis, the immunophenotypic expression of the following markers was investigated by flow cytometry in a PB sample (40999 cells/ mmc), at the cell surface (s): CD45, CD14, CD71, CD5, CD10, CD19, CD33, CD13, HLA-DR, CD34, CD117, CD4, CD8, CD3, CD16+56, CD20, CD22, IgM CD38, and intracellularly (ic): CD20, IgM, TdT, CD79a, myeloperoxidase – MPO. When the patient relapsed, the expression of CD15, CD36, CD11b, CD64, CD16 and all of the markers stated above was evaluated by flow cytometry in a PB sample (101850 cells/ mmc).

Based on the cytomorphological, molecular, and immunophenotypic assays performed at admission, the initial diagnosis was ALL with B-cell precursors and aberrant expression of the myeloid marker CD33. The patient was assigned to the high-risk group, being considered a "poor responder" to prednisone. The patient attained morphological and molecular remission subsequent the induction chemotherapy.

One year after diagnosis, the patient was readmitted with fever. Biological assay showed leucocytosis with a high number of blasts in the PB (~80%). Morphological and immunophenotypic blast description attributed to both, the monocytoid lineage (predominant) and the lymphoid lineage (subdominant).

At this point the diagnosis was acute leukemia of ambiguous lineage, MPAL subtype, with rearranged MLL and early medullary relapse. Although treatment was initiated promptly, no remission was obtained and the patient succumbed two months later.

Molecular tests performed at admission revealed the occurrence of a MLL-AF4 fusion transcript. None of the other transcripts evaluated was found to be positive. The MLL-AF4 amplified product was around 800 bp in size, which was nearly 370 bp larger than the e9e4 amplicon used as standard in the assay and about 120 bp larger than the biggest expected amplicon, e11e4 (Table 1.2.). The presence of the uncommonly larger MLL-AF4 fragment was confirmed with a second amplification, based on additional primer pairs MLL-1/AF4 and MLL-2/AF4, and with three different MLL-AF4 standards used for comparison (e11-e5, e10-e4, e9-e5) (Fig. 1.4).

As all amplified fragments were clearly size-fractionated by electrophoresis on the agarose gel, the MLL-AF4 fragments of interest were easily cut and purified, in order to allow for the analisys of their particular sequence. Sequence analysis revealed a MLL-AF4 product resulting from in-frame fusion between exons 12 and 4 of the MLL and AF4 genes, respectively (Fig. 1.5.).

One year later, at relapse, molecular tests were performed in order to detection any ETV6-RUNX1, BCR-ABL(p190), E2APBX1, and MLL-AF4 fusion transcripts. Due to the lineage

switch noted at relapse, the presence of an additional set of transcripts (routinely evaluated when a myeloid lineage is involved), was also investigated: PML-RARa, AML-ETO, and CBFB-MYH11.

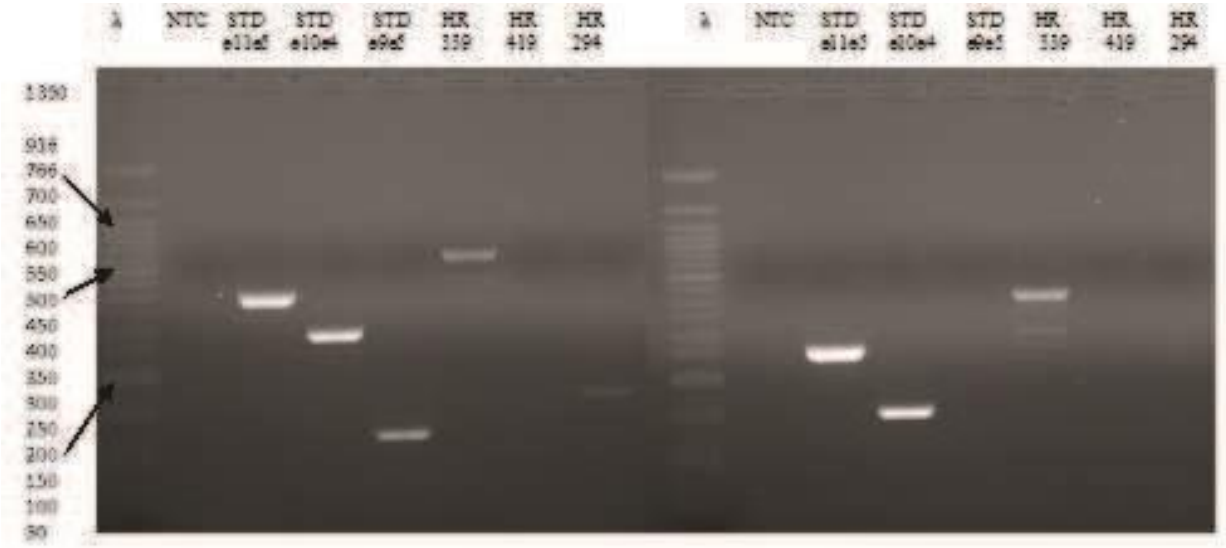

Figure 1.4. The second PCR amplification of the MLL-AF4 fusion transcript for MLL1-AF4 (left) and MLL2-AF4 (right).

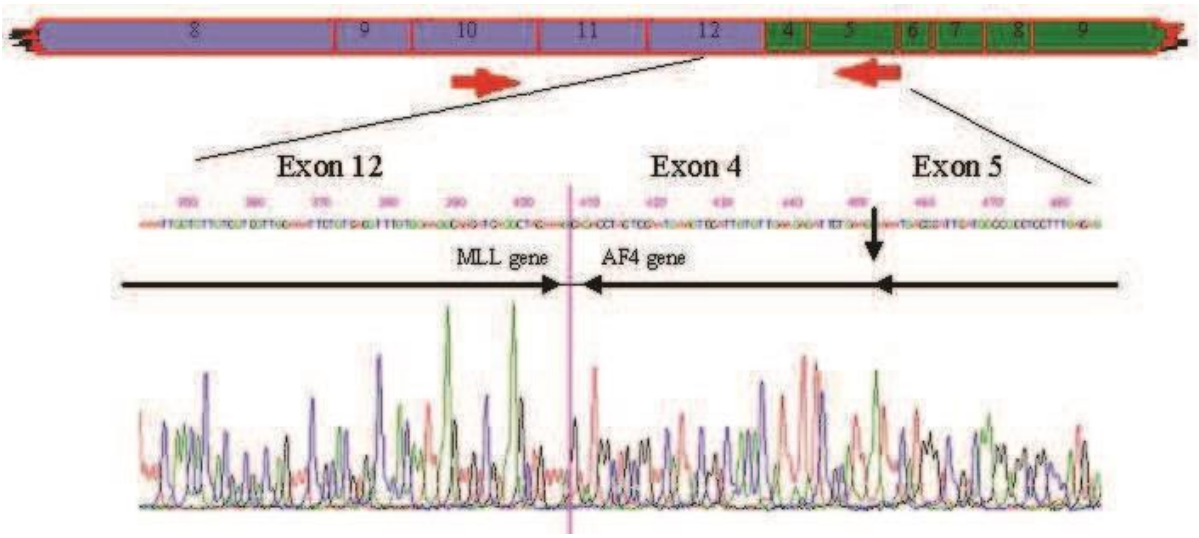

Figure 1.5. The amplicon sequence of the uncommon MLL-AF4 product idendified

The MLL-AF4(e12e4) transcript was the only one found to be present among the evaluated transcripts (either with a lymphoid, or with a myeloid lineage association). The lineage switch at relapse - both immunophenotypic (Fig.1.6.), as well as cytomorphologic assays performed at diagnosis revealed the presence of approximately 50% of B lymphoid precursors in a PB sample with 40000 WBC/mmc. The immunophenotype of these cells, as assessed by flow cytometry, was suggestive for a pro-B blockage: CD45+low CD19+ HLA/DR+ CD10- CD34+ CD22+/- CD20-

CD20ic+ low IgMs+ic - CD79a+ TdT+ CD38+int. Among the three myeloid antigens investigated at diagnosis (MPO, CD13, and CD33), only the CD33 antigen was partially positive (on more than 60% of the blast population), indicating either an illegitimate/aberrant expression of myeloid markers, or a mixed phenotype. Note should be made that no CD15 or CD64 immuno-staining was performed at diagnosis. Subdominant adult (8%) and less-differentiated (9%) monocytoid cell populations were also noticeable in the PB sample evaluated by flow cytometry at diagnosis (Fig. 1.6.).

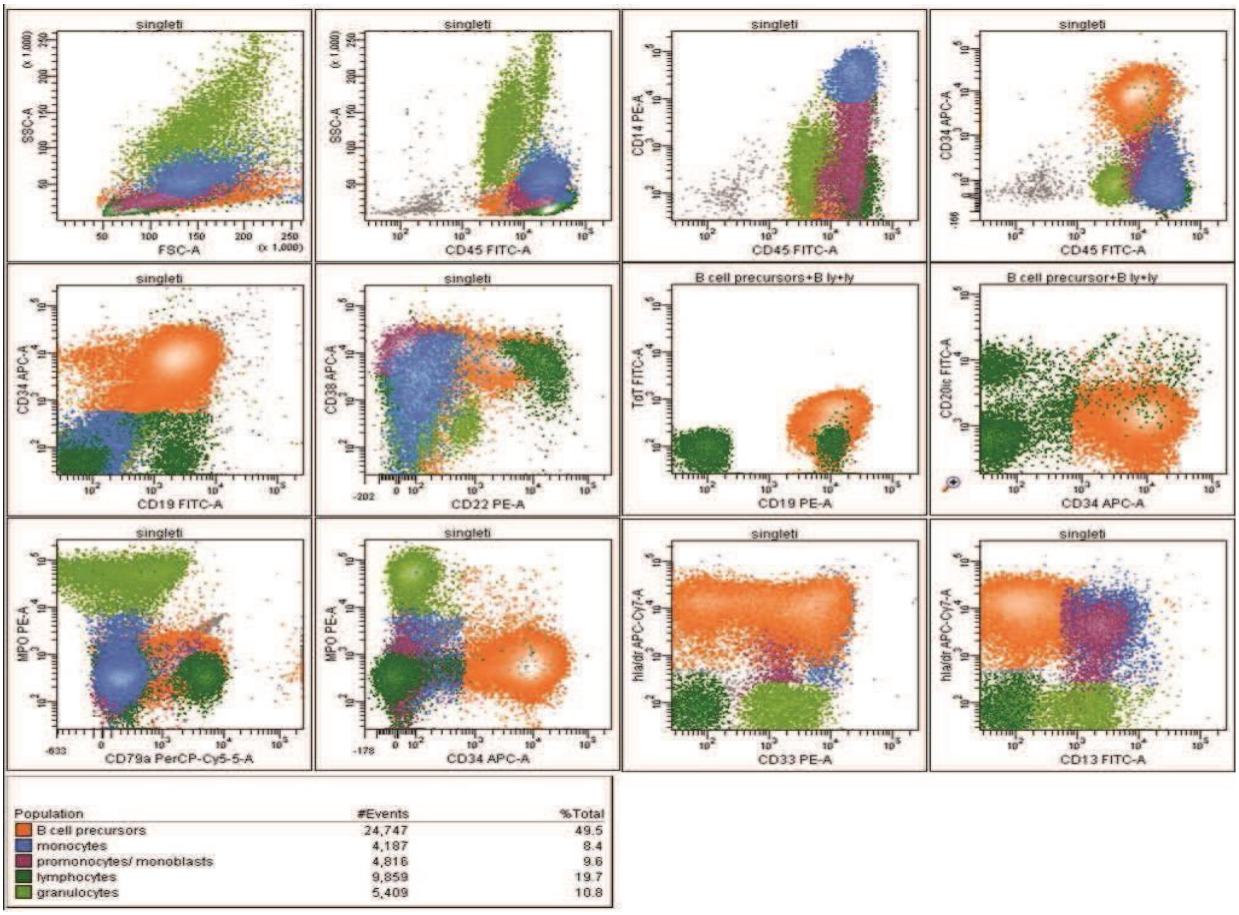

Figure 1.6. The immunophenptypic profile of cell populations detected by flow cytometry in a perpheral blood sample from an infant acute leukemia case (diagnosis). B cell precursors (orange) were found to be the dominant (49.5%) cell population and assigned as the malignant clone at diagnosis. Other cell populations may also be distinguished with the marker combinations used: lymphocytes (dark green), monocytes (blue), promonocytes (violet), granulocytes (light green).

Immunophenotypic examination carried out at relapse revealed the presence of mixed malignant lineages (both lymphoid and monocytoid) in a PB sample, with the predominance of the latter: 62% monocytoid cells (promonocytes and monocytes: CD45+ HLA/DR+ CD33+ CD13+ CD117- CD34- CD14+/- CD64+high CD36+ CD11b+/- CD2- CD15+ CD16- MPO-); 17% B lymphoid precursor cells (CD45+low CD34+ HLA/DR+ CD19+ CD20s- CD22-/+

MPO- TdT+low with mixed lineage phenotype: CD64+low CD15+int CD33+/-); 11% granulocytes; 7% lymphocytes (Fig. 1.7.).

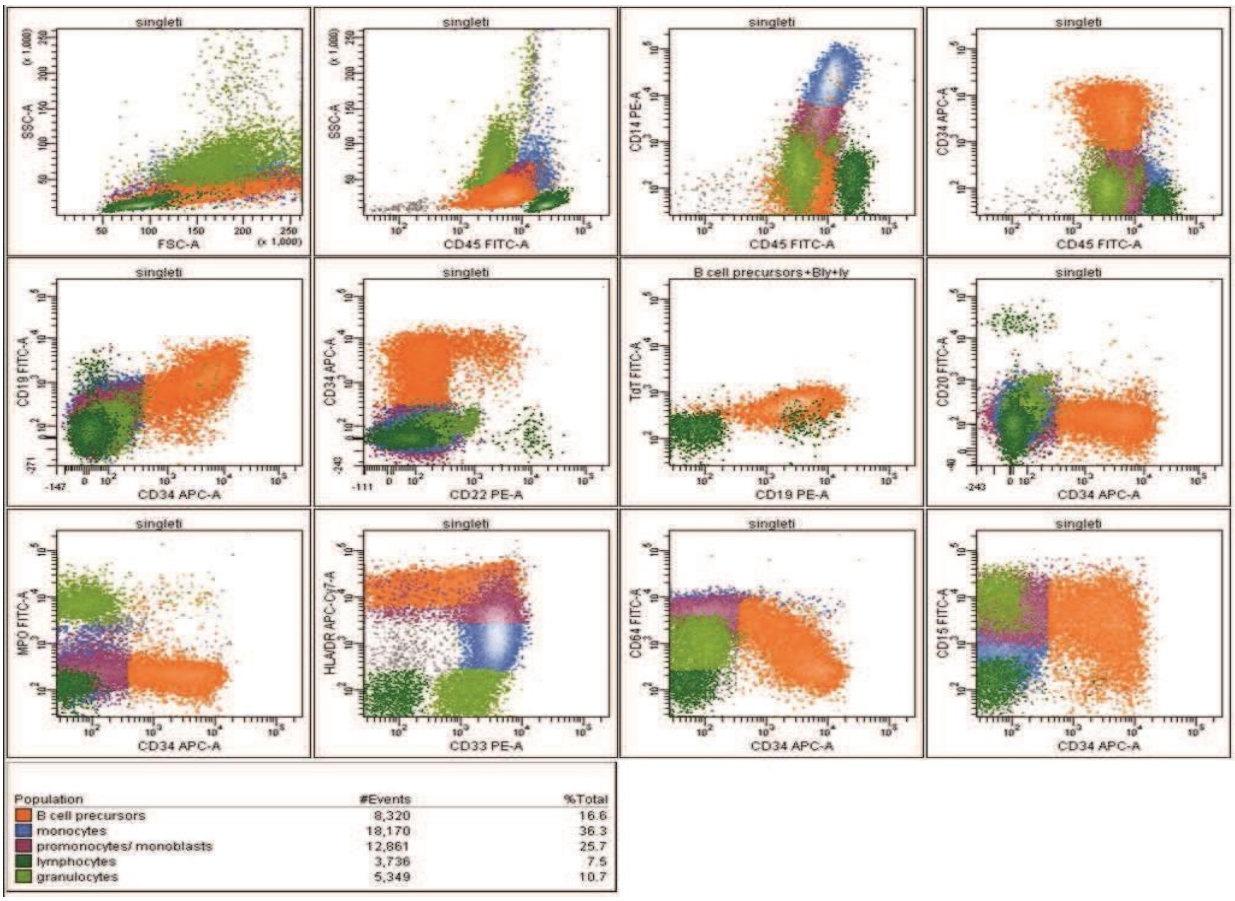

Figure 1.7. The immunophenptypic profile of cell populations detected by flow cytometry in a perpheral blood sample from an infant acute leukemia case (relapse). Monocytoid cells, monocytes (blue) and promonocytes (violet), were found to be the dominant (36% and 26%, respectively) cell populations at relapse. A subdominant (17%) population of B cell precursors (orange) was also identified as having an aberrant phenotype (co-expression of myeloid markers: CD15, CD64, CD33). Other cell populations may also be distinguished with the marker combinations used: lymphocytes (dark green) and granulocytes (light green).

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We continued to research ALL on more incoming patients in the years that followed, and we published the results internationally in 2020. For this more recent cohort of 125 patients, the median age at diagnosis was 5 years, and ALL was most prevalent in the 1-4 age group (44.8%) and among boys (male to female ratio of 1.84:1). The characteristics of the patients with T-cell acute lymphoblastic leukemia and of those with precursor B-cell acute lymphoblastic leukemia are summarized in Table 1.3. The mean age at diagnosis was higher in T-ALL patients compared with BCP-ALL at $p = 0.008$. The WBC was found to be much higher than the normal range in T-ALL (median $70.61*10^3/\mu L$). The most frequent clinical features were hepatomegaly and splenomegaly

in both BCP-ALL and T-ALL. The presence of initial CNS involvement was observed in only 4.8% of patients.

Table 1.3. Clinical and biological characteristics of 125 patients diagnosed with acute lymphoblastic leukemia.

Characteristics	Overall *n* = 125	BCP-ALL *n* = 107	T-ALL *n* = 18	*p*-Value
Age in years (mean ± SD) §	6.78 ± 4.83	6.38 ± 4.77	9.17 ± 4.57	0.009 *
Age, median (range) **	5 (3; 11)	4 (3; 10)	8 (6; 13)	
age 1–4 years	56 (44.8%)	54 (50.5%)	2 (11.1%)	0.011 *
age 5–9 years	34 (27.2%)	26 (24.3%)	8 (44.4%)	
age 10–14 years	21 (16.8%)	15 (14%)	6 (33.3%)	
age 15–17 years	14 (11.2%)	12 (11.1%)	2 (11.1%)	
Gender (male/female) ‡	81/44 (64.8/35.2%)	69/38 (64.5/35.5%)	12/6 (66.7/33.3%)	0.857
White blood cells, median (range), (*10^3/μL) †	11.14 (3.59; 44.30)	8.32 (3.40; 32.82)	70.61 (14.00; 192.64)	0.001 *
<10.0	61 (48.8%)	57 (53.3%)	4 (22.2%)	0.003 *
10.0–50.0	33 (26.4%)	29 (27.1%)	4 (22.2%)	
>50.0	31(24.8%)	21 (19.6%)	10 (55.6%)	
Initial CNS involvement ‡ (absent/present)	119/6 (95.2/4.8%)	102/5 (95.3/4.7%)	17/1 (94.4/5.6%)	0.871
Initial mediastinal mass ‡ (absent/present)	114/11 (91.2/ 8.8%)	104/3 (97.2/2.8%)	10/8 (55.6/44.4%)	<0.001 *
Hepatomegaly ‡ (absent/present)	37/88 (29.6/70.4%)	35/72 (32.7/67.3%)	2/16 (11.1/88.9%)	0.044 *
Splenomegaly ‡ (absent/present)	38/87 (30.4/69.6%)	36/71 (33.6/66.4%)	2/16 (11.1/88.8%)	0.037 *
Prednisone response ‡ (PGR/PPR)	105/20 (84/16%)	93/14 (86.9/13.1%)	12/6 (66.7/33.3%)	0.030 *
Risk stratification (*n* = 122) † Standard/High	98/24 (80.3/ 19.7%)	87/17 (83.7/ 16.3%)	11/7 (61.1/ 38.9%)	0.022 *

§ Mann–Whitney U test; † Pearson Chi-square test; ‡ Yates Chi-square test; ** values presented as median (range: Q25–Q75); * marked effects are significant at $p < 0.05$; T-ALL—T-cell acute lymphoblastic leukemia; BCP-ALL—precursor B-cell acute lymphoblastic leukemia; SD—standard deviation; PGR—prednisone good response; PPR—prednisone poor response; CNS—central nervous system.

Risk assessment was possible in 122 patients; four of the seven patients who died before day 33 assessment had previously fulfilled the high-risk group criteria. A significant number of PPR and HR patients were noticed in the T-ALL group.

Immunophenotype data were available for all patients: 107/125 (85.6%) were precursor-B cell ALL and 18/125 (14.4%) were T-ALL. Multiplex RT-PCR assay to determine the presence of the most common ALL fusion genes was performed in 111 of 125 patients.

The clinical, hematological, and prognostic characteristics were analyzed (Table 1.4.).

Table 1.4. Molecular subgroups and other parameters at diagnosis, as well as during and after treatment

Parameters *n* = 111	ETV6-RUNX1 *n* = 21	E2A-PBX1 *n* = 4	BCR-ABL *n* = 3	ETV6-RUNX1 and E2A-PBX1 *n* = 1	NONE *n* = 82	*p*-Value
% of total	21 (18.9%)	4 (3.6%)	3 (2.7%)	1 (0.9%)	82 (73.9%)	
Age, years ^ median (range) **	3(3–5)	9.5(4.5–13.5)	9(5–15)	3(3–3)	6(3–12)	0.031 *
Age 1–4 years †	15 (71.4%)	1 (25%)	0	1 (100%)	31 (37.8%)	0.041 *
Age 5–9 years	5 (23.8%)	1 (25%)	2 (66.7%)	0 (0%)	25 (30.5%)	
Age 10–14 years	1 (4.7%)	1 (25%)	0 (0%)	0 (0%)	17 (20.7%)	
Age 15–17 years	0 (0%)	1 (25%)	1 (33.3%)	0 (0%)	9 (10.9%)	
Initial WBC (*10^3/μL) ^ median (range)	8.32 (34.0–28.0)	89.96 (40.86–140.0)	20.57 (4.87–41.74)	140.00	10.37 (3.39–55.29)	0.320
<10.0	11 (52.4%)	0 (0%)	1 (33.3%)	0 (0%)	41 (50%)	0.246
10.0–50.0	5 (23.8%)	2 (50%)	2 (66.7%)	0 (0%)	19 (23.2%)	
>50.0	5 (23.8%)	2 (50%)	0 (0%)	1 (100%)	22 (26.8%)	
Response to Prednisone † (PGR/PPR)	20 (95.2%) 1 (4.8%)	3 (75%) 1 (25%)	3 (100%) 0 (0%)	0 (0%) 1 (100%)	65 (79.3%) 17 (20.7%)	0.033 *
Response to induction therapy † CR Induction Failure Unknown	18 (85.7%) 0 (0%) 3 (14.3%)	4 (100%) 0 (0%) 0 (0%)	2 (66.7%) 1 (33.3%) 0 (0%)	1 (100%) 0 (0%) 0 (0%)	73 (89%) 0 (0%) 9 (11%)	<0.001 *
Relapse † No Yes Unknown	16 (76.2%) 2 (9.5%) 3 (14.3%)	2 (50%) 1 (25%) 1 (25%)	1 (33.3%) 1 (33.3%) 1 (33.3%)	1 (100%) 0 (0%) 0 (0%)	58 (70.7%) 10 (12.2%) 14 (17.07%)	0.553

^ Kruskal–Wallis test; † Pearson Chi-square test; ** values presented as median (Q25–Q75); * marked effects are significant at $p < 0.05$; WBC—white blood cells; PGR—good prednisone response; PPR—poor prednisone response; CR—complete remission.

The most common rearrangement was ETV6-RUNX1, found in 21 patients (18.9%), whose median age at diagnosis was 3 years. Twenty patients with ETV6-RUNX1 had good prednisone response (95.2%) and eighteen achieved complete remission (85.7%).

We also detected four patients with E2A-PBX1 translocation. In this subgroup, the median age at diagnosis was 9.5 years. At the end of the induction phase, all these patients were in complete remission and one relapsed later during the maintenance phase of the protocol.

Three cases made up the BCR-ABLl subgroup; these patients' median age upon diagnosis was 9 years. Although all three presented PGR, they were all lost to the disease: one relapsed 13 months after the initial diagnosis, one was a non-responder, and one succumbed to chemotoxicity. In addition to the above, one patient presented both ETV6-RUNX1 and E2A-PBX1 translocations;

he achieved complete remission and was subject to no other significant events during the study follow-up period.

MLL-AF4 rearrangement was absent in all the patients.

Treatment Outcomes

- ***Detection of fusion gene--integral part of the assessment of children with acute leukemia***

In this study, published in 2011, we evaluated the incidence of fusion genes at diagnosis and during the induction phase. Of the 30 patients diagnosed, 2 died before the study was completed. Also, 2 patients were discharged at the parents' request without receiving treatment and, in the other cases, induction treatment was initiated. Seven patients were poor prednisone responders and one was chemoresistant.

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Based on this experience, we then sought to collect data covering a longer period of time and a larger number of patients in order to achieve a more comprehensive and accurate understanding of the patients' treatment outcomes.

This is reflected in the study published in 2020, in which we analyzed 125 cases presenting over the course of 6 years, treated according to the ALL IC-BFM-2002 protocol, and followed up for an average of 60 months.

Regarding the efficiency of prednisone treatment, 93 patients diagnosed with BCP-ALL (86.9%) were good responders, and 12 patients diagnosed with T-ALL (66.6%) responded poorly (p = 0.030). At the end of the induction protocol, 112 (89.6%) patients achieved complete remission (Table 1.5.).

The overall relapse rate across all the studied cases was 11.2%. The majority of relapsed patients experienced an early relapse (11/14, 78%). The site of relapse was BM in nine patients (64%) and CNS in the other five (36%), and there was no combined relapse. 28.5% of relapsed patients were from the BCP-ALL high-risk group and relapsed early.

Also, the length of time from diagnosis to relapse, resistance to chemotherapy (non-response), recurrence, or death (event free survival) was evaluated.

For the BCP-ALL patient group, the median EFS value was 56 months, while in the T-ALL patient group, the median EFS was of 40.5 months (Table 1.5.). The median overall survival rates from diagnosis until death were the same. This may be because of the small number of T-ALL cases, but it is also worth mentioning that death has often been noticed to follow shortly after relapse, resistance to chemotherapy, or recurrence (Table 1.5.).

Death occured in 19 cases (15.2%) and the causes were represented by infections in 11 (57.8%), followed by relapse-progressive disease in 5 (26.3%) patients and chemotherapy related toxicity in 2 (10.5%) patients (Table 1.5.)..

It is also worth mentioning that, whenever it occurred, death seemed to follow shortly after relapse, resistance to chemotherapy, or recurrence.

Table 1.5. Response to chemotherapy, relapse, and outcome. Univariate analysis.

Parameters	**BCP-ALL** ***n* = 107**	**T-ALL** ***n* = 18**	**Overall** ***n* = 125**	***p*-Value**
Relapse: (14 of 125 cases) ‡ Early Late	12 (11.2%) 9 (8.4%) 3(2.8%)	2 (1.9%) 2 (1.9%) 0 (0%)	14 (11.2%) 11 (8.8%) 3 (2.4%)	0.894
Death before CR ‡	6 (5.6%)	1 (5.6%)	7 (5.6%)	0.842
Death after CR	9 (8.4%)	3 (16.7%)	12 (9.6%)	
Event free survival rate § median (range) (months) 95% CI for median	56 (36–74) 41–67	40.5 (10.5–42) 29.5–41.5	52 (26–59) 40.5–56.5	0.327
Overall survival §, median (range) (months) 95% CI for median	56 (38–75) 43–66	40.5 (10.5–44) 29–42.5	52 (21–63) 39.5–58	0.278

† Pearson Chi-square test; ‡ Yates Chi-square test; § Kaplan–Meier method—log-rank test; ** values presented as median (Q25–Q75); * marked effects are significant at $p < 0.05$; PGR—good prednisone response; PPR—poor prednisone response; CR—complete remission. 95% CI—95% confidence interval; T-ALL—T-cell acute lymphoblastic leukemia; BCP-ALL—precursor B-cell acute lymphoblastic leukemia.

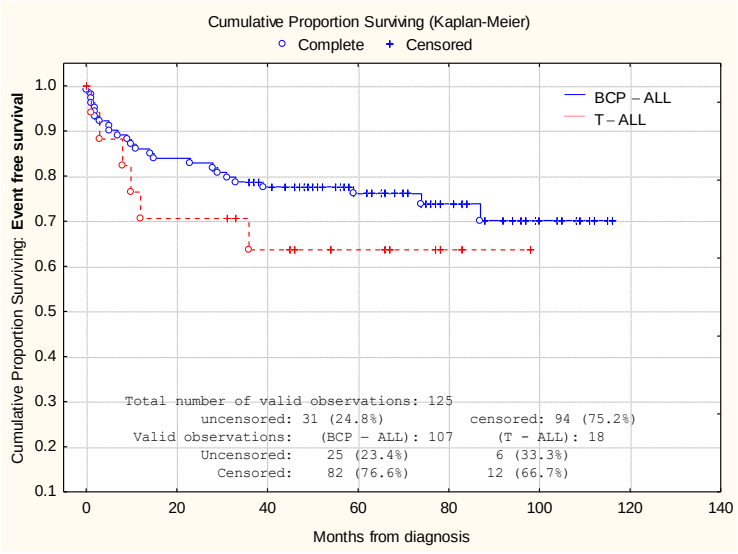
Based on the Kaplan–Meier analysis, we were able to compare the 1-year EFS rates between BCP-ALL cases (86.3%) and T-ALL cases (71.4%), as well as the 3-year EFS rates between the two groups (78.9% and 64.9%, respectively) and 5-year EFS rates: 76.1% in BCP-ALL vs. 64.9% in T-ALL (Fig.1.8a). Similarly, we analyzed the 1-year OS rates for BCP-ALL cases (87.2%) vs. T-ALL cases (71.4%), the 3-year OS rates (82.9% vs. 71.4%, respectively), and the 5-year OS rates (81.6% vs. 71.4% respectively) (Fig.1.8b).

1.2.5. Discussion

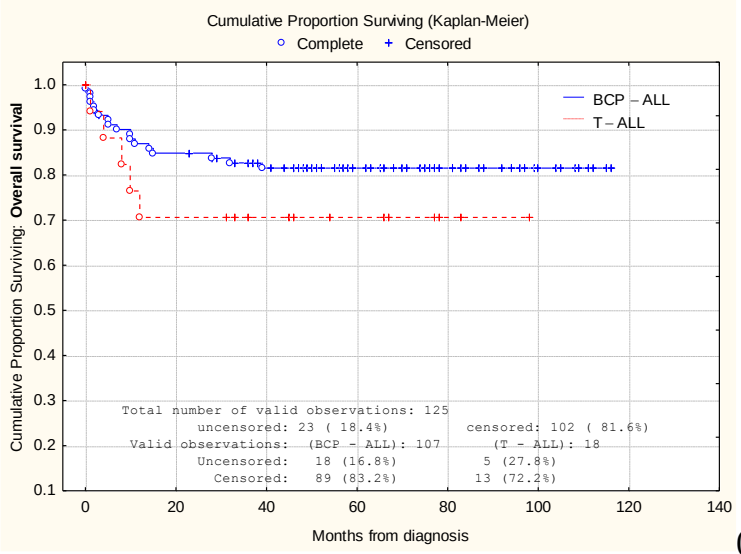
In the absence of prompt and effective treatment, children with acute lymphoblastic leukemia can quickly succumb to the disease. Fortunately, complete remission is possible in a majority of cases, depending on medical and non-medical factors. In developed countries, cure rates are now as high as 80% to 90% (Pui et al., 2012). By contrast, middle-income countries (MICs) report both more cases and lower cure rates, even when MICs have the necessary capabilities to adhere to and follow international therapeutic protocols. In these countries, specialized cancer care centers are fewer and farther between, chemotherapy medication can be inconsistently available, patients may delay presentation and treatment, compliance is more difficult to maintain, and related toxicity is more consequential and even lethal (Friedrich et al., 2016; Kato and Manabe, 2018).

In Romania, the Berlin–Frankfurt–Munster for pediatric ALL is the most used protocol. This protocol is known internationally to lead to complete remission in about 85–95% of pediatric ALL patients (Pui et al., 2012). While access to the latest diagnostic and therapeutic approaches have greatly improved, some methods remain largely unavailable in Romania(e.g., MRD testing).

The clinical and biological features of our patients from both studied periods seem to be consistent with other published data.



(a)



(b)

Figure 1.8. Comparative analysis of Kaplan–Meier curves between precursor B-cell acute lymphoblastic leukemia (BCP-ALL) cases and T-cell acute lymphoblastic leukemia (T-ALL) cases. Comparison of event-free survival (EFS) (a) rates and overall survival (OS) rates (b).

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High rates of pediatric ALL were found in the 1–4 age group, while the mean age of our patients was 5 years, the same as that in the ALL-BFM 2002 study (Halalsheh et al., 2011). The male to female ratio observed in our more recent research was 1.84:1, higher than U.S. reported data (1.56:1) (Siegel et al., 2017), Nordic countries (Hjalgrim et al., 2003), or Surveillance, Epidemiology, and End Results (SEER) database (Barrington-Trimis et al., 2017). Similar male preponderance was reported in Pakistan (66.1%) and in some areas of India (Fado et al, 2015; Arora et al, 2009).

The importance of genetic events in the classification, therapy, and prognosis of ALL has gained recognition among researchers as well as clinical practitioners. Most of the clinical signs and symptoms have been shown to correlate with particular biological or phenotypic properties, and these connections are now known to have significant prognostic implications (Park et al., 2011). The fusion gene positivity occurred in 26% of the patients we reported on this study. The most prevalent molecular modification was ETV6-RUNX1: it was confirmed in 21 (18.9%) patients and had a significant prognostic role, as the patients responded well to the treatment protocol. The frequency of other fusion genes such as BCR-ABL1 and E2A-PBX1 was much lower in our patients and similar to that found by other published studies (Rose-James et al., 2016). Both BCR-ABL1 and E2A-PBX1 had some adverse characteristics such as hyperleukocytosis and older age at diagnosis. In high-risk molecular modifications such as BCR-ABL1, CR was achieved in two of three cases; one of these patients suffered an ALL relapse 13 months after diagnosis and died, confirming the adverse prognostic role of this translocation in pediatric ALL. Also, E2A-PBX1 has been associated with a poor outcome, but this can apparently be improved with more intensive chemotherapy (Gaynon et al, 1997). In our experience, all our E2A-PBX1 positive patients (3.6%) achieved complete remission on day 33; one of them had an early relapse. The frequency of E2A-PBX1 gene in our patients was similar to that other published studies reported in the United States, but much lower compared with Europe (Izraeli et al., 1993; Meyer-Monard et al, 2006; Pui et al., 2004). Interestingly, one patient presenting both ETV6-RUNX1 and E2A-PBX1 fusion genes achieved complete remission and did not require treatment in the 2 years that followed.

We believe studies such as ours from 2020 provide important information with regard to the treatment outcomes and follow up of pediatric patients who share the characteristics of those presenting at our Hematology and Oncology Department. Similar research from Romania is currently scarce in the international literature, which could be regarded as an opportunity for our scientific community.

More specifically, by comparing our results with other published data from middle and high-income countries, the outcomes in our study group were poorer. This may be due to the incidence of high-risk leukemia (BCR-ABL1) and unfavorable factors such as increased WBC (>50.0 $*10^3/\mu L$), T-cell immunophenotype, and older age (15–17 years) (Goldberg et al., 2003; Matloub et al., 2015). Also, a study performed by EUROCARE that included pediatric patients diagnosed with ALL during 2000–2007 showed a 5-year survival rate in Eastern Europe, varying from 70% in Bulgaria to above 80% in Poland, compared with more developed countries where the 5-year survival far exceeded 80% in all countries. Romania, however, was not included in this research (Gatta et al., 2014). According to our findings, at the 5-year mark, the OS and EFS of our Romanian patients were 81.6% and 76.1%, respectively, for BCP-ALL and 71.4% and 64.9%, respectively, for T-ALL. The difference between OS and EFS might be attributed to the small number of T-ALL patients in our cohort (18 patients vs. 107 patients in the BCP-ALL group).

The mortality in pediatric ALL depends not only on the disease itself, but also on the occurrence of complications such as infections and chemotherapy-related toxicities. Rubnitz et al.

concluded that the main cause of death (80%) during the induction phase was related to infectious causes (Rubnitz et al., 2004). In our cohort, 57.8% of patients died of infectious causes. Other causes of death included chemotherapy-related toxicities or bleeding. Moreover, 5.6% of patients died before achieving complete remission, which is more than in developed countries, where the induction mortality rate is below 2% (Ma et al., 2015).

Last but not least, is worth revisiting here the uncommon case of infant ALL reported in ***Infant acute leukemia with lineage switch at relapse expressing a novel t (4; 11)(q21; q23) MLL-AF4 fusion transcript*** Its diagnosis, treatment and clinical management were complex and challenging. The genetic anomaly was initially thought to be a result of either different breakpoints or the insertion of an intronic fragment, but it was finally found to involve novel breakpoints within exon 12 of MLL and exon 4 of AF4. This particular translocation had not been reported before, since the most frequent breakage points in infant ALL were known to occur between intron 11 in MLL and exon 4 in AF4 (De Braekeleer, 2005; Felix et al., 1998). Although not mandatory for terminal myeloid differentiation, MLL function was found to influence the survival and expansion of multipotent progenitors. Similar to other reports, our case had an aggressive clinical evolution (Stasik et al., 2006; Jiang et al., 2005; Park et al., 2011; Lou et al., 2010; Sakaki et al., 2009). The immunophenotypic analyses also revealed an infrequent development, showing a lineage switch at relapse. The bilineage nature of the case suggests that the t(4;11) transforms an uncommitted, multipotential progenitor. According to some authors, the retention of the same genetic anomaly at relapse may be an indication that the switch involved the original leukemic clone and did not reflect the selection of a co-existing, sub-dominant subclone (Stasik et al., 2006). As acute leukaemias of ambiguous lineage are rare subtypes, the pathogenic mechanisms triggering their development have remained obscure. It is still unclear whether the presence of two distinctive malignant clones represents connected disease entities or the consequences of different degrees of maturation from a common precursor. Cases of infant ALL that demonstrated a switch to a monocytoid lineage have been previously reported, harboring distinct genetic lesions, such as the MLL gene translocation with the CREP-binding protein gene, or MLLT10 gene, or other types of MLL rearrangements, with some of these authors implying that the MLL gene rearrangement occurred in precursor cells having a double differentiation potential (towards either B lymphocytes or monocytes) (Sakaki et al., 2009; Jiang et al., 2005; Lou et al., 2010).

1.2.6. Conclusion

Our research experience and results confirm the importance of understanding the genetic underpinnings of pediatric ALL in developing and tailoring therapies for optimal outcomes. We welcome the improved access to innovative, low-cost approaches which help identify and analyze genetic anomalies efficiently. Sensitive detection of MLL rearrangements, accurate lineage assignment, and early lineage switch prediction can have life saving clinical benefits, informing the clinician in treatment-related decisions and increasing the precision of minimal residual disease detection techniques. With regard to the response to therapy and follow up of pediatric patients with ALL, taking into consideration both (para)clinical characteristics and genetic profiling, we

find that day 33 remains a key reference point in evaluating outcomes and predicting prognosis. Although our patients' survival rates were inferior to similar reports from high-income countries, the difference was smaller than expected, which is an encouraging result.

1.3. Approaches to the Management of Pediatric Lymphoma

1.3.1. Introduction

Hodgkin's lymphoma is a clonal malignancy of the lymphatic system that arises from B-cells of germinal and postgerminal centres. Based on differences in the histological picture and the neoplastic cell phenotype HL can be divided into two distinct subgroups: classical HL (cHL) which is recognized in majority of patients (95%) and nodular lymphocyte-predominant HL (5%). cHL type can be further divided into four subtypes: lymphocyte-rich lymphocyte-predominant (LR-LP), nodular sclerosis (NS), mixed cellularity (MC), and lymphocyte-depleted (LD) (Swerdlow et al., 2008). Typical for all subtypes of cHL is the presence of neoplastic Reed-Sternberg (RS) cells, which are not observed in any other neoplastic diseases. Tumor is comprised of RS cells in minority, while the majority is an inflammatory background, crucial for growth and survival of cancer cells (Bonadonna et al., 2004). Microenvironment is composed of various cell types including lymphocytes, eosinophils, histiocytes, and plasma cells, which interact with numerous cells including CD4+ and CD8+ T cells, B lymphocytes, plasma cells, or dendritic cells, through secretion of different cytokines and chemokines. The complex microenvironment interactions are unique among lymphomas and are responsible for initiation and progression of HL. The maximum incidence according to the data from the specialized literature is between 10 and 15 years, being rare under the age of 5 years. The 5-year survival rate reaches 95-98% (Swerdlow et al., 2008; Connors, 2009; Bonadonna et al., 2004).

1.3.2. Aim

Limited data Hodgkin lymphoma in Romanian population is available. Therefore, we carried out these studies to analyze the outcome of Romanian children suffering from HL and treated with different regimens. The aim of these studies was to investigate the clinicopathological characteristics, prognostic factors, treatment and follow-up results of children who were diagnosed with Hodgkin lymphoma in our Pediatric Oncology Department. A special attention was paid for the patients under 5 years old and diagnosed with HL.

1.3.3. Material and methods

Patients and (Para)Clinical Data

- ***Statistical evaluation of clinical characteristics and therapeutic management of Hodgkin disease in children over a 10 year period***

This article presents a clinical retrospective study conducted over a 10 year period, between 1994 and 2005. A number of 57 patients aged between 2 to 17 year admitted to Pediatric Oncology Department of "St Mary" Children Hospital Iasi were included.

The patients were diagnosed with Hodgkin lymphoma and treated in our center. Patients epidemiological characteristics, onset symptoms, histopathological type of the disease, treatment results and treatment toxicity were assessed.

The staging was performed according to Ann Arbor classification. Imagistic studies such as chest Xray, abdominal ultrasound, computer tomography (CT) were used for staging purposes. Also, laboratory tests such as complete blood count, erythrocyte sedimentation rate (ESR), lactate dehydrogenase (LDH) were used.

- ***Clinical features and therapeutics in Hodgkin disease in children over a 25 year period***

This article comprise a study conducted in the Hematology-Pediatric Oncology Department of the Emergency Clinical Hospital for Children beween January 1980 to December 2004. A number of 48 patients diagnosed with Hodgkin's lymphoma, under the age of 5 year-old, out of a total of 129 patients were included.

The following were evaluated: clinical signs, histological type, stage and risk group classification, treatment response, post-radiotherapy and chemotherapy complications.

Biological tests such as complete blood count and blood smear, ESR, LDH, renal and liver function, bone marrow puncture, imagistic test (chest xray, abdominal ultrasound, CT) and lymph node biopsy were used for initial diagnosis and staging of the disease.

The staging was performed according to the modified Ann-Arbor classification. Systemic symptoms (B symptoms): weight loss more than 10% in the last 6 months, recurrent fever > 38 ° C, night sweats were evaluated.

Treatment protocol

All patients included in both studies underwent chemotherapy. The most common regimens used until 1991 were COPP (Cyclophosphamide, Oncovin, Procarbazine, Prednisone) or MOPP (Nitrogen Mustard (Mechlorethamine), Vincristine, Procarbazine and Prednisolone). Further, the combination of ABVD (Adriamycin (Doxorubicin), Bleomycin, Vinblastine, and Dacarbazine) and OEPA (Vincristine, Etoposide, Prednisone, Doxorubicin) for boys or OPPA (vincristine, procarbazine, prednisone, and doxorubicin) for girls were preferred for first line treatment and ICE regimen (Ifosphamide, Carboplatin, Etoposide) for second line treatment. Radiotherapy was used in selected patients.

Surveillance of treatment efficiency and monitoring of disease progression were performed continuously by imaging and laboratory methods. Recurrences were demonstrated by repeat biopsy and anatomopathological confirmation.

1.3.4. Results

- ***Statistical evaluationof clinical characteristics and therapeutic management of Hodgkin desease in children over a 10 year period***

The average incidence of disease was 5.7 case per year. The group included 31 boys (54.38%) and 21 girls (45.62%).

The average age at diagnosis was 10 years, with a slightly asymmetrical unimodal distribution, with values close to the average and a slight tendency towards the lower values of the age parameter. Gender distribution revealed a male/female ratio of 1/1.19.

Regarding the histological type, we identified 2 cases with lymphocyte-rich lymphocyte-predominant (3.50%), 18 with nodular sclerosis (31.58%), 32 with mixed cellularity (56.14%) and 5 patients with lymphocyte-depleted (8.78%).

The disease staging according to the Ann Arbor classification revealed a high incidence of stage II (66.66%), whereas in 52 cases the enlargement of the cervical nodes and a large mediastinal mass in 29 cases were noted. In 35 patients B staging symptoms were identified. According to staging and risk group criteria, twenty-six cases (40.35%) were treated with a chemotherapic protocol and in 34 cases (59,65%) a combined radio-chemotherapy protocol was used. COPP or MOPP protocols were used until 1991, subsequently replaced by ABVD and OPPA/ OEPA, and the second-line protocol used was ICE. Post-treatment early follow-up revealed 96.49% remission rate.

An early relapse was noted in 8.77% of the cases and 10.52% of the patients had a late relapse. The mortality analysis indicated a survival rate of 98% at the end of the study period, with the specification that 7 patients were unaccounted for.

The Pearson correlation between late evolution and the risk group indicated a statistically positive average relation with p=0.005, accordingly we may state that a high risk is at diagnosis is associated with a high tendency towards unfavorable evolution.

The complex logistic regression and four parametric predictors were considered: age group, gender, histology and risk group. It was proved that only 2 of the 4 included differentiate to a certain extent the three evolution modalities. Evolution without relapse was recorded in 97.6% of the cases, whereas relapse occurred in less than 12% of the cases.

The final regression model indicated that the early relapse probability decreases in children under 10 years of age, as well as in those included in the lower risk group.

Early complications were: medullary toxicity (15 cases), lung tuberculosis (7 cases), metabolic complications (3 cases). Late respiratory complications such as pulmonary fibrosis were recorded in 8 cases and cardiac complications occurred in 7 cases.

- ***Clinical features and therapeutics in Hodgkin disease in children over a 25 year period***

In this study, 48 children were under the age of 5 and accounted for 37% of the total 129 cases of Hodgkin lymphoma.

The sex distribution revealed a boy/girl ratio of 41/7 (boys 85.42% and girls 14.58%). According to histopathological type, 7 cases (14.58%) were diagnosed with lymphocyte-rich lymphocyte-predominant, 6 cases (12.50%) with nodular sclerosis, 31 cases (64.58%) with mixed cellularity, 4 cases (8.34%) with lymphocyte-depleted.

Staging of the disease (Ann-Arbor classification) in patients with Hodgkin's disease in our study revealed a high incidence of stage II (27 cases 56.25%) compared to stag I (15 cases - 31.25%), stage III (3 cases -6.25%) and stage IV (3 cases - 6.25%).

B staging symptoms were present in a small number of patients, 9 of 48 patients presented fever, night sweats or weight loss.

The most common regions involved was the cervical region (92%), followed by supraclavicular region and mediastinal region.

The correlation between histology and the location of Hodgkin's disease revealed an increased incidence of histological type with CM at all locations.

The treatment regimes were used according to the stage of the disease and risk group. Thus, chemotherapic regimens such as MOPP, ABVD, hybrid MOPP ABV were used in 35 cases (72.91%) and radio-chemotherapy treatment was used in 13 cases (27.09%). An interruption of treatment due to the family's non-compliance was noticed in 3 patients.

Survival assessment at 3 years follow up for our patients in correlation with events (recurrence, pulmonary or cardiac complications, death) that occured revealed:

• Stages I and II of the disease, without the presence of systemic signs, are favorable prognostic factors with a 96% event free survival at 60 months compared to Stage III 48% event free survival at 60 months and Stage IV with a 18% event free survival at 60 months. The differences in survival rates between HL stages are statistically significant ($p < 0.05$) conferring this parameter a prognostic value;

• The primary location of the disease at one or two lymph nodes groups, excluding hilar lymph nodes and mediastinal mass, are favorable prognostic factors (EFS of 94% compared to 48% for Stage III B and IV).

The histological type with lymphocyte-rich lymphocyte-predominant is a favorable prognostic factor compared to nodular sclerosis and mixed cellularity. There are no statistical differences between forms with nodular sclerosis and those with mixed cellularity.

Complete remission was achieved in 38 (79%) cases; in 3 cases incomplete remission was revealed during post treatment assessment. In seven patient posttreatment assessment wasn't performed due to non-compliance. Regarding the long term follow-up, 20 patients mentained CR, 15 patients relapsed: 5 patients presented an early relapse, respectively a late relapse in 10 patients. Thirteen patients were lost to follow up.

The following early complications were identified in studied patients: medullary toxicity in 3 cases, pulmonary tuberculosis in 3 cases and metabolic complications in 4 cases. As a late complication, one case with pulmonary fibrosis and respiratory dysfunction was diagnosed.

The functionality of the heart was monitored by echocardiography and electrocardiography with no adverse event.

1.3.5. Discussion

- ***Statistical evaluationof clinical characteristics and therapeutic management of Hodgkin desease in children over a 10 year period***

In literature are described two age peakes for HL: between 5 and 6 years and between 10 and 15 years, with rare occurrences below 5 years of age (Lanzkowsky, 2010; Pinkerton et al., 2004). Our study indicated lower age ranges at diagnoses, with an increased male: female ratio.

The favorable prognosis factors were the histological type - stages I and II, the absence of systemic symptoms and the primary localization of the disease into one or two peripheral nodal regions, except for hilar adenopathy and mediastinal mass (EFS 84% as compared with 48% for stages III B and IV).

The non-cross resistant chemotherapy has become a standard treatment approach (Orbelin et al., 1992, Weiner et al., 1997, Ruhl et al., 2001). In our study, we applied an MOPP, ABVD, hybrid MOPP ABV chemotherapy scheme. Although this was a forced choice in our case, it provided the standard treatment and allowed the assessment of treatment success. We determined the risk adapted treatment approach based on stage, the presence of B symptoms, and especially the number of chemotherapy cycles. The most common late complication was pulmonary toxicity secondary to bleomycin administration, which required a risk group adapted therapeutic regimen.

The event-free survival rate at 3 years after the diagnosis was 98% in our patients. The treatment outcome of pediatric patients treated for HL in the North-Eastern of Romania is similar with prior published data in middle income countries (Lanzkowsky, 2005; Stoneham et al., 2007).

- ***Clinical features and therapeutics in Hodgkin disease in children over a 25 year period***

The study published revealed an increased incidence of HL in pediatric patients below 5 years of age: 48 (37.20%) patients of a total of 129 cases. There were no similar data found in the specific literature.

An increased number of boys were diagnosed in this group age (85.41%). Hodgkin lymphoma patients in the 15 year old or younger age group have a male/ female ratio of 3:1 (Hsu et al., 2007). The epidemiological characteristics of HL are different in developed countries compared to developing countries.

Nodular sclerosis was the most common subtype seen in developed countries, while MC was the most frequently seen subtype in developing countries (Huang et al., 2004; Hsu et al., 2007). Similar data was reported in our patients.

Numerous studies determined a number of poor prognostic factors on the overall survival rate, such as: male gender, advanced age, stage IIIB or stage IV disease, nodular sclerosis type, the presence of B symptoms, bulky mediastinal mass, extranodal disease, the number of lymph nodes involved, elevated ESR (Schellong at al., 1999; Ruhl et al., 2001). The classification of patients in risk groups and the identification of prognostic factors, offered the chance of multimodal therapy, so that the overall outcome exceeds 96% for survival at 5 years in the case of stages I and II. For stages III B and IV 5 year survival rate was 48%.

1.3.6. Conclusions

The clinico-epidemiological pattern of Hodgkin's lymphoma in our studies is different to that observed in other western Europe’s countries, with a high rate in younger age. Male predominance, mixed cellularity as the commonest histological type, the stage at presentation are similar to other studies presented. The stage IV disease, ESR elevation, associated with "B" symptoms and bulky disease are prognostic factors of 5-year event-free survival rate. Our

treatment policy was succesful regarding the similar survival rates. The treatment outcome of pediatric patients treated for HL and presented in our studies is similar with prior published data in middle income countries.

Chapter 2. Studies in Pediatric Solid Tumors

2.1. A Profile of Pediatric Solid Tumors – Background

There are fundamental differences between pediatric cancers and those that occur in adulthood. Genetics, risk factors, etiology of cancers are just some of them. A large heterogeneity of pediatric cancers in noted across continents. While in the adult population, approximately 80% of neoplasms are located in the respiratory, gastrointestinal and reproductive organs, in childhood only <5% of neoplasms affect these organs. Moreover, the histopathology of pediatric neoplasia differs significantly from adults: in children, embryonic and immature cells can be found at different stages of development (Greaves and Maley, 2012; Armstrong et al., 2014). Thus, the diagnosis and therapy must be individualized according to the clinical picture and the extent of the tumor (Oeffinger et al., 2006).

According to the estimations of the International Agency of Research in Cancer over 175.000 new cases of pediatric cancer will be diagnosed worldwide every year. The incidence rates of pediatric cancer range between 96-136/ million children/ year in males and between 70-116/million children/year in females (Siegel et al., 2017).

The first edition of the International Classification of Childhood Cancer classifies tumors into 12 diagnostic groups: leukemias, lymphomas, central nervous system tumors, sympathetic nervous system tumors, neuroblastoma, renal tumors, liver tumors, bone tumors, soft tissue sarcomas, germ cells tumors and unspecified malignant cancers.

The most common types of cancers depends on the socioeconomic spectrum – in high income countries: leukemia, lymphoma, brain tumors and in low income countries – NHL (Linet et al., 1999; Siegel et al., 2017).

Depending on the location and the histology of the neoplasm, the treatment can last between 1 - 3 years, followed by periodic follow-up for 3 - 7 years. Due to the high potential for secondary malignancies due to chemotherapy, follow-up may be necessary throughout life. In high income countries, 5 years survival rates as 80% were attained(Oeffinger et al., 2006).

Central nervous system tumors

Central nervous system tumors are the most common solid tumors that occur in pediatric age and are the leading cause of cancer-related mortality in children. It occupies, after acute leukemias, the second place in frequency (approximately 25%) in the malignancies of children under 16 years.

The incidence in the USA, reported by CBTRUS (Central Brain Tumor Registry of the United States), in 2013, was 3.3 cases / 100,000 children, with approximately 4,100 newly diagnosed cases annually (Dolocek et al., 2012). The disease may occur at any age, with the highest incidence in the 0-4 years age group (5.77 cases / 100,000 children) and the lowest in the 10-14 years age group (4.78 cases / 100,000 children). Statistics show that boys are more affected than girls, with a ratio of 1.25 to 1 (Ostrom et al., 2013).

The most common tumors encountered in the 0-14 age group are pilocytic astrocytomas (20%) and medulloblastomas (16%). Gliomas are found in over 50% of brain tumors in children under 15 years of age. In the age group 15-19 years, the incidence of pilocytic astrocytomas and pituitary gland tumors predominates. Also, in this age group, gliomas represent 45% of CNS tumors (Louis et al., 2007).

The diagnosis of brain tumors is often difficult because the clinical symptoms may mimic those of common childhood diseases and the diagnosis might be delayed (Wilne et al., 2007). The tumor clinical signs may vary depending on age, location, and aggressiveness. Correct diagnosis and staging are required for the selection of the most appropriate therapy. Multidisciplinary approach of multiple pediatric specialists in the fields of neurosurgery, radiatiotherapy, neurooncology, endocrinology, psychology and rehabilitation is required (Albright, 1993; Villanueva-Meyer et al., 2017).

Pediatric rhabdomyosarcoma

Soft tissues sarcomas (STS) are a group of non epithelial extraskeletal cancers with an annual incidence of 2-3 cases/100.000 and comprise 1% of all pediatric cancers. The classification is based to the adult tissue remebling: Rhabdomyosarcoma (RMS) – about 50% of STS and non-rhabdomyosarcoma soft tissue sarcomas (NRSTS) – very rare tumors that affects mostly the teenagers, with a pooor response to chemotherapy (Ognjanovic et al., 2009; Yohe at el., 2019).

Rhabdomyosarcoma is a highly malignant mesenchymal tumor that may occurs at any age. One of their characteristics is the local invasiveness and a high risk of metastasize. The classic stratification of RMS divides into 2 groups: the favorable histologic group – embryonal subtype and unfavorable histological group – alveolar subtype (Amer etal., 2019; Yohe at el., 2019).

The site of the disease can be anywhere in the body, with the most common location on head and neck region and genitourinary tract (bladder, vagina, parastesticular). The site can be associated with a tumor histologic subtype: botryoid histology is common on genital mucosa of females or on head-neck region. The extremities are associated with a alveolar subtype. Given the propensity to metastasize, 15-20% of present distant metastasis at diagnosis (Yohe at el., 2019).

Multimodal treatment strategies are necessary in these patients, should be planned according to patients risk stratification and involve surgery, chemotherapy, radiotherapy (Walterhouse and Watson, 2007; Gosiengfiao et al., 2012; Mandeville, 2019). The gold standard in pediatric RMS is a combination of vincristine, actinomycin and cyclophosphamide (VAC regimen) or a combination of ifosfamide, vincristine, actinomycin-D (IVA regimen), with a 6-12 months duration and similar survival rates. Numerous toxicities should be taken into account: neutropenia, peripheral neurophaty, veno-oclusive disease(Gosiengfiao et al., 2012).

Due to local progression or relapse rate, radiotherapy with a dose between of 40-55 Gy plays a major role in RMS treatment. Radiotherapy is necessary in all RMS patients except for certain embryonal RMS (Mandeville, 2019).

Surgery is one component of the multimodal therapy in RMS management and the indication depends on the site of the tumor, on tumor size and may be an aggressive or a more conservative surgery, with organ sparing. Wide resections are necessary for a better disease control. It can be used after primary chemotherapy (Walterhouse and Watson, 2007).

Multiple factors, such as socioeconomic factors, the delay of the diagnosis, the advances stages at diagnosis, the treatment abandonment, treatment toxicities, the non optimal multimodal approach have a specific role in RMS pediatric patients outcome (Punyko et al., 2005). As a result of the multimodal approach of these patients an improvement of the 5 year survival rate more than 80% was noted in localized disease cases. However, not the same can be said about advanced stages where de 5 year survival rate is less than 30% (Crist et al., 2001).

The main preoccupation that I had in this direction of research was materialized in the next papers:

Published papers:

1. Diaconescu S, Burlea M, **Miron I,** Aprodu SG, Mihăilă DO, Olaru C, Miron L. Childhood rhabdomyosarcoma. Anatomo-clinical and therapeutic study on 25 cases. Surgical implications. *Romanian Journal of Morphology and Embryology*. 2013 Jan 1;54(3):531-7. **IF=0.723**
2. Ciobanu A, **Miron I**, Tansanu I. Features of brain stem tumors in children. *The Medical-Surgical Journal.* 2012;116(1):56-61.
3. Ciobanu A, **Miron I**, Tansanu I, Dumitrescu G, Indrei A. Pediatric Brain Tumors-Morphological Findings And Prognostic Factors. *Romanian Journal of Functional & Clinical, Macro-& Microscopical Anatomy & of Anthropology*. 2011 Oct 1;10(4).
4. **Miron I,** Miron L, Dumitraş S, Aprodu G, Ciobanu A, Tansanu I. Statistical study of the evolution over ten years of the clinical and therapeutic approach in childhood soft tissue sarcoma. *Medical Surgical Journal*. 2007;111(2):358-62.

2.2. Pediatric Rhabdomyosarcoma – histopathology and therapeutic approach

2.2.1. Introduction

Rhabdomyosarcomas is a highly malignant childhood cancer and the most common pediatric soft tissue sarcomas in pediatric age, constituting 5–8% of all malignancies during

childhood (Lanzkowsky, 2010). The annual incidence is between 4.5 per million in children less 18-year-old.

Histologically, RMS are classified into embryonal tumors with botryoid and spindle cell variants, typically described in young children, alveolar RMS occurring in teenagers and young adults and undifferentiated (pleomorphic) RMS arising in adults. Several distinct histologic groups have prognostic significance, including embryonal rhabdomyosarcoma, which occurs in 55% of patients; the botryoid RMS (5%); alveolar RMS (20%); undifferentiated sarcoma in 20% of patients (Enzinger and Weiss, 1995; Dagher and Helman; 1999; Lanzkowsky, 2010). The other category of non-rhabdomyosarcoma tumors in children and teenagers represents 3% of the solid malignancies under 18 years old (Lanzkowsky, 2010; Enzinger and Weiss, 1995; Stout, 1946; Childs, 1949).

The most common sites involved are the head and neck (42%), followed by genitourinary tract (34%) and extremities (11%). Signs and symptoms vary of the anatomic site of the tumor. The tumor size, histology, localization, clinical variant and cytogenetic characteristics are prognostic factors in pediatric RMS (Dagher and Helman; 1999; Lanzkowsky, 2010; Gallego Melcón and Sánchez de Toledo 2007).

In 1972, through Constitution of the Intergroup Rhabdomyosarcoma Study (IRS) a multimodal treatment approach of these tumors is enabled (Maurer et al., 1988; Maurer et al., 1993; Crist et al., 1995; Breneman et al., 2003) and the TNM system, clinical grouping system based on therapeutic decision, risk stratification and identification of favorable or unfavorable appurtenance of the tumors contributed to an unitary and better supportive care and systematic application of increasing effective surgery, chemo- and radiotherapy and have dramatically improved five years survival rates over the last two decades from 10–20% exceeding 70% nowadays (Lanzkowsky, 2010; Hayes-Jordan and Andrassy, 2009; Dasgupta and Rodeberg, 2012).

2.2.2. Aim

Our study aims to evaluate the epidemiological characteristics and treatment outcome the of 25 consecutive cases managed according to contemporary protocols and to evaluate whether the therapeutic standards achieved in RMS in developed countries can be reproduced in our activity.

2.2.3. Material and methods

A retrospective analysis was performed on 25 medical records of pediatric patients diagnosed with RMS who were admitted, treated and followed up "St. Mary" Children Emergency Hospital Iasi, Romania, during a 12 years period, between 2000 to 2011. The medical records were reviwed for personal data, presenting symptoms and signs, primary tumor site, staging, post-surgical grouping and risk stratification of the lesions, imaging studies and therapeutic protocols were obtained from the patients medical records.

In order to establish the diagnosis and staging, standard radiological exams, ultrasound / computed tomography / magnetic resonance imaging were performed.

Pathological examination completed by electron microscopy established the RMS type.

The study group is numerically limited but still relevant. Univariate analysis was performed using the chi-square test considered to be significant when the *p*-value was <0.05.

2.2.4. Results

The study group included a number of twenty-five patients, 12 boys and 13 girls with a median age of 6.7 years. The age distribution peaked between three and nine years. The most frequently affected site was genitourinary tract in 12 (48%) patients, followed by the trunk and extremities in eight (32%) patients, the head and neck and retroperitoneum was involved in two cases each and biliary tract one case (Fig. 2.1, Fig. 2.2).

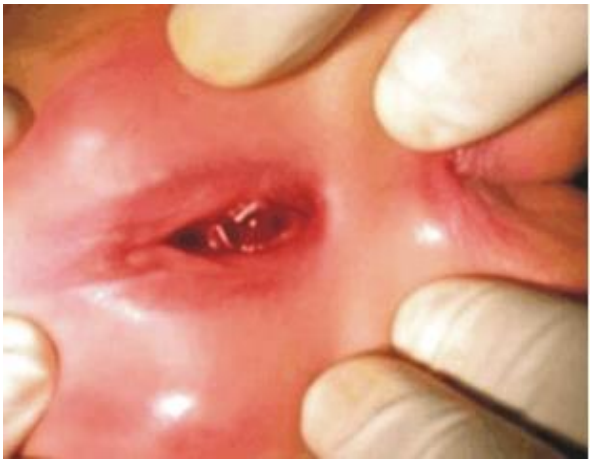

Figure 2.1. Vaginal botryoid RMS in a 10 months female.

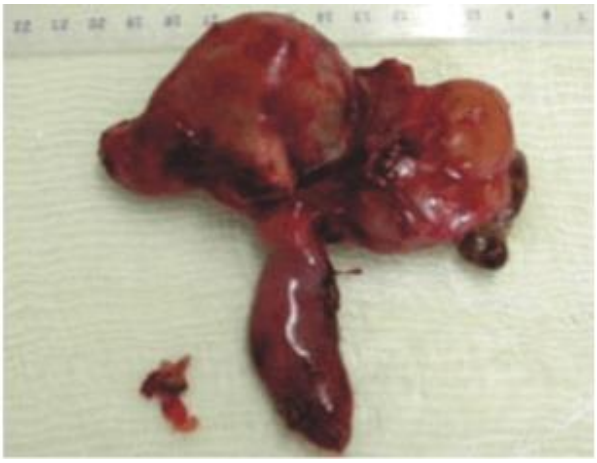

Figure 2.2. Biliary tree botryoid RMS in a 4-year-old female.

The presenting features included swelling or painless mass in varying sites in 15 (60%) cases, local pain and features of organ compression in seven (28%) cases each and local bleeding in two (8%) cases. The biliary tract tumor developed early jaundice. The non-specific pattern of presentation in our group of subjects delayed the hospital admission from two to six months, especially for the four patients aged 12–17 years. Four cases between 1–5 years were brought to the hospital in maximum two weeks after onset. Seventy-five percent of children had been checked and eventually received different treatment for other common pediatric conditions. Pretreatment TNM staging system included four (16%) patients in stage I, also four in stage II, eight (16%) in stage III and nine (36%) cases in stage IV.

According to the IRS post-surgical-based grouping system, only three (12%) patients were group I (complete excision with “clear” margins) and 12 (48%) in group II (macroscopic excision) and III (residual disease). The biopsy was performed in the remaining 10 patients with advanced or metastatic disease.

Regarding the risk group stratification, three patients were included in low - embryonal RMS, in intermediar risk group embryonal or non-embryonal RMS in unfavorable sites 12 (48%) patients and in high risk group - inoperable or metastatic RMS 10 (40%) patients.

Embryonal RMS was the most common histological subtype in 20 (80%) cases (including five botryoid and three spindle cell tumors). At the same time, our study differs from many other papers by the presence of only one (4%) alveolar RMS but especially of three cases of pleomorphic RMS rarely reported in children (Wijnaendts et al., 1994; Furlong and Fanburg-Smith, 2001). The embrinonal RMS 20 (80%) cases showed round to spindle small, medium to poorly undifferentiated cells with hyperchromatic nuclei having a known favorable outcome (Fig. 2.3).

The botryoid subtype was observed in five patients and spindle cell RMS in three patients. Botryoid subtype account for 20% of all cases of RMS being particularized by the formation of polypoid masses demonstrating malignant cells in the myxoid stroma with caveat that a cambian layer is essential for diagnosis (Fig. 2.4., Fig. 2.5., Fig. 2.6.).

Fusiform RMS account for 12% for all cases containing scattered or polygonal spindle cells with abundant brightly eosinophilic cytoplasm and collagen (Fig. 2.5, Fig. 2.6).

Alveolar RMS only one (4%) case typically presented fibrous septa separating clusters of small round cells in an alveolar growth pattern with eccentric, small nuclei and scant eosinophilic cytoplasm.

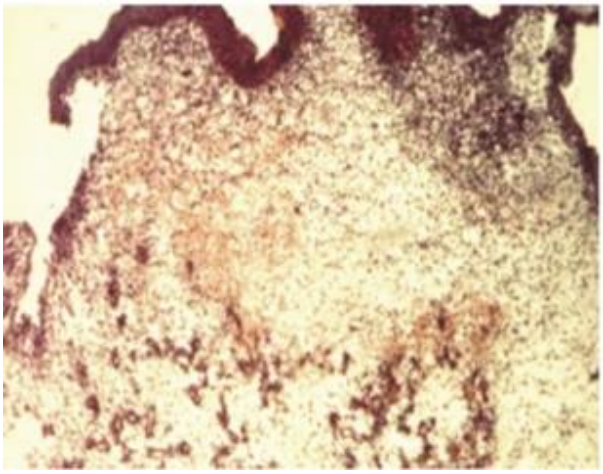

Figure 2.3. Embryonal RMS (Goldner – Szekely staining, x40).

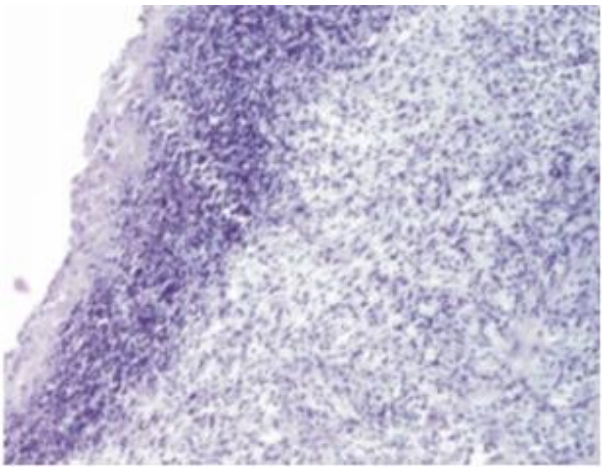

Figure 2.4. Botryoid RMS; Suburothelial cambian layer (HE staining, x40).

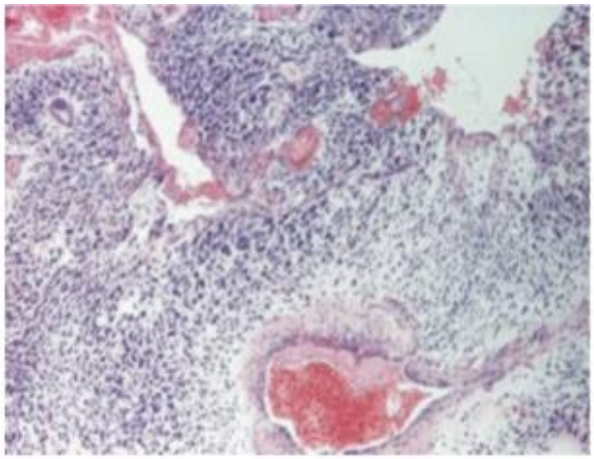

Figure 2.5. Embryonal RMS proliferate by spindle cells (HE staining, ×40).

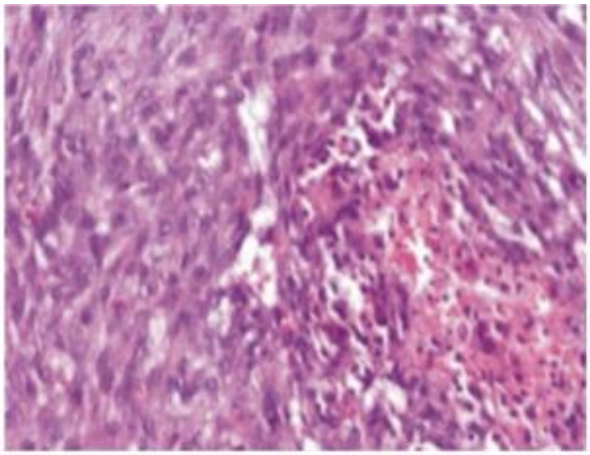

Figure 2.6 – Spindle cell RMS (HE staining, ×100).

Rare rhabdoid features were present (cytoplasmic bodies, eosinophilic nucleoli) (Fig. 2.7., Fig. 2.8.). We also noted three pleomorphic RMS, very rare described in children, a high-grade sarcoma with "bizarre" polygonal, round or spindle cells with abundant cytoplasm, hyperchromatic nuclei and atypical mitosis but without embryonal or alveolar cellular elements (Fig. 2.9., Fig. 2.10.).

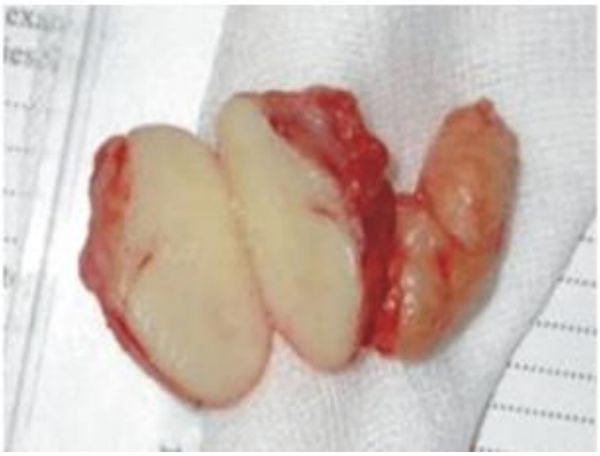

Figure 2.7. Alveolar RMS; operative specimen with lymphnode.

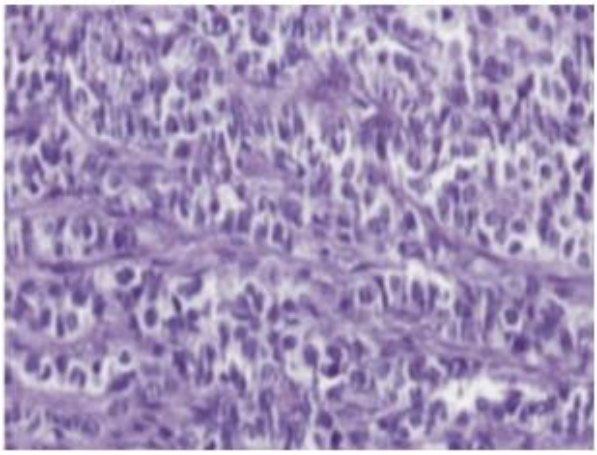

Figure 2.8. The same case: microscopy (HE staining,×100).

Immunohistochemical IMC staining for myogenin (extremely sensible and specific for rhabdomyoblastic differentiation), desmin, vincristin, CD45 in lymphocytes but no in tumor, CD34 in vessels but no in tumor (CD99 and NSE) contributed to a better diagnosis support and treatment stratification in these patients (Fig. 2.11., Fig. 2.12.).

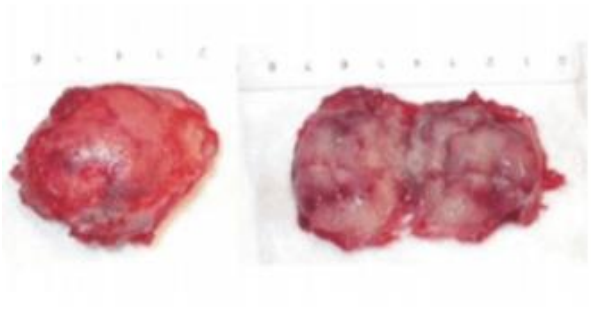

Figure 2.9. Pleomorphic shank's RMS; operative specimen.

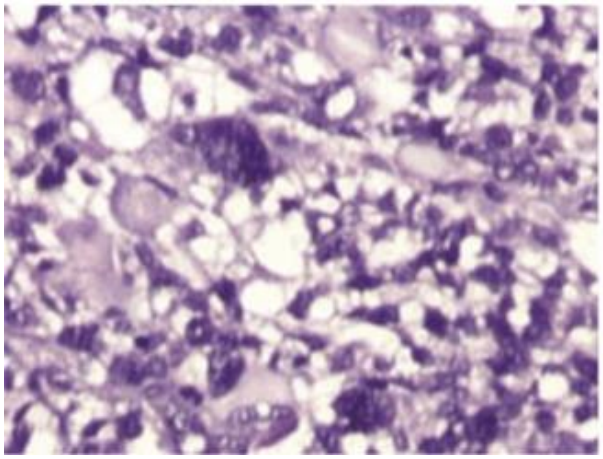

Figure 2.10. The same case: microscopy (HE staining, ×200)

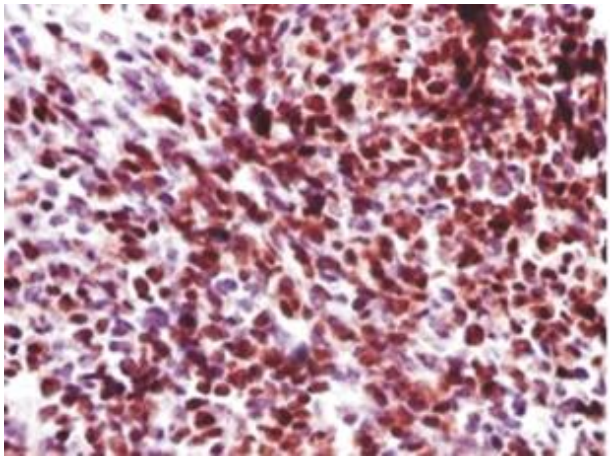

Figure 2.11. Embryonal RMS; myogenin, ×100.

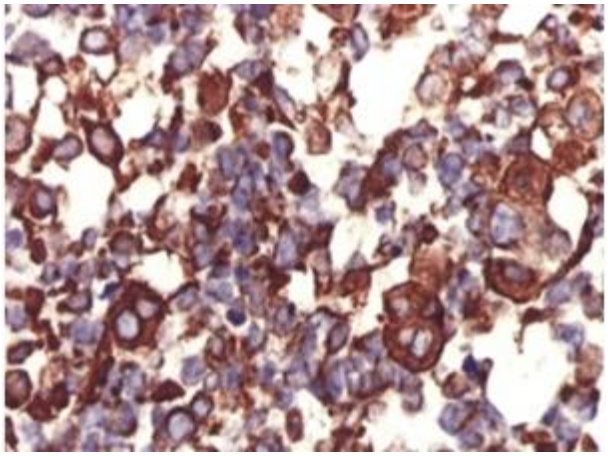

Figure 2.12. Pleomorphic RMS; desmin, ×200.

In our experience, the gradual introduction of the contemporary multidisciplinary approach using complete surgical resection, prolonged courses of multiagent chemotherapy but sporadic radiation therapy improved the therapeutic results in the srudied cohort. Complete macroscopic surgical removal of the tumor was obstained in 9 of 15 patients and depended of the tumor initial site (bladder or prostate), whilst other six cases located on the head and neck, retroperitoneal or biliary and genitourinary tract presented residual disease. All patients were treated with chemotherapy, four of them also performing radiotherapy (Table 2.1.).

The French SIOP (International Society of Pediatric Oncology) protocol MMT 95-2001 depending upon the clinical stage TNM, SIOP pTNM and IRS group, histology and primary site was used. Radiotherapy followed by surgery to control local microscopic or gross residual disease was prescribed in only four patients (one in stage/group II and three in more advances cases) with unfavorable sites (two of them in head and neck), alveolar histology and huge lesions incompletely resected, the low number being also due to the reticence of radiation oncologist to perform this

medical attendance in young children. Radiotherapy was performed 8 to 12 weeks after chemotherapy initiation and continued for five to six weeks. The dose per fraction ranged from 150 to 200 cGy depending to tumor site, extend and staging. However, the advanced tumor stages hindered a lasting beneficial effect, three of patients dying in less than one year.

Table 2.1. Pediatric RMS surgically removed

No	**Case**	**Sex/Age**	**Site**	**T≈ 5 cm**	**Lymph node**	**Histological subtype**	**IRS group**	**CR**	**CT**	**RT**	**Result**
1.	**F.I.**	♀/9 years	BT	<	-	E	I	-	+	-	Alive
2.	**Z.M.**	♀/5 years	H&N	<	+	E	II	+	+	+	Dead
3.	**I.E.**	♀/7 years	Extr	<	-	E	I	-	+	-	Alive
4.	**G.M.**	♂/8 months	UG	<	-	B	I	-	+	-	Alive
5.	**U.A.**	♀/17 years	HN	>	+	A	III	-	+	+	Dead
6.	**Z.S.**	♂/1 year	UG	<	-	B	I	+	+	-	Alive
7.	**G.D.**	♀/10months	UG	>	-	E	II	-	+	-	Alive
8.	**B.B.**	♀/4 years	BT	>	+	E	III	-	+	-	Dead
9.	**R.O.**	♀/3 days	Extr	<	-	F	I	+	+	-	Alive
10.	**M.I.**	♀/15 years	Extr	>	-	F	II	+	+	-	Alive
11.	**L.E.**	♀/11 years	UG	>	-	F	III	+	+	+	Dead
12.	**C.C.**	♂/8 years	UG	>	-	E	III	+	+	-	Dead
13.	**R.Y.**	♂/4 months	UG	<	-	B	II	-	+	-	Alive
14.	**M.C.**	♂/6 years	FI	<	-	F	I	+	+	-	Alive
15.	**M.D.**	♂/1 year	UG(P)	<	-	B	II	+	+	-	Alive

BT – biliary tree; Extr – Extremities; FI – Flank; H&N – head and neck; P – prostate, UG – urogenital; T – Tumor size; A – alveolar; B – Botryoid; E – Embryonal; F – Fusiform; CR – complete resection; CT – Chemotherapy; RT – Radiotherapy

Our treatment results were inferior compared to most international studies, the overall survival rate being 37.5% (nine cases alive at the time of this audit without evidence of disease and mean survival of 65.5 months). Significant favorable prognostic factors as estimated by

univariate analysis were age less nine years = 0.005, histological type = 0.001, primary site = 0.02, tumor size <5 cm = 0.04, IRS stage = 0.003, complete surgery = 0.002, suitable chemotherapy = 0.003. We recorded 16 deaths (one immediately postoperatively, others at 1–10 years after surgery with tumor recurrences and/or dissemination.

Mortality in our setting is still unacceptably high, late presentation, huge volume and advanced stage of the lesion and withholding from radiotherapy being the main contributing elements.

2.2.5. Discussion

Our study presented the clinical and pathologic features in a series of cases gradually better managed according to contemporary guidelines and to evaluate whether the therapeutic standards achieved in RMS in developed countries can be reproduced in our activity. There is a paucity of reports on the pattern of these tumors occurrence, diagnosis and therapy in countries with limited resources and even in Romania (Huh and Skapek, 2010; Shouman et al., 2005; Uba and Chirdan, 2008; Company et al., 2011; Badr et al., 2012).

Therefore, demographic analysis show an equal male to female ratio compared to most published studies, which generally indicates a prominent male presence (Van Der Schyff and Stefan, 2010; Missaoui et al., 2010). Most of our patients were under the age of nine years (two within one month of birth), age which appeared to be a clear boundary between two phases in biologic behavior of childhood RMS. The age distribution showed two peaks at 0.1–1 year and between 4–6 years. RMS prognosis was far worse in children under one year and over nine years (Maurer et al., 1993; Leaphart and Rodeberg, 2007; Sultan and Ferrari, 2010).

Clinical characteristics were dominated by delayed presentation with advanced stage of disease and large asymptomatic masses, numerous observations having tumor diameter larger than 5 cm. In our series, genitourinary tract was the most common affected primary site followed by extremities and head and neck. These results are different from many statistics where the most common location is the head and neck. The clinical course and prognosis was noteworthy better in localized lesions situated in favorable sites. Seven cases presented regional lymph node but nine patients have distant metastases at the initial consultation (Shouman et al., 2005; Uba and Chirdan, 2008).

The embryonal RMS was the most common pathological subtype described in related series having the better clinical behavior, but together with the alveolar subtype declined with age. Pleomorphic tumors rarely described in childhood was however present in three patients all of them marked by a fast poor course with reduced survival (Ognjanovic and Linabery, 2009; Sultan and Ferrari, 2010).

Management of childhood RMS tended to a multidisciplinary risk adapted approach including surgery, chemotherapy but in much lesser extent radiation therapy, each of them having its own specific role. The indications and order that these treatments were applied depended on site, size, histological diagnosis and subtype, extent of disease (stage) and risk stratification, which relied on tumor characteristics before use and results of surgery. All tumors should be subclassified

based on the histology into favorable embryonal/botryoid/spindle cell subtypes and unfavorable alveolar/pleomorphic forms (Ognjanovic and Linabery, 2009; Sultan and Ferrari, 2010; Leaphart and Rodeberg, 2007).

The guiding principle of RMS surgery was complete tumor removal providing that it do not cause mutilating or cosmetic damages. The role of extensive surgery however has become less important with advances of chemo- and radiotherapy. Surgery was done in noninvasive even bulky lesions, which can be largely extirpated initially or after clinical response with chemotherapy. Operation included an en block complete resection of the primary tumor with surrounding margins of uninvolved tissue during initial procedure. In three cases an adjacent lymph node dissection was done. Also, in two embryonal RMS, re-resection of cure was indicated since neoadjuvant therapy obtained a notable lesion shrinkage allowing a second-look operations. The bulking surgery was avoided, excisions of residual or recurrent disease founded after first operation or chemotherapy and amputations in extremity RMS (Leaphart and Rodeberg, 2007). In many sites biopsy was the only feasible surgical procedure, and visceral exenterations, radical cystectomies, hysterectomies/vaginectomies were considered already historical procedures. Therefore, surgery conserved a capital prime line importance in local control and also in establishing risk stratification in order of subsequent RMS therapy.

RMS are chemosensitive lesions and most our cases received combination chemotherapy as there were ample evidence that adjuvant and neoadjuvant therapy significantly improved remissions and even survival. Its main indication was to strength the healing in conveyable operated or even those not completely resected cases but also as upfront treatment in inoperable or metastatic cases (Leaphart and Rodeberg, 2007; Ognjanovic et al., 2009; Sultan and Ferrari, 2010; Huh and Skapek, 2010).

Our current standard frontline chemotherapy consisted of vincristine and actinomycin in combination with an alkylating agent namely ifosfamide or another regimen associating IVA with carboplatin, epirubicin and vincristine and two courses of IVE, both recommended to initially I–II stages/groups, sites, embryonal and low risk lesions. In advanced cases from any site, any stage/group, any nonembryonal type, local invasion, incomplete resection or recurrences (stages II–III) as in metastatic disseminations (stage IV), repeated courses of IVA+VAC+IV Etoposide completed by radiotherapy in few cases were used. Even if few patients initially had mixed response or apparently stabilized condition, after 4–6 months irreversible progressive disease was noticed for all this cases. Although radiotherapy is an indispensable component of the current therapeutic “gold” triad of childhood RMS, it was used only in few cases from our series. Beside anatomo-clinical characteristics, this was due mainly to the dificulties of our external collaborators to apply radiotherapy in children under seven years or due to some technical difficulties. However, radiotherapy is an essential modality of treatment for local control of RMS eradicating residual tumoral cells and excepting first-grade embryonal lesions all histological subtypes should receive it to achieve longtime disease remissions (Ognjanovic et al., 2009; Sultan and Ferrari, 2010; Huh and Skapek, 2010).

Newer methods of delivering radiation therapy included intensity modulated and protonbeam radiotherapy and also brachytherapy maintaining efficacy but reducing long-term sequelae of this method (Leaphart and Rodeberg, 2007; Sultan and Ferrari, 2010; Huh and Skapek, 2010; Terezakis and Wharam, 2013; Wand, 2012; Van Der Schyff and Stefan, 2010).

Even if most our patients achieved substantial remissions for 1–10 years, the current series registered a 5-year survival overall and 5-year event free survival of 37.5% and 33.3% respectively constituting a modest long-term outcome comparing with the results in countries entirely applying verified therapeutic guidelines.

2.2.6. Conclusions

RMS remains one of the most common soft tissue sarcoma in childhood characterized by a significant morphological, clinical and prognostic heterogenity with great differences between their genetic type, clinical features, multimodal treatment response.

The epidemiological characteristics of our patients are similar to the worldwide data. The application of the intensive-risk-based protocol for treating our patients had led to improvement in the curability of the disease.

The poor outcome in the study group was related to non specific symptoms that determined a late presentation, in advanced stages, associated with impossibility of complete surgical removal of the tumoral mass in many cases and also lack of radiotherapy in specific cases.

2.3. Childhood Pediatric Brain Tumors

2.3.1. Introduction

Brain tumors are the most common pediatric solid tumor with a suboptimal long-term outcome in many of their subtypes being the leading cause of cancer related death of solid tumors (Gupta et al., 2010). The World Health Organisation classifies CNS tumors in astrocytomas, medulloblastomas, ependymomas, pineal tumor, meningiomas, brain metastasis and other types of tumor (Kleihues et al, 1993, Lanzkowsky et al., 2005). Clinical signs and symptoms depend on tumor site, aggressivity, birth weight or age (Harder et al., 2008, Ortega-Aznar et al, 2001, Warren et al., 2004).

The onset may take several days to even several years, depending on the growth rate of tumor (Pancucci et al., 2007, Klein et al., 2007, Polkinghorn et al., 2007, Klein et al., 2007). The clinical aspects are dominated by progressive neurological deficits, signs and symptoms caused of increased intracranial pressure, visual disturbances, behavioral disorders, seizures and endocrine disorders. It is well established the link between pediatric brain tumors and certain genetic syndromes: type I neurofibromatosis, Li-Fraumeni syndrome, sclerosis tuberosa, Von Hippel-Lindau, Turcot and Gorlin syndrome (Gupta et al., 2010, Biegel, 1999).

Imaging evaluation includes computed tomography and magnetic resonance imaging that complement each other and describe the tumor location, local extension, compression in the neighborhood structures, superjacent hydrocephalus. Positron emission tomography can

differentiate recurrent tumor from necrosis, postoperative scarring or post-therapy edema (Marcus et al., 2005, Halperin et al., 2005, Kalapurakal and Thomas, 1995).

The surgery aims to remove the tumor as complete as possible and is followed by pathological examination (Kleihues et al, 1993, Lanzkowsky et al., 2005, Burzynski, 2006). Multimodal treatment (chemo-radiotherapy) is based on histopathological tumor type (Gupta et al., 2010, Warren and Packer, 2004). Despite multimodal treatment, the relapse rate is increased and is linked to ablative surgery, histology and individual factors (gender, age, genetic status) (Gupta et al., 2010, Zuzak et al., 2008).

2.3.2. Aim

There are only few reports on pediatric brain tumors in countries with limited resources, and, if existent, there are only single institution experiences. Thus, the incidence of brain tumors and treatment outcome is difficult to be estimated in these countries. As we know, few data are published regarding pediatric central nervous system tumors in Romania. The aim of the papers is to present the situation of patients diagnosed with brain tumors in Pediatric Oncological Department and their outcome. Also, due to the initial non-specific symptoms, we paid special attention to brainstem tumors.

2.3.3. Material and methods

- ***Features of brain stem tumors in children***

This article review the patients admitted between 2003-2010 diagnosed with brain stem tumors. Eight children (4 girls and 4 boys) aged 2-13 years (mean age 6.82 years) met the inclusion criteria.

Disease history, onset symptoms, complete physical, laboratory and imaging investigations, and individualized therapeutic approach have been reviewed. Family history was considered to be of particular clinical importance.

Monitoring the disease progression was possible until the time of death (when it occurred in hospital) or by information provided by the family and family physician in cases where death occurred at patient's home.

- ***Pediatric Brain Tumors-Morphological Findings And Prognostic Factors***

This paper review a number of 102 cases of central nervous system tumors diagnosed betwen 1990 and 2007 at the Hemato Oncology Department “Sf. Maria” Emergency Hospital for Children Iasi.

The diagnosis of primary or metastatic brain tumors was established by clinical, biological and imagistic studies. The clinical signs and symptoms, biological and imaging investigations followed by surgery and histopathological examination were examined. The treatment protocol consisted in a multimodal treatment (radiotherapy and/or chemotherapy) according to the international protocols.

The statistical analyse was performed using SPSS 15 program.

2.3.4. Results

- ***Features of brain stem tumors in children***

Histopathological examination diagnosed one pilocytic astrocytoma (grade I), one fibrillary astrocytoma (grade II), one anaplastic astrocytoma (grade III), and one glioblastoma multiforme (grade IV). In the remaining 4 cases imaging was suggestive for glial tumors.

Multimodal therapy was used in 2 patients, 7 received adjuvant chemotherapy, and in 1 case no therapy was administered because the tumor rapidly progressed to death.

Seven of our patients died at 6.28 months after the diagnosis (range 2 to 9 months). A family history of brain tumors positive in 2 (25%) of our cases supports the hypothesis of genetic involvement.

- ***Pediatric Brain Tumors-Morphological Findings And Prognostic Factors***

Following the analysis of the study group an increasing incidence over the years was noticed, with a maximum number in 2006 - of 18 cases. The epidemiological characteristics of the studied patients revealed a male predominance (65 cases) with sex ratio of 1.7:1. Most of the patients were from country side area (61.94%).

The histopathological exam showed: astrocytomas in 44 (44.66%) patients, medulloblastomas 23 (22.33%) patients, ependymomas 17 (16.50%) patients, craniopharyngiomas 3 (2.91%) patients and pineal tumors 3 (2.91%) patients. One case (0.97%) presented a choroid plexus carcinoma, 5 (4.85%) patients presented brain metastases from other primar tumors (osteosarcoma, neuroblastoma, hepatoblastoma, rhinopharingeal carcinoma) and 4 (4.46%) patients other tumoral types: intraspinal extradural neuroblastomas, non Hodgkin lymphomas, hypophiseal and IV-th ventricle vascular tumor.

An increased intracranial pressure at admission was noticed in 70 cases, neurological difficulties in 74 cases and visual impairment in 24 cases. Seizures were an onset sign in 7 cases, two patients were admitted in comatose state and unspecific general symptoms in 14 cases. The median age at diagnosis was 8,5 years with a range between 3 months and 17 years.

Another factor reviewed was birth weight: 85 (82.52%) cases had a normal birth weight (2,500g - 4,000 g); 7 (6.79%) patients had below 2,500 g and 10 (9.72%) patients had above 4,000 g. Nutritional status was normal in 93 cases, 2 patients were overweight and 7 had nutritional deficits. Total tumor resection was performed in 35 cases (33.97%), a subtotal resection in 27 cases (26,21%), a partial resection in 20 cases (19.41%) and in 7 cases only surgical biopsy. For 13 patients (12.62%) surgical intervention wasn’t indicated.

Surgery followed by chemotherapy in 86 cases, radiotherapy in 47 cases and both in 40 cases. A relapse rate of 53.39%(55 patients) and 68 of 102 cases (66.01%) died; death in the first year was noticed in 24 cases: astrocytomas grade II 6, grade III 3, grade IV 7, medulloblastomas 2, ependymomas 2, brain metastasis 3 and other tumor 1.

2.3.5. Discussion

In childhood CNS tumors, clinical signs and symptoms depend on tumor site, the aggression, and patient's age. Progressive neurological deficits, signs and symptoms caused by

increased intracranial pressure, visual disturbances, behavioral disorders, seizures, endocrine disruption, failure to thrive may occur in various combinations. In only 50% of our cases the tumor could be removed. Imaging proved highly suggestive for a brain stem tumor.

We analysed a group of 102 children treated for CNS tumors between 1990 and 2007. There is an increasing incidence over the years with a peak in 2006; these represent a real increasing incidence associated with improvements in diagnose. Similar to others studies we noticed the predominance of male and country side origin.

The mean age was similar for boys and girls. The astrocytoma group account for 44.66% of all CNS tumors, followed by medulloblastomas and ependymomas; other types of tumor represent only 16.51% of total cases.

The most common presenting symptoms were headaches, nausea, vomiting, neurological disturbances and visual impairment. The clinical signs and symptoms were broad; from almost asymptomatic to a progressively deteriorating clinical course even to coma. Birth weight was in normal range in most of the cases. Previous studies have suggested that high birth weight is associated with an increased brain tumor risk; in our study 10 from 102 cases had high birth weight and seemes to confirm this association. There is no implication of nutritional status in tumor relapse.

Treatment strategies (according to international protocols) included surgical remove of the tumor followed by chemotherapy and/or radiotherapy. Only one third 33.97% of the patients had total resection; two third had different types of partial resection or no surgery at all, leading to a poor prognosis. Half of the patients relapsed with a total number of deaths of 68 (66.01%) after relapse; one year survival rate was similar to other studies, except from grade II and IV astrocytomas in whom we found a lower survival rate.

2.3.6. Conclusions

Tumors of the CNS in pediatric population exhibit a large range of behavior; the prognosis of these patients is influenced mostly by the histological features of the tumor and less by the age and the personal status. The type of surgery remain the most important factor linked to long term survival. As multimodal treatment strategies (surgery, radiotherapy and chemotherapy) have been developped and used, the median survival time has improved, but not to the extent observed in other pediatric neoplasms.

Chapter 3. Chemotherapy – related Toxicities and Late Effects of Childhood Cancer Therapy

3.1. Background

Over the last decades, the survival rates for childhood cancer has increased due to the improvements of multimodal treatments and many children become long-term survivors of cancer (Gatta et al., 2009)). On the other hand, the patients are burdened with unique severe side effects

or complications of the treatment (Millan et al., 2018). Aggressive chemotherapy protocols, as well as radiotherapy are in most cases associated with many early, delayed or/and late side effects, complications or sequels which may result in multiorgan impairment and deterioration of the quality of life. Chemotherapy's adverse effects and complications are varying of severity, clinical relevance and include gastrointestinal toxicity, mucositis, cardiotoxicity, nephrotoxicity neurocognitive impairment, neuropathy, musculoskeletal morbidity, endocrine dysfunction (Schmiegelow et al., 2017). Unfortunately, all these acute events may lead to treatment delay, dose reduction or treatment abandonment with a direct impact on patient outcome (Yeoh et al., 2017; Hough and Vora, 2017). It is the responsibility of the physician to anticipate, early recognize, correctly assess, and properly manage these events.

Children treated for cancer are significantly immunocompromised by the changes that occur in both cell mediated and humoral immunity, thus infections are an expected sequel and a major cause of morbidity and death in these patients (El-Mahallawy et al., 2005). Most drugs causing immunosuppression increase the risk for bacterial, viral or fungal infections. Infection prophylaxis is possible by the identification of risk factors: prematurity, prolonged hospitalization, intensive care admission,mechanical ventilation, prior anticiotherapy. Prompt empirical broad spectrum anti-microbial therapy even in the absence of clinical or radiological signs is crucial in cancer patients (Zhu et al. 2020; Babay et al., 2005). However, this immediate empirical therapy may result in the over treatment of pediatric cancer patients, especially of neutropenic patients. Hence, a predictive models of numerous large studies managed to stratify these patients into risk groups and to develop suitable preventive and therapeutic strategies (Garrido et al., 2019).

Treatment related late effects may have the potential to result in a severe disabling, life-threatening or fatal illness such as cardiovascular disease. Major risk factor as a late effect for cardiovascular impairment is the anthracycline based treatment regimen. Anthracycline (doxorubicin, epirubicin, daunorubicin, idarubicine) is a cornerstone in the treatment of childhood hematological malignancies (lymphoma, ALL, AML) and solid tumors (rhabdomyosarcoma, neuroblastoma, osteosarcoma), with an important implication in irreversible cardiovascular complications defined as anthracycline-induced cardiotoxicity (myocardial injury, left ventricular dysfunction and heart failure) (Lipshultz and Adams, 2010; Sobczuk et al., 2020). Based on review of literarure, there are cardiac biomarkers used in predicting and detecting early cardiac dysfunction, before the occurrence of clinical symptoms or echocardiographic signs. These plasma biomarkers include N-terminal pro brain natriuretic peptide, cardiac troponin T, creatine kinase MB and more specific such as myeloperoxidase, topoisomerase II beta, interleukin-6 (Lakhani et al., 2021; Horacek et al., 2007)

On the other hand, obesity, metabolic syndrome and type 2 diabetes mellitus are an acknowledged late effect in childhood cancer survivors. Cranial and abdominal radiotherapy, total body irradiation, chemotherapy or long term steroids treatment are implied in alteration of the body composition, of the leptin and adiponectin mechanism in oncological patients. These cancers survivors are at increased risk for early cardiovascular disease. (Teixeira et al., 2016; Barnea et al., 2015). Additionally, with the increasing in the general population becoming obsese, recogniziting of the predictive signs and markers of early cardiovascular dysfunction in pediatric

obesity may prevent heightened risk of cardiovascular morbidity and mortality in adulthood (Abaci et al., 2009; Umer et al., 2017).

In this direction of research, the relevant papers and a research grant are listed and revisited below.

Research grant

Grant CNCSIS no. 1219 Mutations in the etiological spectrum of systemic infections in patients with malignant hematological diseases. **Director: Prof. dr. Dumitru Buiuc, Microbiology Department**

Published articles:

1. Trandafir LM, Cojocaru E, Moscalu M, Leon Constantin MM, **Miron I**, Mastaleru A, Teslariu O, Datcu ME, Fotea S, Frăsinariu O. Predictive Markers of Early Cardiovascular Impairment and Insulin Resistance in Obese Pediatric Patients. *Diagnostics*. 2021; 11(4):735. **IF=3.706**

2. Trandafir LM, Russu G, Moscalu M, **Miron I**, Lupu VV, Leon Constantin MM, Cojocaru E, Lupu A, Frasinariu OE. Waist circumference a clinical criterion for prediction of cardio-vascular complications in children and adolescences with overweight and obesity. *Medicine (Baltimore)*. 2020 Jul 24;99(30):e20923. **IF=1.889**
3. Gavrilovici C, Luca AN, Antoniu SA, Gallaby KA, Stefanescu R, Starcea MA, **Miron I**, Bild V. How nephrotoxic is the cancer therapy in children. *Farmacia*. 2018 Mar 1;66(2):197-208. **IF=1.527**
4. Mandric CG, Dimitriu AG, Petrariu FD, Frasinariu OE, **Miron I**. The Utility Of Serial Evaluation Of Ctni And Bnp In Early Detection Of Anthracycline-Induced Cardiotoxicity In Children. *The Medical-Surgical Journal*. 2017 Apr 1;121(2):245-52.

5. Sarbu I, Socolov D, Socolov R, **Miron I,** Trandafirescu M, Diaconescu S, Ciongradi CI. Hydrocephalus secondary to chemotherapy in a case of prenatally diagnosed giant immature grade 3 sacrococcygeal teratoma: A case report and literature review. Medicine (Baltimore). 2016 Oct;95(43):e5244. **IF=1.804**

6. **Miron I,** Maria SM, Lucaci L, Arsenescu-Georgescu C, Miron L, Ciubara A, Burlea M. Chemotherapy-related toxicity in childhood neoplasia. *Journal of the Balkan Union of Oncology*. 2014;19(4):1070-5. **IF=0.741**

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3.2. Toxicity Profile During Childhood Cancer Therapy

3.2.1. Introduction

Cancer accounts for 10% of all deaths in chilhood, and leukemia is the most frequent malignancy in children (20-30% of childhood neoplasias) (De Braekeleer et al., 2012).

After the first temporary remission in acute lymphoblastic leukemia obtained by Farber et al. using a folic acid antagonist in 1948 (De Braekeleer et al, 2005), agents like corticosteroids, 6-mercaptopurine, vincristine, methotrexate (MTX), L-asparaginase and anthracyclines (doxorubicin and daunorubicin) were discovered in 1950s and 1960s. Thereafter, the first clinical trials demonstrated that combinations of two or more agents were superior to single-agent chemotherapy (Drexler et al., 2004).

The major drugs included in the chemotherapy regimens in children are alkylating chemotherapy agents (ifosfamide, cyclophosphamide), platinum agents (cisplatin, carboplatin), antimetabolites (methotrexate, clofarabine), nitrosoureas and other cytotoxic drugs (mithramycin, 5-azacytidine, anthracycline, actinomycin D, asparaginase).

Alkylating chemotherapy agents as ifosfamide and cyclophosphamide are important agents used for the treatment of many paediatric solid tumours like soft tissue sarcomas, rhabdomyosarcoma and Ewing's sarcoma (Faught et al., 2015; Skinner, 2010). Platinum agents as cisplatin (CDDP or CIS), carboplatin have a well-defined role in the treatment of several paediatric solid malignancies like brain tumours, osteosarcoma, neuroblastoma, germcell tumours and liver tumours (Skinner, 2010).

Carboplatin has been used as an alternative to cisplatin in several solid tumours (e.g., brain tumours, neuroblastoma or germ-cell tumours).

Antimetabolites Methotrexate has a wide range of use due to its anti-proliferative and immunomodulatory effects (Schiotis et al., 2017). In paediatric oncology it represents an effective treatment for acute lymphoblastic leukaemia (Skinner, 2010).

*Clofarabine, a purine nucleoside analogue***,** is approved for the treatment of refractory paediatric acute lymphoblastic leukaemia.

Carmustine (BCNU), Lomustine (CCNU), Semustine (methyl-CCNU) and streptozocincan are alkylating nitrosourea agents used for especially in solid tumors treatment.

Therapeutic regimens used for the treatment of hematologic malignancies and solid tumors create complications like febrile neutropenia, nausea, local toxicities, mucositis, diarrhea, anemia/pancytopenia from medullary aplasia, neuropathy, arthropathy, cardiomyopathy, second malignancy, iatrogenic Cushing's syndrome, toxic hepatitis, digestive candidiasis, kidney injuries with variable incidence and severity.

Nephrotoxicity has been defined as the ability of an agent to cause structural kidney damage or functional impairment: glomerular and/or tubular dysfunction, impairment of blood pressure regulation and renal endocrine dysfunction (Gavrilovici et al., 2018). Nephrotoxicity in children with cancer depends on many factors including: the pre-existing renal damage due to the malignancy itself (by tumour infiltration or urinary tract obstruction), the patient's age and,

respectively, the nature, duration and dosage of the nephrotoxic treatment. Chronic renal impairment in children with cancer may be due to a renal malignancy (e.g. Wilms tumour) or to the adverse effects of the treatment: chemotherapy, radiotherapy, surgery, immunotherapy or supportive treatment (Skinner, 2010).

Different forms of chemotherapy-induced kidney disease, like toxic acute tubular necrosis, thrombotic microangiopathy (TMA), crystal nephropathy, proteinuria/nephrotic syndrome, minimal change disease, focal segmental glomerulosclerosis (FSGS), membranous nephropathy, interstitial nephritis and tubulopathies have been reported (Giucaneanu et al., 2015). Severe ifosfamide-induced renal damage has been reported also in patients with prior unilateral nephrectomy or kidney tumour infiltration (Faught et al., 2015; Rossi et al., 1994). Both ifosfamide and cyclophosphamide might be responsible for haemorrhagic cystitis and for syndrome of inappropriate antidiuretic hormone secretion (SIADH) and nephrogenic diabetes insipidus. Chemotherapy induced nausea may also play a contributory role, since nausea stimulates the release of ADH. Chronic ifosfamide-induced nephrotoxicity in children may result in hypophosphatemic rickets, renal tubular acidosis, renal glycosuria (in the absence of hyperglycaemia) leading in severe cases to Fanconi syndrome (Skinner, 2017).

Platinum agents accumulates in the kidney at higher concentrations than in the blood and other organs, thereby contributing to kidney injury (Bardi et al., 2004), causing both acute and chronic glomerular and tubular toxicity (Jiménez-Triana et al., 2015; Skinner, 2017). However the most frequent manifestation of cisplatin`s nephrotoxicity is acute kidney injury (AKI) (Miller et al., 2010). When cisplatin is administered with bleomycin or gemcitabine, it may cause TMA mainly due to direct endothelial injury with secondary platelet activation (Malyszko et al., 2017; Volovat et al., 2014). Chronic magnesuria, hypomagnesemia, hypocalciuria, with normokalaemia or mild hypercalcemia may result from dissociation of magnesium and calcium due to tubular lesion (Skinner, 2010). On the other hand, if hypomagnesaemia occurs, the release of parathyroid hormone is inhibited, leading to potentially severe hypocalcaemia (although these occur less commonly) (Cooper and Gittoes, 2008; Hanly et al., 2013). Other renal manifestations include salt wasting (Miller et al., 2010), a Fanconi-like syndrome (Oeffinger et al., 2004), anaemia (due to erythropoietin deficiency) (Lager et al., 2005), chronic renal failure (Koch et al., 1998) and TMA (Blake-Haskins et al., 2011). Hypertension may occur, most likely by way of renal or/and vascular toxicity (Nechita et al, 2016; Skinner, 2010; Skinner, 2017). CDDP has several side effects like nausea, vomiting, neurotoxicity, myelosuppression and ototoxicity. The dose-limiting effect however is the nephrotoxicity that accompanies the treatment (Howard et al., 2016).

High dose regimens with Methotrexate (over 500 mg/m2) are susceptible to result in AKI, with an overall incidence between 2 - 12% (Howard et al., 2016) and is mainly due to precipitation of MTX and its metabolite in the distal tubular leading to tubular obstruction, decreased GFR, ARF and tubular cell death (Perazella and Moeckel, 2010). MTX can also be responsible for SIADH and high MTX concentrations reduce folate concentrations within normal cells resulting in toxicity. Typical renal histology findings include glomerulosclerosis, tubular loss and interstitial fibrosis (Skinner, 2010). Risk factors include hydration status and urinary pH, with those who are

dehydrated or have an acidic pH being at greater risk. Currently, high dose leucovorin is administered 24 - 36 h after MTX, with the purpose of saving normal tissues from MTX toxicity by reloading folate concentrations (Vredenburg et al, 2015).

Clofarabine shows dermatological toxicity, gastrointestinal toxicity, CNS toxicity, haematological toxicity and hepatotoxicity, myelosuppression is the dose-limiting factor. The elimination is performed through renal excretion. Current studies on clofarabine have revealed various degree of nephrotoxicity, mostly AKI, ranging from 10 to 36%. The mechanism of nephrotoxicity is not completely understood (Malyszko et al., 2017).

Alkylating nitrosoureas may cause a slowly progressive, chronic interstitial nephritis which is generally irreversible (Mousavi et al., 2014). Streptozotocin use is known to results in mild ARF(acute renal failure), mild proteinuria and/or tubular toxicity, manifested by aminoaciduria, phosphaturia, uricosuria, glycosuria, bicarbonaturia or Fanconi syndrome (Haubitz et al., 2002; Kintzel, 2001; Skinner, 2010).

Anthracyclines (ANT) are one of the most effective and commonly used antineoplastic agents in treatment of cancer in children. Their use has positively influenced the outcome, increasing substantially the survival rate. However, this benefit is encumbered by a high rate appearance of anthracycline-induced cardiotoxicity (Stevens and Lenihan, 2015).

Over the years, many researchers have used various methods to assess the cardiotoxicity, for an early detection and initiation of preventive measures. Cardiotoxicity detection by imaging methods (echocardiography, radio-nuclear angiography) has major limitations, emphasizing late left ventricular dysfunction and objectifying toxic effects too late, when enough damage on the heart have already caused functional disorders. Recent studies on cancer patients showed that elevated levels of B- type natriuretic peptide (BNP) and troponin I (cTnI) may serve as a predictor of infraclinical phase of cardiotoxic effect of chemotherapy, allowing the stratification of cardiac risk in a very early phase, long before heart function decreasing and symptoms appearance, when many of preventive therapeutic strategies are effective (Stevens and Lenihan, 2015).

Up to 80% of children undergoing chemotherapy will develop mucositis, its incidence varying with the type of cancer and treatment (Cheng et al., 2004)**.** Out of all haematological malignancies, acute limfoblastic leukemia is the most frequently linked to mucositis in children due to the fact that ALL is the most frequent malignancy in this age group and treatment includes chemotherapy medications that have been shown to lead to mucositis such as doxorubicin, cyclophosphamide and methotrexate. Other chemotherapy agents linked to mucositis are 5 fluorouracil (5-FU) and etoposide (Miller et al., 2012). The complete phisopathological mechanism of oral mucositis in not completely known. Sonis, 2004 proposed a five biological stage model of progression for oral mucositis: initiation, primary damage response, signal amplification, ulceration and healing. (Sonis, 2004). Clinical manifestations of oral mucositis may include erythema and generalized oedema of the mucosa, ulcerative lesions, thickening of the saliva, oedema of the gums, pain that varies in intensity, bleeding gums. This may lead to refusal of solid diet or/and liquid diet and can become lifethreatening. The MASCC/ISOO Clinical Practice Guidelines analyse the following prophylactic measures: basic oral care, growth factors,

antiinflamatory agents, antimicrobials, lowlevel light therapy/laser and other natural agents (Zinc, honey) (Lalla et al., 2014). Oral cryotherapy involves placing ice cubes or ice chips in the mouth and periodically refreshing them during the period of cytotoxic treatment. The biological mechanism behind this effect is presumed to be the reduced distribution of the cytotoxic agent to the oral mucosa due to vasoconstriction. It is only feasible if chemotherapy is administered as a short infusion and has a short half-life, as in the case of 5-fluorouracil, melphalan, etoposide, cisplatin, mitomycin-C, vinblastine. Another limitation is age, as it may prove to be a choking hazard for very young children (Sung et al., 2017).

Chemotherapy-induced diarrhea (CID) is a common problem and can occur in 50–80% of patients depending on the chemotherapy regimen (Benson et al. 2004; Gibson and Stringer, 2009). Some chemotherapeutic agents seem to have involvement in the pathogenesis. Among these anthracyclines and cytosine arabinoside have been cited to induce mucosal injury, and vincristine to lower peristalsis. The patients' exposure to corticosteroids, especially dexamethasone regimen before of the episode was precipitating the disease (Gibson and Stringer, 2009).

One of the life-threatening gastrointestinal complication seen in oncological patients is *neutropenic enterocolitis* (NE) or necrotizing enterocolitis. NE occurs during periods of severe neutropenia (Rizzatti et al., 2010; Wagner et al., 1970). NE is a necrotizing inflammatory condition of the mucosal barrier of the intestine as a result of chemotherapy or neutropenia and can rapidly progress to intestinal perforation, bacteremia, sepsis and death. It is characterized by typical triad fever, abdominal pain and neutropenia, but other clinical features such as diarrhea, abdominal distention and tenderness, vomiting, occult or frank blood in the stools may occur. Aggressive chemotherapy used in leukemia treatment such as cytarabine, vincristine, etoposide (VP16), doxorubicin, cyclophosphamide and daunorubicin could be involved in the development of NE (Kaste et al, 1997; Sundell et al., 2012) The data from the current literature suggest an increased incidence of NE among patients with hematologic malignancies compared with those presenting solid tumors (McCarville et al., 2005; Gil et al., 2013).

Despite the availability of a wide range of broad-spectrum antibiotics and advanced therapeutic choices, systemic infections are still responsible for a high rate of mortality and morbidity especially in patients with malignant hemopathies. Because of their main disease and the immunosuppressive therapy, these patients run a higher risk of bacteraemia. Antibiotic prophylaxis administered promptly at the first symptoms of fever has shown a decrease in the mortality rate. However, new issues and modifications in the etiologic spectrum of systemic infections have occurred and the emergence of microorganisms previously regarded as non-pathogens or commensal are presently reported as etiologic agents of serious invasive infections in neutropenic patients (Ellis, 2004; Woo et al., 2001; Onwuamaegbu et al., 2005). Oncological patients were chosen because of their susceptibility to infections. Neutropenia, hypogammaglobulinemia, T-lymphocyte dysfunctions, lesions on the mucous membrane is the ground for these patients.

3.2.2. Aim

The aim of the performed studies estimated the incidence and the various types of chemotherapeutic complications for hemathological malignancies and solid tumors in pediatric patients.

3.2.3. Material and Methods

- ***The utility of serial evaluation of ctni and bnp in early detection of anthracycline-induced cardiotoxicity in children***

This is a prospective study and included 34 patients, 16 girls and 18 boys, aged between 3 and 18 years, admitted in a pediatric oncology-hematology clinic for one year period. The children have followed various therapeutic protocols that included anthracyclines as a main chemotherapic drug for different cancers types.

The median dose of anthracyclines (doxorubicin) administered during the study was 226 mg/m², with the minimum-maximum range of 50–920 mg/m². Additional to anthracycline treatment, patients received other cardiotoxic chemotherapy, which increased the risk of cardiotoxicity.

Cardiac assessment included clinical examination, cardiothoracic radiography (with cardiothoracic index calculated), electrocardiogram (EKG), echocardiography (ECHO) 2D/Doppler and biochemical markers dosing (cTnI, BNP).

Echocardiographic evaluation was performed before treatment and after each cycle of chemotherapy and at 6 and 12 months after initiation of chemotherapy. The followed parameters were: ejection fraction (EF) and shortening fraction (SF) for systolic function, the peak early filling (E-wave) and late diastolic filling (A-wave) velocities, the E/A ratio, isovolumic relaxation time (IVRT), peak systolic (S) velocity, peak anterograde diastolic (D) velocity, the S/D ratio, isovolumic contraction time (ICT) for diastolic function, mixed parameters for systolic and diastolic function and myocar-dial performance index - Tei index. An average of 6 echocardiography exams were performed for each patient. BNP and cTnI biomarkers assessment was done before initiating therapy and in dynamic, depending on the time of administration of anthracyclines - before and after administration of cytostatic infusion, for a period of 12 months. No cardioprotective treatment was administered during the study, except for two patients who developed heart failure, in which specific treatment required.

Statistical analysis was performed with *SPSS*, version 15.0. Descriptive and inferential statistical analysis was done. Corre-lations between variables were calculated using the correlation coefficient test of Pearson's. Regression analysis was also effectuated. Statistical significance was set for a p-value <0.05.

- ***Mutation in the ethiological spectrum of systemic infections in patients with hematological malignancies***

A prospective study comprising 115 blood cultures from 76 patients with febrile response admitted into the Pediatric Oncology and Hematology Department in Iași between May 2004 - December 2005 was performed.

For the blood cultures were used diphasic osmotically protective media *(Hemoline Performance duo bioMerieux),* while for passaging were used agar Columbia plates - chocolate. In case if no positive cultures occurred within 48 hours we appealed to blind passaging and fluorescent microscopy with acridine-orange dye.

In case L forms were found, we passaged the blood cultures on reversion environments recommended by Dominigue (Mattman, 2000).

The clinical significance criteria of the isolates were: the number of positive blood cultures in the same patient, the time to the positive result, the identity of the isolate, the isolation of the same species from another anatomic site, imagistic particularities (X-ray, CT, scan), the clinical condition of the patient (predisposing factors and therapeutic evidence included).

The identification of isolates was performed on API *bioMerieux* galleries, antibiotic susceptibility was established through the breaking points method *(*API *bioMerieux* galleries*)* or disk disfussion antibiotic susceptibility tests according to NCCLS2002 standards.

The statistic processing of data was made with the SPSS program, using non-parameter tests (*Chi square*).

- ***Chemotherapy-related toxicity in childhood neoplasia***

We performed a retrospective and prospective study covering a 4-year period which involved 100 children admitted to the Hematology-Oncology Department of Children's Emergency Hospital in Iasi.

The data included cancer type and the time lapse (months) until one or more of the following chemotherapy adverse reactions occurred: Cushing's syndrome, alopecia, mycositis, medullary aplasia, diarrhea, toxic hepatitis, candidiasis, emetic syndrome, herpes or thrush. Time to disease remission (months) was also evaluated.

Statistical analysis was performed using SPSS 16.0 (SPSS Inc, Chicago, IL). The Kaplan-Meier method to estimate the probabilities for events to appear and the time interval from the beginning of chemotherapy administration until 25%, 50% or 75% (quartiles) of the patients developed the side effect in question. The Kaplan-Meier method allowed us to measure the time intervals and to extrapolate data even if information is incomplete (not all patients developed all side effects, therefore quartiles might not be possible to be estimated in some cases). Comparisons of Kaplan-Meier curves were performed using log-rank test. Standard error and 95% confidence interval (95% CI) were also used. Statistical significance was set at $p<0.05$.

3.2.4. Results

- ***The utility of serial evaluation of ctni and bnp in early detection of anthracycline-induced cardiotoxicity in children***

From the study group, 27 patients were alive at the end of study; death of seven patients was determined by the disease progression. Patients characteristics are presented in Table 3.1. The cardiotoxicity manifestations were divided in two major groups: clinical and subclinical type.

Table 3.1. Characteristics of the study group

Parameters		**Number**	**Percent %**
Sex	Female	16	47.1%
	Male	18	52.9%
Age	< 6 years	16	47.1%
	6-10 years	8	23.5%
	11-18 years	10	29.4%
Diagnostic	ALL NHL	18	52.9%
	Sarcoma	6	17.6%
	AML	2	5.9%
	HL	2	5.9%
	others	5	14.7%
		1	2.9%
Echocardiographic Changes	Yes	26	76.5%
	no	8	23.5%
Decreased EF >10%	Yes	18	52.9%
	no	16	47.1%
Decreased SF >10 %	Yes	23	67.6%
	no	11	32.4%
EKG changes	Yes	25	73.5%
	no	9	26.5%
BP changes	Yes	6	17.6%
	no	28	82.4%
Cardiotoxicity	No	10	29.4%
	infraclinical	22	64.7%
	clinical	2	5.9%

In the clinical type group were included patients with clinical signs and symptoms of heart failure and modified paraclinical markers (echocardiogram, EKG, cardiac biomarkers) and in the subclinical type, asymptomatic patients, but with important modifications in systolic or diastolic function. Thereby, 22 cases (64.7%) were asymptomatic and in 2 cases of heart failure occurred.
EKG alterations were represented by changes in left ventricle repolarization, QT prolongation, supraventricular tachycardia (24 patients) and 1 case of AV block grade III.
Changes in diastolic function were more common, 26 cases and Tei index increased was found in 14 cases (Table 3.2.).

Table 3.2. Cardiac modification after anthracyclines

Cardiac Assessment	Cardiac modification	Number	Percent %
Echocardiography		8	23.5
	normal decreased EF by > 10% decreased SF by>10%	18	52.9
	modification of LV diastolic function	23	67.6
	modification of LV systolic function	26	76.5
		16	47.1
EKG	normal	9	26.5
	modified	25	73.5
BNP levels	< 100 pg/mL	16	47.1
	100 - 200 pg/mL	6	17.6
	200 - 500 pg/mL	9	26.4
	> 500 pg/mL	3	8.8
BNP categories	A- normal	16	47.1
	B - transient increase further normalization C- increased	12	35.2
		6	17.6
cTnI levels	< 0,04 ng/mL	34	100
	> 0,04 ng/mL	0	0
BP	Normal	28	82.4
	High	6	17.6
Cardiotoxicity	no infraclinical	10	29.4
		22	64.7
	clinical	2	5.9

Echocardiographic changes were seen in 76.5% of cases; most of them were transient, occurring immediately after taking cytostatic, later return to normal baseline. Decrease by> 10% of the EF or SF was found in 53%, respectively, 67.7%of cases, although the actual value of the FE and/or SF has not dropped below the lower limit of normal. In 1 case EF was <28% (Table 3.2.).

BNP's elevated levels in plasma, above the cut-off of 100 pg/mL, were funded at 52.9% of cases. According to the BNP values changes during the study, they were divided into 3 groups: group A normal levels, group B - BNP levels were elevated, but later returned and were maintained in the normal range, and group C- BNP levels have remained elevated. The increased plasma levels of BNP were positively cor-related with the administered dose of anthracyclines and type of chemotherapy followed protocol (Fig. 3.1., Fig 3.2.).

Serum cTnI levels did not exceed the cut-off value of 0.04 ng/mL in any patient in the study group, both baseline and in dynamics. Only 3 patients had cTnI value of 0.04 ng/mL, considered be the normal value.

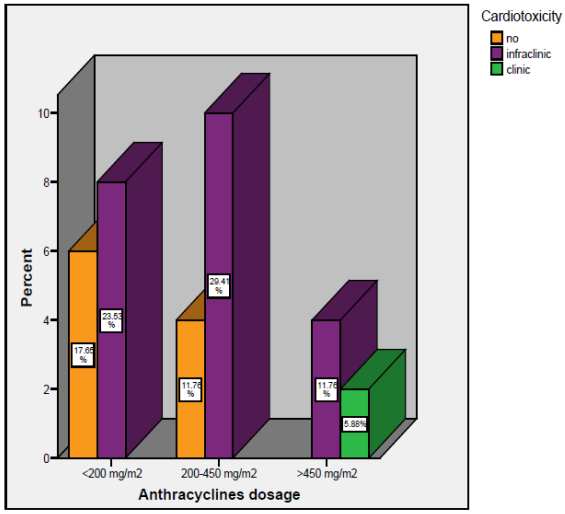


Figure. 3.1. Correlations between the cumulative dose of anthracycline and emergence of cardiotoxicity

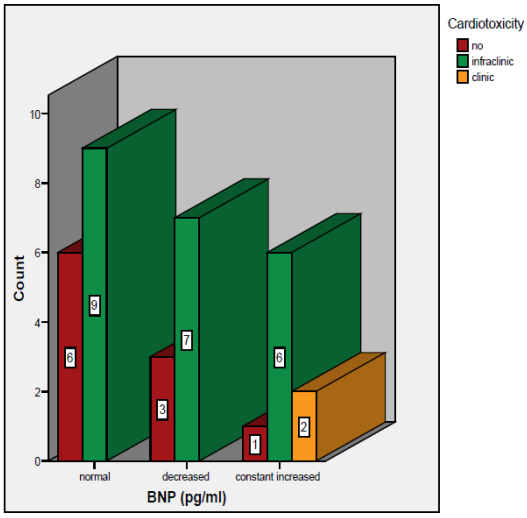


Figure. 3.2. Correlations between the BNP dynamics and emergence of cardiotoxicity

In the current study, cardiotoxicity was significant correlated with a decreased EF> 10% (r = - 0.457, p = 0.007), decreased SF >10% (r = -0.508, p = 0.002), pathological BP (r = - 0.341, p = 0.048), higher cumulative anthracycline dose (r = 0.505, p = 0.02) and higher serum BNP levels (r = 0.465, p = -0,006).

Scatterplot diagram of the relationship between the cumulative dose of anthracy-clines and BNP value suggested a positive linear correlation. Accurately predicting the BNP levels depending on the cumulative anthracyclines dose is made by the relation: Y = - 49 657 (1,099 x) in which x = the individual cumulative dose of anthracy-clines and Y = the best predictive value for the individual BNP (Fig.3.3.).

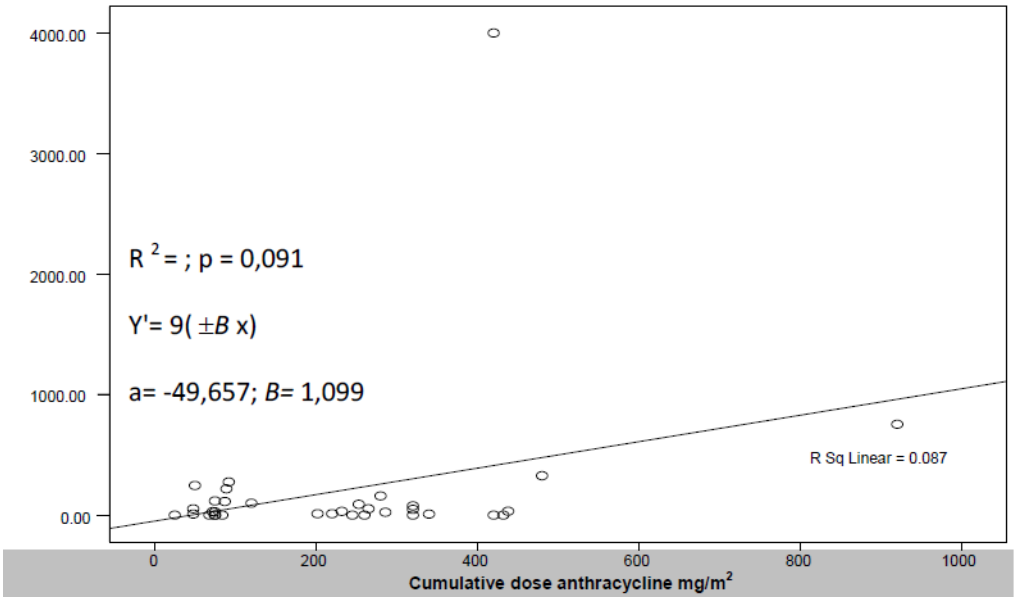


Figure 3.3. Linear regression analysis between BNP levels and cumulative anthracycline dose

- ***Mutation in the ethiological spectrum of systemic infections in patients with hematological malignancies***

The patients cohort were diagnosed with the following background conditions: acute lymphoblastic or myeloblastic leukemia in 35 (46.05%) of cases, solid tumors in 14 (18.42%) cases, lymphoma in 13 (17.10%) cases, other conditions in 14 (18.42%) cases such as chronic leukemia, multiple myeloma, constitutional bone marrow aplasia, Langerhans cell histiocytosis.

The study group included 76 patients; 40 (52.63%) male patients and 36 (47.37%) female patients with a median age of 26 years (the youngest patient was 1 year old and the oldest was 76).

There were registered 39 febrile episodes with positive hemocultures and 47 febrile episodes with negative hemocultures (54.65%). During the 39 febrile episodes in the 76 patients, in 45.35% cases occured positive blood cultures; bacterial isolates represent 89.74%, while the mycotic isolates represent 10.26% (Tabel 3.3.).

The most frequent encountered germs were cocci gram positive, followed by bacilli gram negative, bacilli gram positive, fungi and anaerobic bacteria.

Gram positive cocci caused 32.5% (n=13) of all bacteremic episodes and staphylococci in 22.5% of the cases, of which 3 isolates (23.07%) were methicillin resistant.

Gram negative bacteria were present in seven cases. Culture proven fungal infection occurred in 12.5% (n=4) cases during the study period. Candida non albicans*(Candida guilliermondi, Candida sake, filament fungi (Alternaria spp, Scedosporium spp)* isolates were identified in these patients. Out of these 4 patients, in 3 cases the patients suffered from acute leukemia, neutropenia and had received antibiotic prophylaxis.

Tabel 3.3. Blood stream infections encountered in the study group

Microorganism	**No**	**Percent(%)**
Gram-positive cocci	13 (6)	32,5
Coagulase-negative staphylococci	8 (4)	20
S aureus	1	2,5
Micrococus luteus	1 (1)	2,5
Coci gram positive unidentified	1 (1)	2,5
Streptococus pneumonia	1	2,5
Streptococus salivarius	1	2,5
Irreversible L shapes	9	22,5
Gram-negative bacilli	7 (3)	17,5
E.coli	3	7,5
Klebsiella pneumonia	1	2,5
Enterobacter cloacae	1	2,5
Non-identifiable gram-negative bacilli	2 (1)	2,5
Gram-positive bacilli	5 (5)	12,5
Cellulomonas spp	1 (1)	2,5
Nocardia spp	1 (1)	2,5
Oerskovia xanthynelitica	1 (1)	2,5
Corinebacterium jeikeium	1 (1)	2,5
Actinomicet	1 (1)	2,5
Fungi	4	12,5
Candida guilliermondi	1	2,5
Candida sake	1	2,5
Alternaria spp	1	2,5
Scedosporium spp	1	2,5
Anaerobic	2	5
Eubacterium limosum	1	2,5
Non identifiable Gram-negative bacillus	1	2,5

- ***Chemotherapy-related toxicity in childhood neoplasia***

The study cohort included pediatric patients diagnosed with hematological malignancies: acute lymphoid leukemia (47%), Hodgkin's lymphoma (9%) and several solid tumors: osteosarcoma (9%), neuroblastoma (8%), non-Hodgkin's lymphoma (8%), Wilms tumor (7%), acute myeloid leukemia (4%), chronic lymphoid leukemia (2%), myelodysplastic syndrome (1%), PNET tumor (1%), ovarian tumor (1%), histiocytosis (1%), fibrosarcoma (1%) and gastric tumor (1%). There were 29 children aged 0-3 years, 21 aged 4-6 years, 13 aged 7-10 years and 37 aged 11-18 years.

Cushing's syndrome All iatrogenic Cushing's syndrome cases appeared during the first 6 months of treatment, except for one case (36 months). Because only 21.5% of the patients developed this complication, the quartiles could not be estimated (Fig.3.4.).

Alopecia All cases of post chemotherapy alopecia appeared during the first 14 months, except one case that appeared at 48 months. Fifty percent of the cases appeared during the first 4 months and 25% appeared during the first 2 months and 75% during the first 11 months. In total 79.3% of the patients developed alopecia **(**Fig. 3.5.).

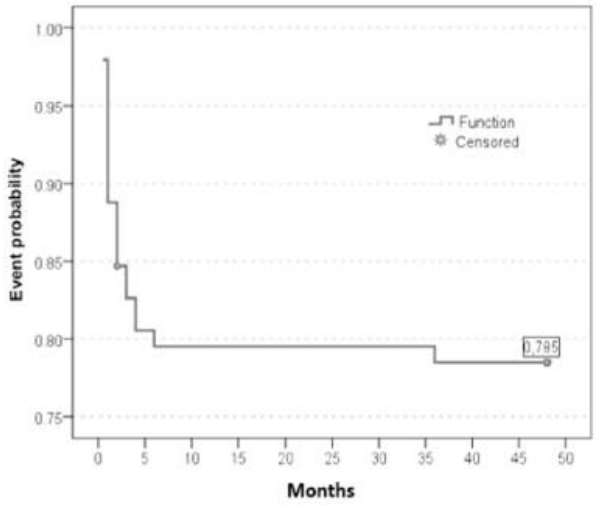


Figure 3.4. Time evolution curve of the event of patients not having Cushing's syndrome (78.5%).

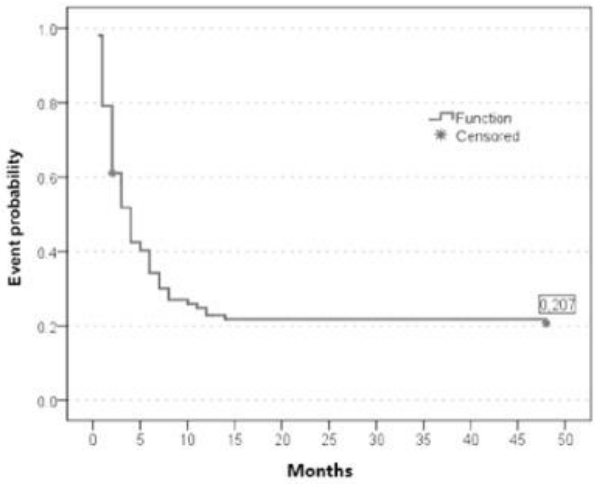


Figure 3.5. Time evolution curve of the event of patients not having alopecia (20.7%).

Mucositis Mucositis was the most frequent toxicity and developed in 65.3% of the patients. This condition appeared during the first 12 months of treatment. Half of the cases appeared during the first 3 months and 25% during the first month **(**Fig. 3.6.).

Medullary aplasia All cases of medullary aplasia appeared during the first 14 months, except a single case, which appeared at 48 months.

Fifty percent of the cases appeared during the first month. The total percentage of this complication was 71.1% (Fig.3.7.).

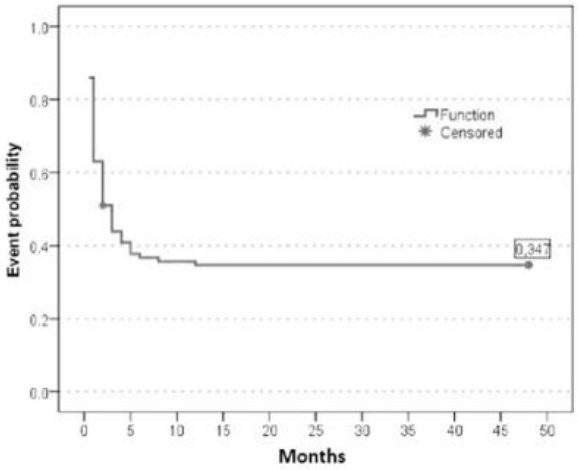


Figure 3.6. Time evolution curve of the event of patients not having mucositis (34.7%).

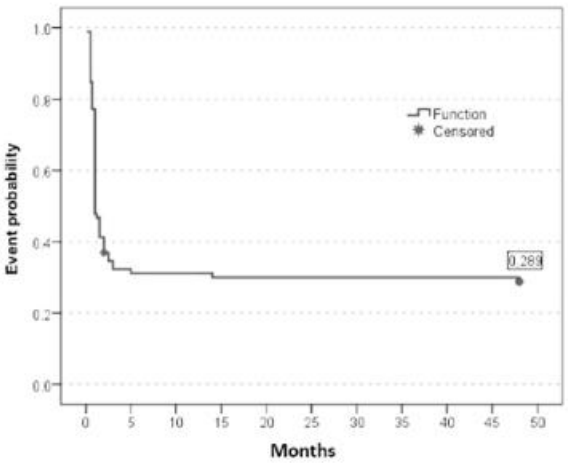


Figure 3.7. Time evolution curve of the event of patients not having medullary aplasia (28.9%).

Diarrhea All diarrhea cases appeared during the first 15 months of therapy. Fifty per cent of the cases developed during the first 6 months and 25% during the first 2 months. Sixty four per cent of all patients developed post chemotherapy diarrhea **(**Fig.3.8.).

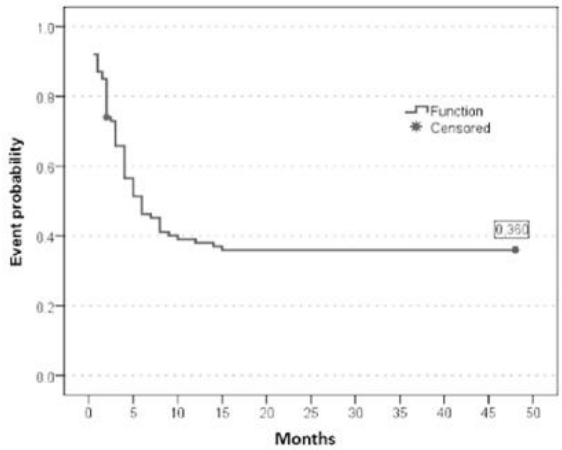


Figure 3.8. Time evolution curve of the event of patients not having diarrhea (36%).

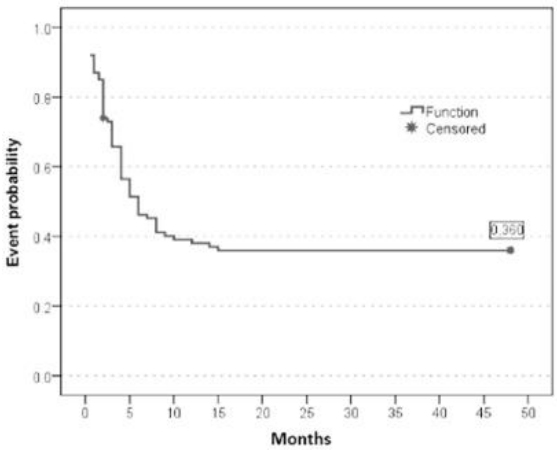


Figure 3.9. Time evolution curve of the event of patients not having toxic hepatitis (39%).

Hepatic toxicity Sixty one per cent of the patients developed toxic hepatitis and all cases appeared during the first 12 months, except one case that appeared 48 months after the beginning of treatment. Fifty per cent of the cases appeared during the first 10 months and 25% appeared during the first 2 months (Fig.3.9.).

Emesis Chemotherapy-induced emetic syndrome appeared in 64% of the patients. All cases appeared during the first 12 months, 50% of the cases during the first 4 months and 25% of the cases during the first 2 months (Fig.3.10.).

Candidiasis All candidiasis cases appeared during the first 14 months of treatment. Only 12.2% of the patients developed this complication, therefore quartiles could not be estimated (Fig.3.11.).

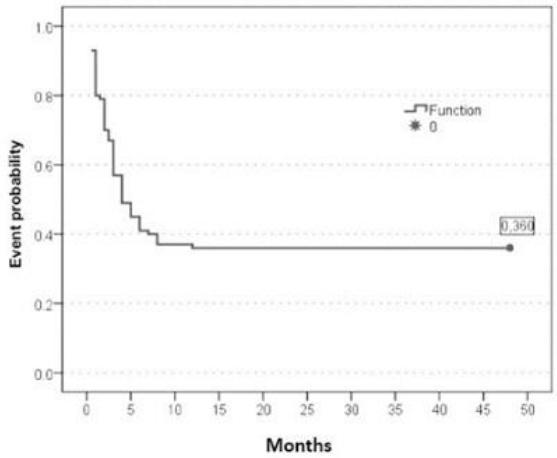


Figure 3.10. Time evolution curve of the event of patients not having emetic syndrome (36%).

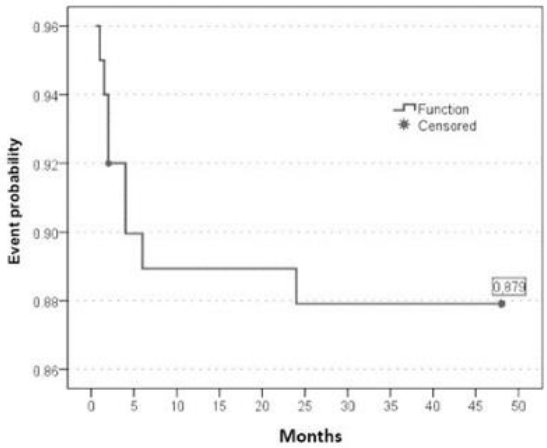


Figure 3.11. Time evolution curve of the event of patients not having digestive candidiasis (87.9%).

Herpetic infection

Post chemotherapy oral herpes or thrush appeared in 13.2% of the patients. All cases appeared during the first 15 months (Fig.3.12.).

Remission Remission of the underlying malignancy occurred in 69.6% of the patients. All cases with remission appeared during the first 4 months of chemotherapy.

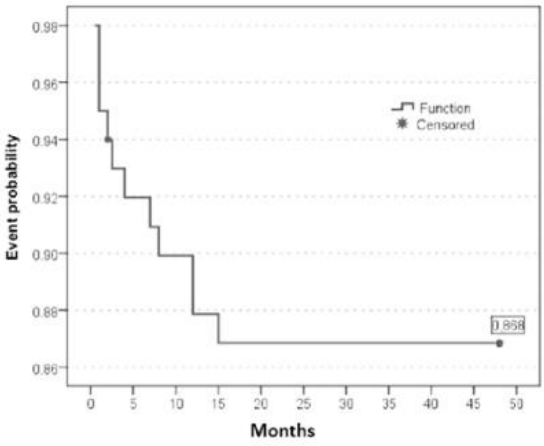


Figure 3.12. Time evolution curve of the event of patients not having oral herpes or thrush (86.8%).

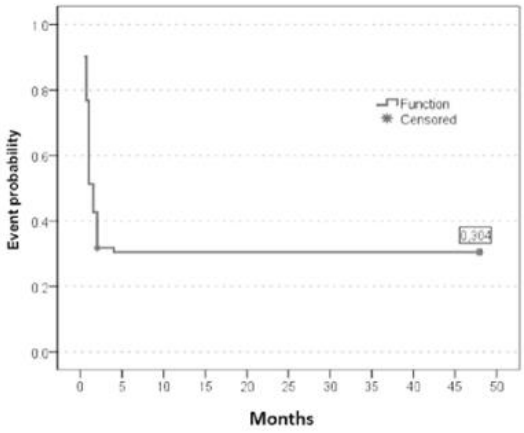


Figure 3.13. Time evolution curve of the event of patients not having remission of malignancy (30.4%).

Fifty per cent of the cases appeared during the first 1.5 months and 25% during the first month of treatment. Remissions occurred in leukemia and lymphoma patients, not in those with

solid tumors, thus, due to study group heterogeneity, the remission rate was relatively low (Fig.3.13.).

Event comparison Cushing's syndrome, emesis and toxic hepatitis were the earliest side effects. Alopecia and medullary aplasia were the most frequent side effects while oral herpes or thrush and oral candidiasis were the most infrequent side effects (Fig.3.14).

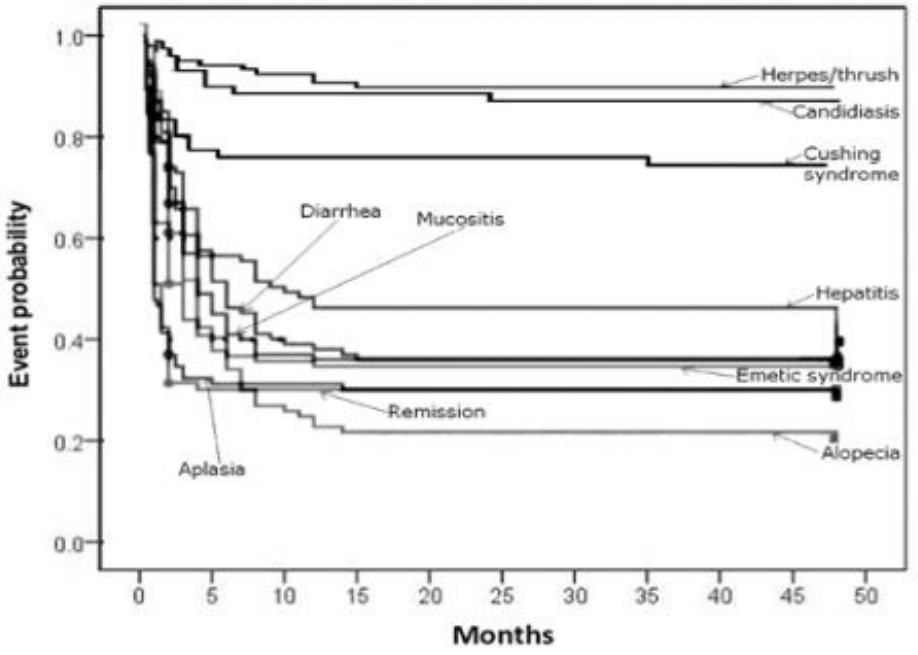


Figure 3.14. Comparison between time evolutions of all studied events.

- ***Hydrocephalus secondary to chemotherapy in a case of prenatally diagnosed giant immature grade 3 sacrococcygeal teratoma: A case report and literature review***

The case of a newborn diagnosed with a sacrococcygeal teratoma and which presented an unusual and unpredictable post chemotherapic complication was published in 2016.

The patient was diagnosed at 24th week of gestation, and was closely monitored by serial ultrasound. The morphology of the lesion was defined by fetal MRI performed at 25th week of gestation. The baby girl was born at 39 weeks of gestation by caesarean section. A large sacrococcygeal lesion was confirmed at postpartum examination, with a diameter of 22 cm. The serum markers showed an elevated level of *a*-fetoprotein (AFP) (>4425.29 UI). On the second day of life, the child underwent surgical excision of the sacrococcygeal tumor and the pathological exam revealed an immature teratoma.

The child underwent a bleomycin, etoposide and cisplatin (BEP) protocol. The postoperative course was complicated by surgical dehiscence and sepsis (blood culture positive for *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) treated by local care and prolonged intravenous antibiotics. Subsequently, the child developed subarachnoid hemorrhage and secondary progressive active hydrocephaly that required a ventriculoperitoneal shunt at the age

of 3 months. The child's outcome was positive after 8 cycles of chemotherapy, with no impairment in micturition or defecation, functional ventriculoperitoneal shunt, normal neurological function, normal values of AFP (4.71 UI/ mL), negative pelvic CT scan, with no signs of local recurrence at the age of 18 months.

3.2.5. Discussion

- ***The utility of serial evaluation of ctni and bnp in early detection of anthracycline-induced cardiotoxicity in children***

The study highlights the presence of anthracycline cardiotoxicity in pediatric oncological patients with a considerable 70.6% incidence. The data obtained in this study correlate with the results referenced studies published in the literature (Christenson et al., 2015; Stevens and Lenihan, 2015; Schultz et al., 2003). It stands high percentage of infraclinical cardiotoxicity (64.7%). Many of the clinical manifestations are nonspecific and may be misinterpreted. They are routinely assigned to the malignant disease, leaving paraclinical investigations to early highlight the changes (Stevens and Lenihan, 2015).

The first echocardiographic changes in our patients were those of diastolic function subsequent appeared and the systolic function. We did not found positive statistically significant correlation between electrocardiographic changes and the emergence of cardiotoxicity; this result is con-sistent with the results reported by other authors (Singh et al., 2015; Lipshultz et al., 2008).

As shown in other studies (Schultz et al., 2003; Lipshultz et al., 2008; Simbre et al., 2005), paraclinical method of choice is to evaluate serum levels of BNP and cTnI, that correlates with echocardiographic assessment. Evaluation of serum BNP and cTnI is a non-invasive, easily reproducible, with substantially reduced cost compared echocardiogram or other imaging method.

Asymptomatic patients with an elevated BNP's during study period can be considered high risk for developing anthracycline cardiomyopathy. In a small number of cases were highlighted elevated BNP levels prior to anthracyclines treatment initiation which may suggest the existence of other factors involved in myocardial wall stress (anemia, tachycardia). The great marginal significance prognosis correlation between anthracyclines cumulative dose and higher levels of BNP ($r = 0.295$, $p = 0.091$), in terms of a strong relationship between elevated BNP and cardiotoxicity, as well as a strong relationship between higher anthracyclines cumulative dose and cardiotoxicity, suggests that other conditions which influence the BNP values need to be considered for further evaluation. In some patients changes in biomarkers and echocardiography were transient. This emphasizes the need of monitoring initial and in dynamic status, to confirm whether patients with subclinical changes will develop cardiomyopathy over time and where is appropriate to start a cardioprotective treatment

The results on studying serum cTnI levels were according to the data obtained from other important studies in the literature (Christenson et al., 2015, Stevens and Lenihan, 2015), but larger studies are needed to establish whether this biomarker can be used to predict cardiotoxicity. An individual variability exists in the relation between cummulative dose - cardiotoxic response, so

efforts should aim to prevent cardiovascular morbidity than in chemotherapeutic protocols modification (Khakoo and Yet, 2008; Steinherz et al., 1991).

- ***Mutation in the ethiological spectrum of systemic infections in patients with hematological malignancies***

Anti-cancer chemotherapies (e.g. cyclophosphamide, methotrexate, 5FU) lead to the occurrence of mature enterocytes (Bultzinggslowen, 2003), an decreased number of intraepithelial lymphocytes and IgA secreting plasma cells, the opening of occluding junctions. In 1985 Tancrede CH (Tancrede, 1985) showed that there is a close connection between neutropenia (0.1 x109/L) and the bacterial infection caused by *Enterobacteriaceae* and *Pseudomonas.* The concordance between the antibiotype, biotype and serotype of bacterial strain isolated from blood and feces demonstates the intestinal origin of bacteraemia. In our study we observed the intestinal origin of the isolates in the blood cultures in two situations (Candida guilliermondii and Streptococcus salivarius), based on the correlation between the antibiotype and the biotype.

In 1978 over 58% of the bacteraemia cases in patients with leukemia were caused by gram-negative bacteria: *E.coli, Pseudomonas spp, Klebsiella spp (*Ellis, 2004*).* From the prevalence of gram-negative microorganisms, starting with the 1990s, an increase in the incidence of systemic infections caused by gram-negative bacteria has been noted. Several causes responsible for the alteration of the etiologic spectrum have been incriminated: the large-scale use of vascular catheters, the active antibiotic prophylaxis upon gram-negative pathogens, but not upon the gram-positive ones, the aggressive chemotherapy treatments that severely alter the digestive mucous membrane (mucositis) (Zinner, 2000). Currently, gram-positive bacteria cause 40% to 75% of the bacteriemia cases in neutropenic patients in the USA (Wisplinghoff, 2003). Coagulaso-negative staphylococcus was the most frequent microorganisms isolated in our study. Although these bacteria belong to the skin microbiota and are usually associated with catheter bacteraemia, they also belong to the digestive tract and the analysis of the plasma pattern of coagulase-negative staphylococcus isolated from the blood indicated that in 70% of the cases they originate from the mucous membrane microbiota. In the patients included in our study we isolated gram-positive cocci in 32.5% of the cases and staphylococci in 22.5% of the cases, of which 3 isolates (23.07%) were methicillin resistant (MRSA).

The use of fluoroquinolones and cotrimoxazole as antibiotic prophylaxis during the period of neutropenia is often cited as an important predisposing factor of bacteremia with streptococcus viridans. In 1978, the first septicemia caused by streptococcal viridans was reported in a child cancer patient, and in 2004 the incidence of bacteraemia with streptococcal viridans in patients with malignancies was 5% (Lyytikainen, 2004). In our study group, we encountered a single bacteraemia episode caused by S. salivarius (2.5%) in a female patient with multiple myeloma who had not previously received antibiotic prophylaxis.

The severity of mucosal damage is a predisposing factor for anaerobic bacteraemia. These are usually polymicrobial, and their low incidence in leukemic patients may also be due to the routine use of antibiotics that are active on anaerobic bacteria (Mathus, 2002). We isolated

anaerobic bacteria in two patients (5%) with acute leukemia and neutropenia; The chemoprophylaxis in these situations included a beta lactam and an aminoglycoside.

Of the most often mentioned predisposing factors of systemic mycotic infections we can mention: neutropenia occurring for over 3 weeks, the selective pressure of antibiotics therapy on the resistant microbiota, the presence of central venous catheters, colonization with *Candida spp.* In 1988 the most frequent isolated fungi were *Candida albicans* and *Aspergillus fumigatus.* In the following years an alternation of the etiologic spectrum of mycotic infections was noted, indicated by the increased prevalence of infections caused by *Candida* non *C. albicans* species and the emergence of other fungi *(Fusarium, Scedosporium, Penicillium marneffei, Zygomycete, Paecilomyces spp)* (Zinner, 2000; Walsh, 2004; Lionskis, 2004). Of the 4 mycotic episodes identified by us (10%), 2 were determined by *Candida* non *C. albicans* and 2 *by filament fungi* (Scedosporium spp, Alternaria spp); in 3 of the 4 cases the patients suffered from acute leukemia, neutropenia and had received antibiotic prophylaxis.

Almost all types of bacteria, both aerobe and anaerobe, were isolated in the L-form stage in bacteraemia and septicemia (Mattman, 2000). This is why it was speculated that when a patient becomes febrile, the pathogen circulates in the blood stream in the L-form stage, regardless of the localization of the infection.

In our study, 95% of the patients with positive blood cultures had received antibiotic therapy before sampling the blood culture (which constantly included a betalactamine). We isolated 23 cell wall deficient bacteria (57.5% of the total number of isolates) of which 9 were irreversible at the normal growth form and 14 revertants (6 gram-positive cocci, 5 gram-positive bacilli, 3 gram-negative bacilli), obtaining thus an approximate percentage of gram-positive bacteria obtained by the reverse of the initial L-forms with the one reported by Woo et al. (Onwuamaegbu et al., 2005). The revertants correlated significantly from a statistical viewpoint just with the gram-positive bacilli (p under 0.05) thus providing a partial explanation for the percentage of isolated gram-positive bacilli (12.5%). When correlating the antibiotic prophylaxis with the microscopic categories we obtained a value of p=0,097 for the L-forms which indicates that it tends to become statistically significant if the number of investigated patients increased.

As the cell wall deficient bacteria do not completely achieve the first and third of Koch's postulates, many microbiologists doubt their capacity of producing lesions in the host. In this context it would be advisable and much safer to consider the possibility that the L-forms are capable of triggering diseases by means of mechanisms that are not completely understood. The therapeutic approaches should include combinations of bactericide and bacteriostatic antibiotics, as without the use of antibiotics that use both versions of the bacterial population the involvement of L-forms in pathogenesis would remain unaccounted for (Onwuamaegbu et al., 2005).

- ***Chemotherapy-related toxicity in childhood neoplasia***

Iatrogenic Cushing's syndrome often has a reserved prognosis due to corticoid resistance (Super et al, 1997). Adrenocorticotropic hormone (ACTH) can be excessively produced within the frame of a paraneoplastic syndrome. Alternatively, Cushing's syndrome may develop as a direct effect of high prednisone dosage included in some chemotherapy regimens. Neoplasm-induced

ACTH may have a different structure from endogenous ACTH and immunohistochemical staining using polyclonal anti-ACTH antibodies may not be useful in tumor cells (Pui and Evans, 1998).

The incidence of post chemotherapy alopecia reported by several authors (Pui et al., 1995), is 65%, this percentage being in accordance with our study. The process begins in weeks 2-4 of treatment and may be complete in 1-2 months (Felix et al., 1988).

Oral mucositis may limit the dose of some chemotherapeutic drugs. The incidence of mucositis is 31-85%, even higher if cervical region radiotherapy is delivered (Reichel et al., 2001) and higher in immunocompromised patients (Jansen et al., 2007). Intestinal mucositis quantification is difficult, with diarrhea as its sole clinical sign, as well as a non-specific one. Oral mucositis is associated with infectious episodes and gastrointestinal mucositis is accompanied by infection and bleeding (Marcucci et al., 1998). An infection of previous ulcerative lesions with bacteria, viruses (herpes or thrush), and fungi (candidiasis) may occur; in our study, the incidence of these complications was 12.2% for oral candidiasis and 13.2% for herpes or thrush.

Medullary aplasia is a feared complication of the chemotherapeutic regimens. For instance, the average duration of febrile neutropenia after induction therapy in AML is 11-31 days (Hagemeijer et al., 1987; Gleissner et al., 2005), depending on the cytotoxic agent administered, hematopoietic growth factors used (Abdelhaleem, 2007), patient age (Supriyadi et al., 2012) and myelodysplasia (Borowitz et al., 2008). Several studies relate the medullary aplasia with a death rate of 3-29% (Bene et al., 1995; Catovsky et al., 1991).

Preexisting conditions, tumors, immunosuppression, hepatitis, nutritional deficiencies or total parenteral nutrition may influence patient susceptibility to hepatic toxic injuries. Therefore, it is difficult to attribute hepatic injury to toxic reactions solely (Cheng et al., 2004). Many hepatotoxic reactions are idiosyncratic, due to immunologic mechanisms of the host (van der Linden et al., 2012) and often they are not dose-dependent. In our study, emetic syndrome did not always appear from the first chemotherapy administration.

Nausea and vomiting may become worse during the treatment (Zhang et al., 2011; Hughes et al., 1991) and may be so severe that the patient requests treatment withdrawal (Gabert et al. 2003; Gerr et al., 2010; Sakaki et al., 2009; Stasik et al., 2006). The most pro-emetic chemotherapeutic drugs are cisplatin, carboplatin and doxorubicin (Jiang et al., 2005).

The global remission rate of malignancies in our study was 69.6%, but one must consider the heterogeneous pathologies. If in the literature the remission rate of ALL could be as high as 85%, (Park et al., 2011), statistics show remission in myelodysplastic syndromes may be as low as 28% (Lou et al., 2010).

The most frequent form of chemotherapy-related toxicity in children was alopecia (79.3%), appearing during the first 14 months of treatment, followed by medullary aplasia (71.1%), occurring in the same time interval, and mucositis (65.3%), occurring within the first 12 months of treatment.

Diarrhea and emetic syndrome appeared in 64% of the cases each, during the first 15 and 12 months, respectively. Toxic hepatitis developed in 61% of the patients, during the first 12 months of treatment and Cushing's syndrome appeared in 21.5% of the cases, all of them during

the first 6 months. Oral herpes or thrush and oral candidiasis were less frequent (13.2% and 12.2%, respectively) and appeared during the first 15 and 14 months, respectively. Remissions of malignancies were obtained in 69.6% of the cases, all of them during the first 4 months of treatment. While alopecia and medullary aplasia were the most frequent side effects, the earliest ones were Cuhsing's syndrome, emetic syndrome and toxic hepatitis.

3.2.6. Conclusions

The clinical management of pediatric cancers will always have the burden of the potential lack of adherence to chemotherapeutic protocols for children and their families. The potential severity, frequency and unpredictability of the toxic treatment in childhood cancer reveal the importance of the presented studies.

Early detection of anthracycline related cardiotoxicity and highlighting the patients at risk is a goal of many researchers in the field. BNP assessment at the initiation of treatment and subsequently in dynamics is useful in highlighting patients at increased risk of developing cardiotoxicity, and to establish therapeutic strategies to prevent installation of severe heart damage, improving the overall outcome of these patients.

Blood stream infections usually occur during febrile neutropenic episodes in patients with hematological malignancies. The involvement of the L-forms in infectious pathology is still a controversial issue. Our aim for the future would be to eradicate both populations of bacteria, the cell wall deficient bacteria and those which present cell wall integrity, and observe if this approach modifies the outcome of the disease. Futher studies should clarify the cost-benefit relationship as well as whether the osmotic protective environments should be incorporated in the routine system of blood cultures.

The most frequent form of chemotherapy-related toxicity in children was alopecia, followed by medullary aplasia and mucositis. Diarrhea and emetic syndrome appeared in 64% of the cases each, during the first 15 and 12 months, respectively. Toxic hepatitis developed in 61% of the patients, during the first 12 months of treatment and Cushing's syndrome appeared in 21.5% of the cases, all of them during the first 6 months. Oral herpes or thrush and oral candidiasis were less common and appeared during the first 15 months of treatment. While alopecia and medullary aplasia were the most frequent side effects, the earliest ones were Cuhsing's syndrome, emetic syndrome and toxic hepatitis. Remissions of malignancies were obtained in 69.6% of the cases, all of them during the first 4 months of treatment. Periodic monitoring should be instituted immediately after the treatment and within a long term follow-up.

3.3. Childhood obesity – From Chemotherapy related Late Effect To Cardiovascular Risk Factor

3.3.1. Introduction

The increased prevalence of obesity among children determined the rising number of its comorbidities in children and adult, too. In children, we are witnessing an upward trend in the

prevalence of obesity globally, from 0.7% in 1975 to 5.6% in 2016 in girls and from 0.9% to 7.8% in boys. With the increasing prevalence of obesity in pediatric age, the number of complications associated with obesity has also increased: dyslipidemias, type 2 diabetes mellitus, fatty liver disease, sleep apnea, microalbuminuria, elevated triglycerides (TG), total and low density lipoprotein cholesterol (LDL-C), and insulin levels, as well as a reduced high density lipoprotein cholesterol (HDL-C) levels. All of them represent risk factors for the occurrence of cardiovascular diseases (Cozzolino et al., 2015; Alpert et al., 2016).

Insulin resistance (IR) and chronic inflammation have an essential role in the pathogenesis of obesity-associated comorbidities (Lopez-Sandova et al., 2018).

The adipose tissues release many inflammatory mediators which predisposing to a pro-inflammatory state and oxidative stress. Among the markers of chronic inflammation, interleukin 6 (IL-6) is an adipocytokine with a pro-inflammatory role and contributes to IR.

Metabolic endotoxemia is related to systemic and local inflammation and therefore may contribute, at least in part to cardio-metabolic disease risk associated with obesity (Kallio et al., 2015).

Other signs of cardiovascular dysfunction in the obese child are: significantly higher arterial blood pressure, changes in the structure and function of the myocardium (left ventricular hypertrophy, left ventricular diastolic dysfunction, and myocardial dysfunction), and the occurrence of long-term epicardial fat (Cozzolino et al., 2015; Alpert et al., 2016; Elshorbagy et al., 2016). Cardiovascular disorders in childhood are serious because they cause heart failure, acute coronary syndrome, and sudden premature death in adult life (Attar et al., 2016; Wong et al., 2004) Screening for early metabolic complications is considered very important, but it is still unclear which parameter would be better to focus on: assessing body mass index or waist circumference as a cardio-metabolic risk factor (Magnussen et al., 2012).

The waist circumference (WC) is an easy-to-determine clinical parameter for assessing the nutritional status of the child, independent of body mass index (BMI) (Bassali et al., 2010). WC correlates with visceral obesity, which is why obese children with elevated WC need to be carefully monitored to prevent long-term cardio- metabolic complications.

Studies have proven that central obesity is an independent risk factor for coronary artery disease, arterial hypertension, dyslipidemia (Umer et al., 2017; Larsson et al., 1984).

3.3.2 Aim

The aim of our papers was to evaluate the presence of certain markers of inflammation and insulin resistance in obese pediatric patients: IL-6, Intercellular Adhesion Molecules (ICAM) and endotoxemia and their correlation with metabolic markers of IR represented by insulinemia, HOMA index (Homeostasis model assessment), plasma cortisol. Also, to establish the involvement of waist circumference as a predictor factor for obesity related cardiovascular complications in children.

3.3.3. Material and Methods

Patient cohort

- ***Waist circumference a clinical criterion for prediction of cardio-vascular complications in children and adolescences with overweight and obesity***

In 2020, we conducted a retrospective study that included 160 children with overweight and obesity, hospitalized between January 1, 2016 and December 31, 2018 in the "Saint Mary" Emergency Children Hospital, Iasi, Romania.

The inclusion criteria were overweight and obesity diagnosis without associated pathologies. Obese patients with associated medical illness (genetic syndromes, diabetes, congenital heart or dyslipidemic diseases, renal, or neurological diseases), eating disorders, chronic medications were excluded.

According to their age, patients were included in 2 groups: group A: children between 6 and 11 years old; group B: adolescents between 12 and 18 years old.

- ***Predictive markers of early cardiovascular impairment and insulin resistance in obese pediatric patients***

We expand in 2021 our research on pediatric obesity in order to identify more predictive markers for early cardiovascular damage. A prospective study of 115 pediatric patients admitted and followed in Children's Hospital "Sf Maria" Iaşi between January 1st and December 31st 2019 was conducted. The patients were divided on two groups: the study group included 85 obese pediatric patients between 6 and 18 years old with obesity without associated pathologies and the control group with 30 pediatric patients with normal BMI.

The newly diagnosed obesity without any dietary and / or pharmacological treatment was the inclusion criteria. The exclusion criteria were smoking, pregnancy, secondary and genetic causes of obesity, cardiovascular diseases in treatment and other chronic diseases, autoimmune diseases, hormonal abnormalities (thyroid diseases, polycystic ovary syndrome, secondary amenorrhea), or and administration of any chronic therapy in the previous three months.

Diagnosis methods

In both published studies, anthropometric data (height, weight, WC, BMI), biologic (total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) high-density lipoprotein cholesterol (HDL -C), triglycerides (TG), glucose levels, alanine aminotransferase values, urea, creatinine) and imagistic studies were performed. IL-6, ICAM, endotoxemia, insulinemia, plasma cortisol, HOMA-IR were used for the evaluation of the inflammatory and metabolic status. We used enzyme-linked immunosorbent assay (ELISA) kits for the quantitative detection of human IL-6, sICAM-1, cortisol.

Interpretation of BMI values was based on BMI Z score and BMI percentile, applicable for age and sex, according to WHO standards, using WHO AnthroPlus software. Depending on the BMI Z score, the patients were classified into overweight (BMI Zscore >+1SD or BMI percentiles between 85 and 97th), obese (BMI Z score >+2SD or BMI percentiles between 97 and 99.9th), and severe obesity (BMI Z score >+3 SD or BMI percentiles>99.9th).

WC was measured using a centimeter, halfway between the coastal rim and the iliac crest,

at the end of the expiration and adapted to tables with specific percentiles for age and sex, developed based on National Health and Nutrition Examination Survey (NHANES) 3 data (Fernandez et al., 2004). Visceral obesity was defined by values over the 90th percentile of WC (Bassali et al., 2010).

The blood pressure (BP) value was interpreted according to the National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents. Children hypertension was defined as systolic BP and/or diastolic BP >95th percentile, adjusted for height, age, and sex, at least 3 separate determination. In our study, BP values ≥90th percentile were defined as ”elevated BP” or vascular impairment (National High Blood Pressure, 2004).

Echocardiography was performed in all patients, the following parameters being thickness of the interventricular septum (IVS), diastolic diameters of the left ventricle (DdLV), left ventricular mass (LVM), the relative thickness of the ventricular wall, the presence of epicardial fat. Diastolic dysfunction was evaluated through the *E*/*A* ratio and the pulmonary venous flow through the *S*/*D* ratio. Left ventricular hypertrophy (LVH) was defined as LVM index >95th percentile for normal children and adolescents. Relative wall thickness (RWT) above >0.41 was considered pathological. Patients with increased LVM index and elevated RWT (>0.41) had concentric LVH; those with increased LVM index and normal RWT (<0.41) had eccentric LVH. Concentric remodeling was defined as elevated RWT, but with normal LVM index.

Pathological epicardial fat quantified by echocardiography was considered over 4.1 mm (Foster et al., 2008; Fang et al., 2019). Patients who had concentric or eccentric LVM hypertrophy, concentric remodeling, and/or epicardial fat with pathological values were considered to have cardiac impairment.

Statistical analyse

Statistical analyzes of the variables were performed using SPSS software v.20 (IBM Corporation, North Castle Drive, Armonk, NY 10504-1785, U.S.A.) type variables were reported as mean with standard deviation and STATA 16 software (StataCorp LLC, Texas 77845-4512, USA). Comparisons between the analyzed groups were performed using Student *t* test or Mann–Whitney *U* test for continuous variables.

The qualitative variables were presented as absolute (n) and relative (%) frequencies, and the comparisons between groups were made based on the results of McNemar, Yates Chi-square, or Fisher exact test. Univariate and multivariate analysis of prognostic factors regarding cardiovascular complications was performed using the Logistic regression model. To verify the correlations between the continuous type variables we applied the Pearson univariate correlation test. Quantitative analysis of the contribution of each biochemical parameter and BMI in the modification of inflammatory markers and metabolic markers of insulin resistance was performed based on the coefficients of multiple linear regression. At the same time, their predictive power was estimated based on the receiver operating characteristic (ROC) curve and the AUC value (area under the ROC curve).

The significance level calculated in the used tests (*P*-value) was considered significant for *P* values <.05.

3.3.4. Results

- ***Waist circumference a clinical criterion for prediction of cardio-vascular complications in children and adolescences with overweight and obesity***

The 2020 study cohort was divided in two study groups: group A included 97 patients aged 6 to 11 years old (mean age 9.82 ± 2.2 years) and group B (adolescents) included 63 patients aged 12 to 18 years old (mean age 14.7 ± 1.6), with a sex distribution male predominance in both groups (59.8% in group A versus 60.3% in group B).

In the study group A, 32.99% of children had severe obesity while in the group B, 44.44% of adolescents were overweight. These results are concordant with the classification of the study groups according to the nutritional status, with significant differences regarding the classification of children and adolescents in overweight, respectively morbid obesity.

Obesity and severe obesity are significantly associated ($P < .001$) with the age of <12 years old. Therefore, the BMI percentile indicated higher mean values for children compared with adolescents ($P <.0001$).

Considering the age criterion and the classification of hypertension, in obese adolescents (8.3% vs 30.2%; $P =.0003$) pre-hypertension and hypertension are significantly more frequent (20.6% vs 14.4%; $P =.0003$).

The assessment of total cholesterol and triglycerides values between children and adolescents revealed no statistically significant differences between the 2 age groups for cholesterol, but statistically significant differences between children and adolescents ($P =.0142$) regarding triglycerides values.

The correlation between epicardial fat and visceral obesity in children and presence of visceral obesity was unquestionably associated ($x^2 = 11.72$, $P =.0006$) with the presence of pathological epicardial fat: from the 46 cases without visceral obesity only 2.17% had pathological epicardial fat, whereas of the 92 cases with visceral obesity, 21.74% presented pathological epicardial fat (Table 3.4.).

Table 3.4. Evaluation of the association of epicardial fat versus visceral obesity.

	Visceral obesity			
	Absent	Present	Statistical test[a]	P-value
Pathological epicardial fat				
No (n/%)	45/97.83%	72/78.26%	11.7281	.0006*
Yes (n%)	1/2.17%	20/21.74%		
Visceral obesity versus pathological epicardial fat		95% CI for AUC		
Area under the curve	0.668	0.562–0.775		.014*

The analysis revealed that BMI is not a significant predictor for vascular impairment for either children or adolescents (AUC= 0.57, $P =.327$ vs AUC= 0.54, $P =.53$).
However, BMI is an important predictive factor for the occurrence of cardiac impairment in

children (AUC= 0.62, *P* =.041) and adolescent (AUC = 0.66, *P* =.036). In the age group 6 to 11 years, among the factors analyzed (BMI, visceral obesity, TC, TG, LDLc, HDLc), none of them are important predictive factors for vascular impairment (*P* > .05), but BMI is a significant predictive factor for cardiac impairment.

Visceral obesity is not a risk factor for vascular or cardiac impairment in this age group. In adolescents, the results showed that visceral obesity is an important predictive factor for the occurrence of vascular (AUC= 0.669, *P* =.021) and cardiac (AUC= 0.697, *P* =.037) impairment. Also, increased levels of TG and LDLc are predictable for the occurrence of cardiac impairment in adolescents (AUC= 0.67, *P* =.044; AUC= 0.66, *P* =.038). Total cholesterol is not a predictive factor for cardiac manifestations.

In our study, visceral obesity was predictive for increased the LMV index values in both children (AUC= 0.594, *P* =.024) and adolescents (AUC = 0.53, *P* =.035). Moreover, concentric LV hypertrophy is significantly influenced by the presence of visceral obesity (AUC= 0.664, *P* =.013 children; AUC= 0.716, *P* =.026 adolescents). Concentric remodeling and the presence of eccentric hypertrophy were not significantly influenced by the presence of visceral obesity (*P* > .05). Regarding diastolic dysfunction, no changes in the *E*/*A* ratio were identified, but the *S*/*D* ratio <1 was identified in 6 patients with severe obesity who also had hypertrophic cardiomyopathy.

- ***Predictive markers of early cardiovascular impairment and insulin resistance in obese pediatric patients***

In this study, published in 2021, based on our previous experience, we intended to complete the biological panel for pediatric obesity in order to achieve a more comprehensive understanding of the early cardiovascular damage and the insulin resistance in these patients.

No significant differences in the two study groups related to age and gender of the children were noted. In the control group, the mean age was 13.4 ± 2.47 years and for the study group, the mean age was 12.1 ± 3.4 years.

A higher values of triglycerides, HbA1c, insulinemia and HOMA index and lower levels of HDL cholesterol in the obese patients was observed. Also, inflammatory markers, IL6, ICAM 1, and endotoxemia were significantly higher in obese patients versus the control group.

A significant correlations between BMI and inflammatory markers of the obese patients was indentified in our patients and are presented in table 3.5.

IL6 correlates significantly with blood glucose (*r* = -0.334, *p* = 0.001) and BMI percentile (*r* = 0.252, *p* = 0.031) (Table 3.5.), these being significant predictive factors for cardiometabolic diseases.

ICAM and serum triglycerides values (*r* = 0.252, *p* = 0.001), plasma glucose level (*r* = -0.145, *p* = 0.044) and with BMI (r = 0.302, p = 0.037). Also, in the context of obesity, the results indicated a significant correlation between endotoxemia and plasma glucose level (*r* = 0.346, *p* = 0.024) but also with BMI percentile (*r* = -0.255, *p* = 0.001) (Table 3.5.).

Table 3.5. Univariate analysis showing correlations between inflammatory markers and biochemical parameters, BMI percentile.

Dependent variable	Independent variable	Correlation Coefficient (Pearson Correlations)	*p*–value
IL6 vs.	Total serum cholesterol	-0.039	0.318
	LDL-cholesterol	-0.0633	0.427
	Triglycerides	-0.034	0.341
	Plasma glucose level	-0.334	0.001*
	BMI percentile	0.252	0.031*
ICAM vs.	Total serum cholesterol	0.121	0.072
	LDL-cholesterol	0.208	0.008*
	Triglycerides	0.252	0.001*
	Plasma glucose level	-0.145	0.044*
	BMI percentile	0.302	0.037*
Endotoxemia vs.	Total serum cholesterol	0.082	0.166
	LDL-cholesterol	-.0754	0.343
	Triglycerides	-0.035	0.335
	Plasma glucose level	-0.346	0.042*
	BMI percentile	-0.255	0.001*

* Marked effects are significant at $p < 0.05$.

We observed that IL 6 was significantly correlated with blood glucose and BMI percentile, these being significant predictive factors for cardiometabolic diseases. We did not find a positive correlation ($r = 0.09$, $p = 0.297$) between the value of IL6 and ICAM in the analysed groups (Fig 3.15.).

We performed a multivariate analysis to evaluate the contribution of each biochemical parameter but also of BMI percentile in the variation of the inflammatory markers, using ROC curve analysis to evaluate the predictive power of BMI percentile, plasma glucose level and serum triglycerides on IL6, ICAM and endotoxemia. The results indicated a significant predictive power of BMI percentile on inflammatory markers: IL6 (AUC = 0.803, 95% CI: 0.72 - 0.88, $p < 0.001$), ICAM (AUC = 0.806, 95% CI: 0.72 - 0.89, $p < 0.001$) and endotoxemia (AUC = 0.762, 95% CI: 0.68 - 0.85, $p = 0.019$) (Fig. 3.16.a,b). Plasma glucose level shows a significant prediction for IL6 (AUC = 0.784, 95% CI: 0.63 - 0.93, $p = 0.019$). Although a significant correlation with ICAM was observed in the case of serum triglycerides ($p = 0.01$), the results did not indicate a significant predictive power on any inflammatory marker (AUC = 0.60; 95% CI: 0.46 - 0.73, $p = 0.129$).

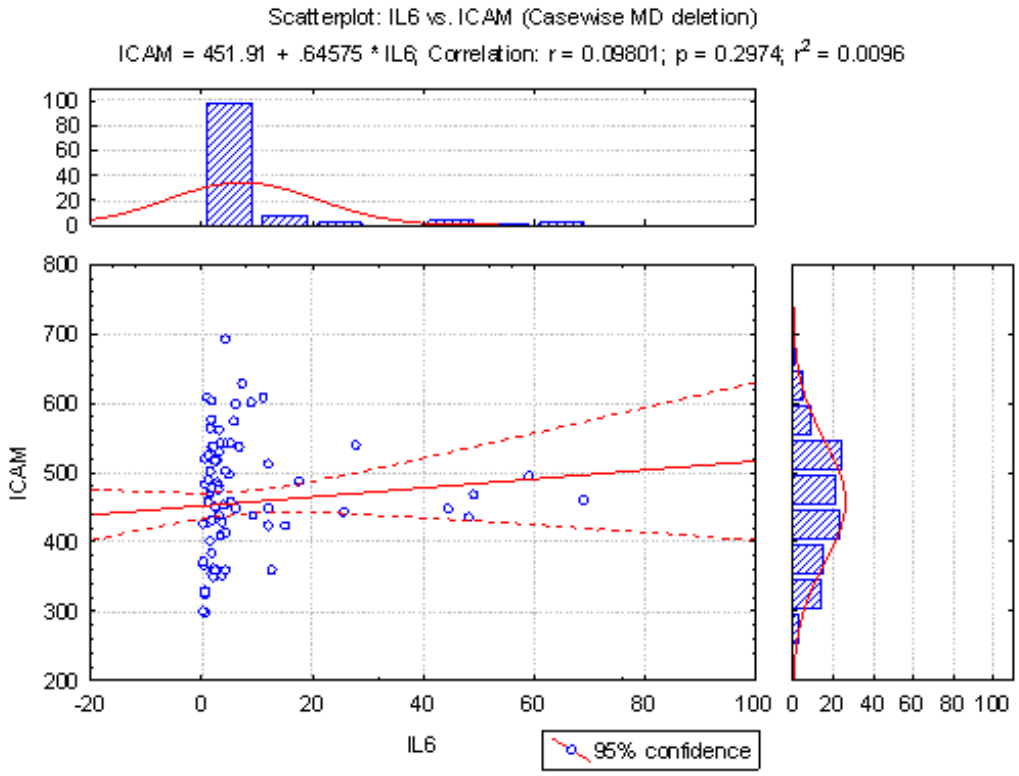
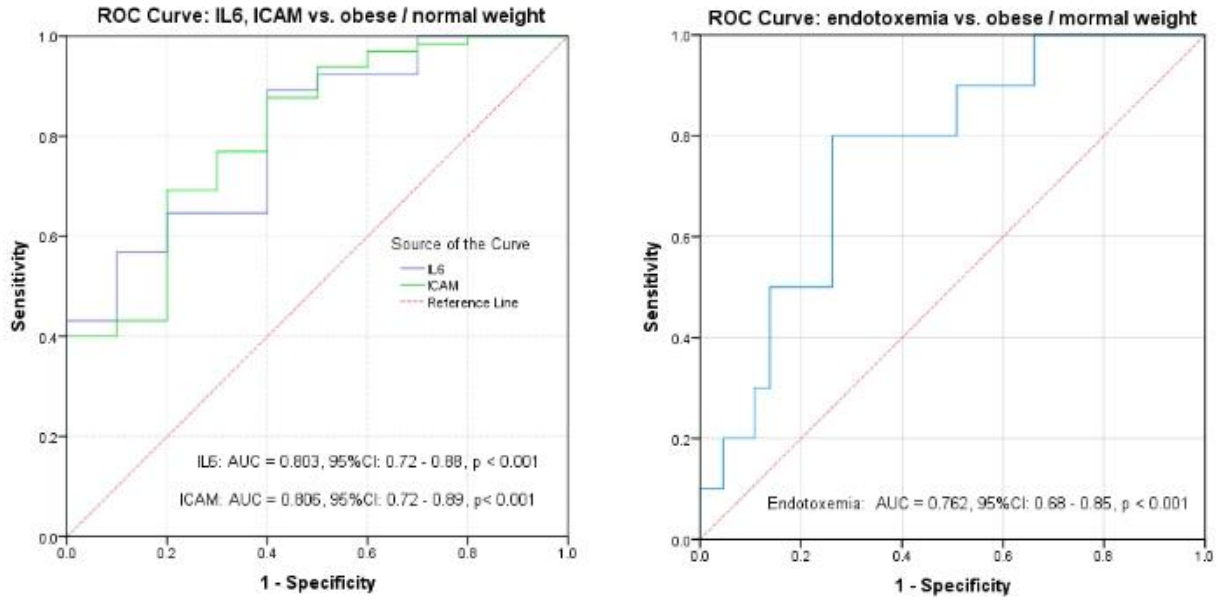


Figure 3.15. The regression line in the correlation of IL6 and ICAM values.



(**a**) (**b**)

Figure 3.16. ROC curves for BMI vs (a) IL6, ICAM, (b) endotoxemia.

The baseline cut-off values for IL6, ICAM, and endotoxemia for obese children and adolescents with early vascular damage are presented in Fig. 3.17.

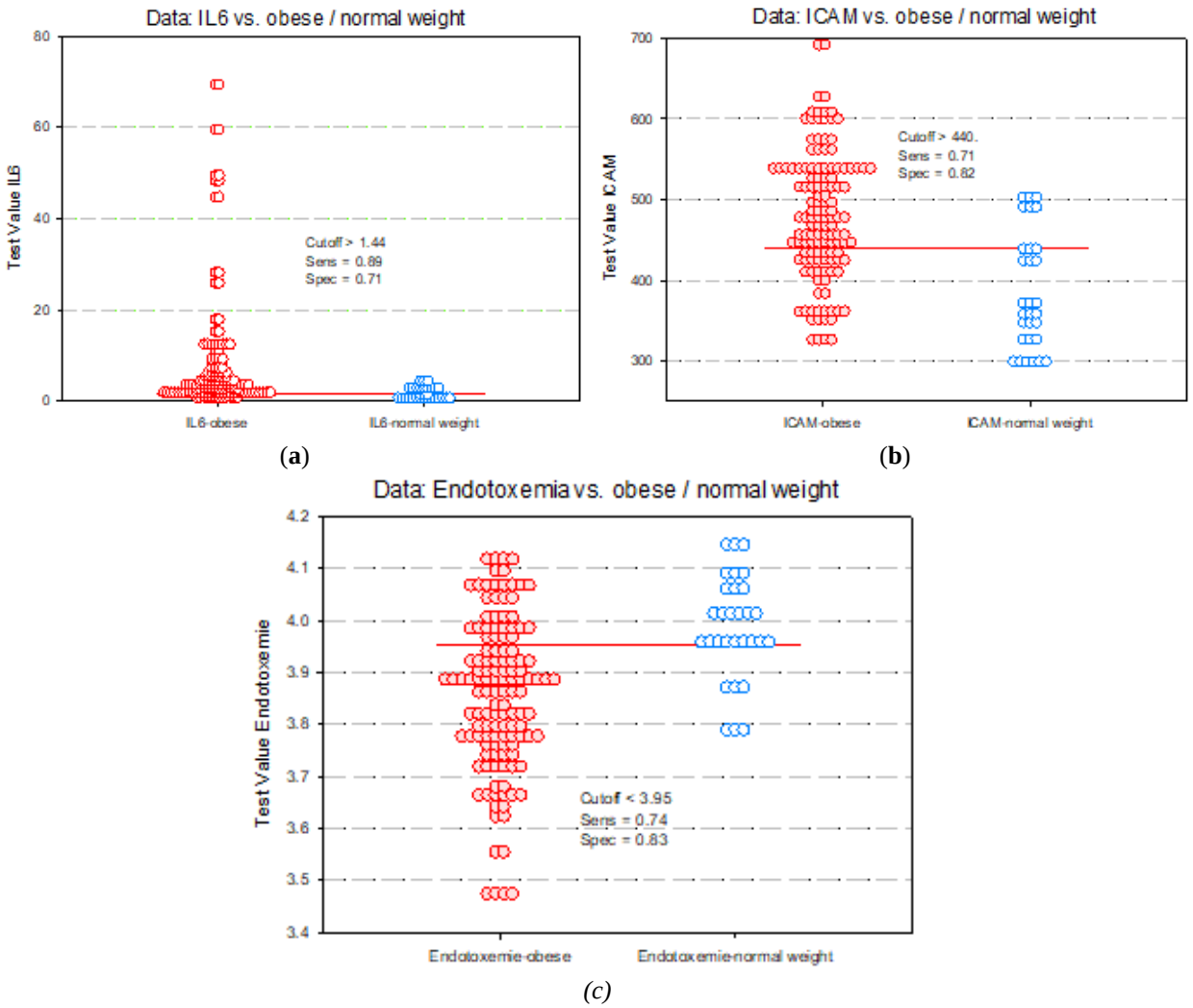


Figure 3.17. Identifying cut-off values predictive for vascular impairment in obese children (dot histogram) for: (a) IL6; (b) ICAM; (c) endotoxemia.

We found that insulin value correlates significantly with BMI (r = 0.52, p = 0.001), total serum cholesterol (r = 0.265, p = 0.022) and triglycerides level (r = 0.228, p = 0.006) (table 3.6.) and that the HOMA index correlates significantly with BMI (r = 0.516, p = 0.001), which is a significant predictive factor for the value of the HOMA index.

HOMA index also shows a significant correlation with total serum cholesterol (r = 0.273, p = 0.017) and with serum triglycerides (r = 0.205, p = 0.009).

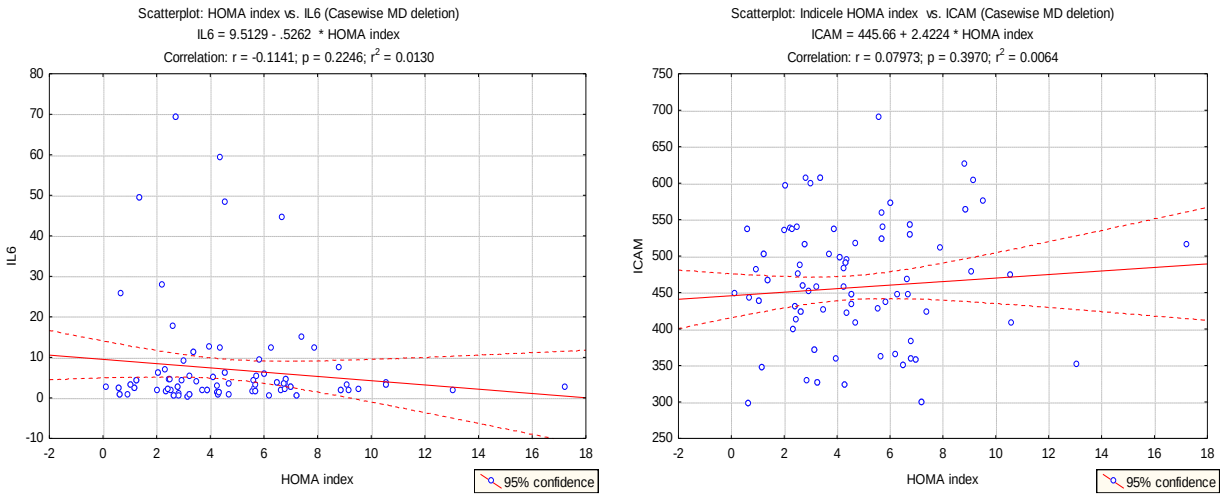
Plasma cortisol did not show significant correlations with total cholesterol, triglycerides, blood glucose levels, body mass index (table 3.6.).

Table 3.6. Univariate analysis showing correlations between metabolic markers of insulin resistance and biochemical parameters, BMI percentile.

Dependent variable	Independent variable	Correlation Coefficient (Pearson Correlations)	p – value
Insulin value vs.	Total serum cholesterol	0.265	0.022*
	Triglycerides	0.228	0.006*
	Plasma glucose level	-0.126	0.142
	BMI percentile	0.522	0.001*
HOMA index vs.	Total serum cholesterol	0.273	0.017*
	Triglycerides	0.205	0.009*
	Plasma glucose level	0.132	0.092
	BMI percentile	0.516	0.001*
Plasma cortisol vs.	Total serum cholesterol	0.037	0.326
	Triglycerides	0.027	0.372
	Plasma glucose level	0.042	0.596
	BMI percentile	0.144	0.067

* Marked effects are significant at $p < 0.05$.

Because the evolution of obesity in our pediatric patients' group is relatively shortly, we noticed that there is no correlation between all these markers (HOMA index and IL6, ICAM, endotoxemia) (Fig.3.18). However, each individually assessed marker has predictive value for the onset of cardiovascular and metabolic impairment in obese children.



(**a**) (**b**)

Figure 3.18. The regression line in the correlation of HOMA index and (a) IL6 values, (b) ICAM.

3.3.5. Discussion

We believe that studies such ours from 2020 and 2021, underline the importance of identifying obese children at high risk for early cardiovascular dysfunction. Childhood-onset of cardiac dysfunction of obese children progress into adulthood, and literature studies show that in overweight and obese adults, heart failure will develop 10 years faster compared with subjects with normal BMI (Abaci et al., 2009; Umer et al., 2017).

BMI and WC were the first cardiovascular risk factors discussed in childhood and adolescence (Janssen et al., 2005), but with certain limitations. WC plays an important role in the early identification of metabolic syndrome and cardiometabolic risk (Flodmark et al., 1994; Maffeis et al., 2008) and provides an indicator of visceral adipose tissue. In obese 12 to 14-year-old children, WC correlates to a potentially atherogenic lipoprotein profile (Bassali et al., 2010; Flodmark et al., 1994). A high BP in children has been associated with increased WC, and childhood obesity is associated with high risk of adult hypertension, especially in boys (Wong et al., 2004). Regarding the predictive power of BMI and WC on elevated BP, literature studies have shown that increased WC is associated with elevated BP even when BMI is normal (Pazin et al., 2017). Visceral obesity induces a variety of structural alterations in cardiac structure and function. Children with severe obesity exhibit a tendency to develop abnormal LV geometry and cardiac dysfunction (Pazin et al., 2017) and furthermore the hypertension related obesity in these patients increase the risk of left ventricular hypertrophy increases (Wong et al., 2004). Most studies agree that obese children have increased LVM index (Fang et al., 2019; Jing et al., 2016). Also, LVM index significantly correlated with BMI in children and adolescents with essential hypertension (Lee et al., 2015).

On the other hand, chronic inflammation in the context of obesity is associated with the development of IR, diabetes, atherosclerosis and hypertension (Head, 2015) and the identifying of children with higher cardiovascular risk and IR at an early stage is crucial in preventing childhood obesity complications (Varda et al., 2020; , Genovesi and Parati, 2020). The role of low-grade inflammation as a link between obesity, IR and endothelial dysfunction is increasingly being discussed in the literature (Bruyndonckx et al., 2013; Bilha et al., 2018). Elevated values of IL 6 is associated with inflammation and promotes the proliferation of vascular smooth muscle tissue, an early component of hypertension and atherosclerosis (Syrenicz et al., 2006; Todendi et al, 2015). ICAM-1 is considered a molecular marker of endothelial dysfunction and increased levels appears to reflect the extent of atherosclerotic lesions and is likely to be a predictive factor for future cardiovascular events in adult (Syrenicz et al., 2006).

Metabolic endotoxemia is a increase in plasma levels of lipopolysaccharides from the intestine with a pro-inflammatory role, increased lipid absorption and development of obesity. The endotoxemia values correlates with the value of BMI (Eworo et al., 2020). Thus, an intervention on the intestinal microbiota through diet and therapeutic measures may be important in reducing inflammation and endothelial dysfunction (Nirmalkar et al., 2018).

The obesity as a major risk factor for the development of insulin resistance was validated in the study of Elnashar et al. which showed that insulin and the HOMA index have a significant increase in obese children (Elnashar et al., 2017).

- ***Waist circumference a clinical criterion for prediction of cardio-vascular complications in children and adolescences with overweight and obesity***

In our study published in 2020, we observed that WC is an important predictive factor for the occurrence of vascular impairment (pre-hypertension and hypertension) only in adolescents, not in children under 12 years. Regarding the predictive value of BMI, this is not a significant predictive factor for vascular impairment neither in children or adolescent in our study. Also, we observed that a WC above the 90th percentile is a predictive factor for increased LVM index and concentric hypertrophy in both children and adolescents. Moreover, concentric remodeling and the presence of eccentric hypertrophy were not significantly influenced by the presence of visceral obesity.

- ***Predictive markers of early cardiovascular impairment and insulin resistance in obese pediatric patients***

In our 2021 study, low grade inflammation was assessed by measuring both IL6 and ICAM. Thus, we observed that IL 6 was significantly correlated with blood glucose and BMI, these being significant predictive factors for IL-6. The correlation between an elevated level of IL-6 and the occurrence of metabolic or cardiac comorbidities in adulthood has been demonstrated. Even if ICAM correlates significantly with serum triglycerides, blood glucose, BMI percentile, only the BMI percentile has significant predictive power over ICAM. We did not find a positive correlation between the value of IL6 and ICAM in the analysed groups. A positive correlation was demonstrated between central obesity/insulin resistance and sICAM-1 levels, with sICAM-1 being considered a prototype inflammatory marker (Elnashar et al., 2017).

Also, a significant correlation of endotoxemia with BMI was noted in our study, BMI being highly predictive of endotoxemia. We investigated the insulin value among children and adolescents, trying to find a correlation with excess weight. We found that the values of insulin is correlated significantly with TG and BMI percentile, but only the BMI percentile has predictive power over insulin.

The tests performed in our study did not identify significant correlations between the value of plasma cortisol and the levels of cholesterol, TG and glycemia, nor with BMI percentile , a fact confirmed by Abraham's study (Marson et al., 2016) which did not find any correlation between BMI percentile or weight and cortisol (either salivary or urinary/24 hours), and no correlation between cortisol and the values of TG, HDL, BP.

The HOMA index has significantly correlation only with BMI percentile, which is a significant predictive factor for the value of the HOMA index. We found a significant correlation between cardiovascular risk factors and insulin resistance defined by HOMA.

The both aforementioned studies had main limitations. In the 2020 study, the impossibility of performing the vascular ultrasound and evaluation of intima media thickness for the evaluation of subclinical atherosclerosis, a marker of morphological vascular damage was a study limitation.

The limitation of 2021 study was represented by the relatively small number of the patients in the both groups. Although BMI is widely used as a surrogate measure of adiposity, it is a measure of excess weight, rather than excess body fat (Freedman et al., 2005), thus the impossibility of evaluating fat mass and fat free mass through densitometry or dual-energy X-ray absorptiometry was a major limitation in both studies.

3.3.6. Conclusions

Our results confirm the importance of identifying and understanting the predictive factors for the occurrence of vascular impairment in overweight and obese children. Important predictive factors such as WC above the 90th percentile as a predictive factor for increased LVM index and concentric hypertrophy in both children and adolescents. Obese pediatric patients with elevated WC need to be carefully monitored to prevent long-term cardiovascular complications. Also, we found a positive correlation between the inflammatory markers studied and the percentile value of the body mass index. We can say that the lower value of the body mass index, more precisely it's percentile, the lower inflammatory markers values will be.

Chapter 4. Ethical and Social Practices in Pediatric Oncology

4.1. Background

The hematology and oncology of childhood is a relatively recent area of study whose development depend upon the evolution of the science. The term of ethical dilemmas is familiar to those who take care of oncological patients. Few domains have so many ethical challenges as pediatric oncology. A diagnosis of pediatric cancer is very distressful for the child and their parents (Bartholdson et al, 2015). The ethical analysis must be made not through the light of classical ethical theories and principles, but by using tangible arguments such as the quality of life, life expectancy, utility / uselessness of treatment, sanctity and dignity of life (Alahmad, 2018; Wiener et al., 2012). Each case presents a unique set of multilayered considerations and may require systematic review to delineate the factors in any ethical dilemma.

The authority of decision is claimed and must be divided among clinician, caregiver and minor patient. In pediatrics, the authority of decision is a right and an obligation that cannot be transferred of parent, where the adult patient may participate. The development of the child's autonomy influences greatly the therapeutic decision. In the societies in transition, the passing from the paternalistic model (dominated by the doctor's authority that makes any decision) to the liberal model (dominated by the patient's sovereignty) is done slowly and with difficulty, determining many conflicts of ethical order, or even legal ones. From the need of avoiding these conflicts and the need of the caregiver's involvement, as well as of the patients, it is opened the issue of informed consent adapted to the sections of pediatrics oncology (Levi et al., 2000). "The doctrine" of the informed consent represents a particular applicability in child and teenager, who

lack the capacity to decide or the legal power of attorney. The concept of the parent's informed agreement (rather than a consent) reflects this distributed decision that customizes the ethical aspects in pediatrics (Levi et al., 2000). At this time, the informed consent represents the basis of the elaboration of the medical decision. Thus, this document becomes one of the main points of the doctor-patient relationship and is the clearest proof of respecting the individual autonomy and of the right of free choice to live in accordance with the personal values and principles. In oncology, there is the issue of the patient's explicit information on diagnosis (Alahmad, 2018, da Silva et al., 2010).

The child with cancer, the parents, and the clinicians face difficult decisions when cure is no longer possible. The relationship developed between doctor and child/parent is important in making judgements and decisions towards the end-of-life of the patients diagnosed with cancer (Valdez-Martinez et al., 2014). According to the American Academy of Pediatrics, the "goal of pediatric palliative care is to add life to the child's years, not simply add years to the child's life". Palliative care offer a greater attention to symptom control needs at the end of life and an improving of health-related quality of life. The process of making the medical decisions enforces balanced and pertinent considerations from a medical, ethical, psychological, social and cultural, religious and, last but not least, legal point of view. Another thorny issue in the Romanian pediatric oncology is the lack of a legal coherent and real frame regarding terminal situations and / or palliative. Although reason and human behavior are based, in principle, on several unwritten laws, creating a correct and adapted legal frame, especially regarding the actions and activities of great responsibility

The main preoccupations that I had in this direction of research were materialized in the next indexed articles:

Published articles

1. Badarau DO, De Clercq E, Wangmo T, Dragomir M, **Miron I,** Kühne T, Elger BS. Cancer care in Romania: challenges and pitfalls of children's and adolescents' multifaceted involvement. *Journal Of Medical Ethics*. 2016 Dec 1;42(12):757-61. **IF=1.529**
2. Dumitras S, Enache M, **Miron I,** Ioan B. Implications Of The Lack Of Romanian Legal Framework On The Medical Attitude Toward Terminal Paediatric Patients-Case Study. *Romanian Journal Of Bioethics*. 2013;11(1). **IF=1.170**
3. Miron L**, Miron I,** Marinca M. Ethical particularities and dilemmas of informed consent in pediatric oncology. Revista *Romanian Journal Of Bioethics*. 2009;7(1). **IF=0.480**
4. **Miron I,** Gavrilovici C, Cucer F. End of life in children non medical decisions in a medical case. *Romanian Journal Of Bioethics* 2009 Jul 1;7(3). **IF=0.480**
5. Tansanu II, **Miron I.** Development of pediatric hemato-oncology in Romania. *Pediatric Hematology And Oncology*. 1995 Jan 1;12(5):429-30.

4.2. Ethical Considerations in Pediatric Oncology and Cancer Care in Romania

4.2.1. Introduction

- ***Development of Pediatric Hematology and Oncology in Romania***

Since the 1960s, the pediatric departments from Bucharest, Cluj, and Iasi have had a special interest in children's tumors because of the changes in pediatric morbidity in Romania.

The first teams to recognize the importance of the problem were the Pediatric Clinic led by Professor A. Rusescu (1960-1975) in Bucharest, the Pediatric Clinic led by Professor Ion Nicolau in Iasi, the Pediatric Clinic of Fundeni led by Professor Dr. Gheorghe Goldis in Bucharest, and the Pediatric Clinic led by Professor Louis Turcanu in Timisoara.

After 1963, the Pediatric Clinic from the Academic Hospital Fundeni represented the working group that developed this subspecialty and trained young physicians. New important centers developed after 1988: the Pediatric Department of the Oncologic Institute of Bucharest, led by Dr. Nicolau, and another working group in Clinical Hospital "Caraiman" led by Dr. C. Palade, and others in Tg. Mures, Craiova, and Oradea. Also, one of the most important working groups treating hematooncologic problems in children is in the Oncologic Institute of Cluj, led by Dr. Stefania Neamtu, which was developed after 1984.

Since 1977, "St. Mary" Children's Hospital in Iasi developed the first Department of Hemato-Oncology, led by Professor Dr. Ioan Tansanu. The building of such centers stimulated professional discussions among staff members, including practitioners and university doctors. The first Oncopediatric Conference, organized in Iasi in October 1977, focused on pediatric lymphoma.

Since 1990, many Romanian pediatricians have become interested in hemato-oncology and have tried to become connected to the West European and American therapeutic attitudes. Young doctors became specialized abroad, in Vienna, Hannover, Paris, Lille, Lyon, and Padova.

- ***Ethical Considerations in Pediatric Oncology – From Informed Consent Particularities to End of Life Decisions***

The doctrine of the informed consent is basically a crucial paradigm in medical decision-making. Ethical dilemmas are to be met at each and every stage when obtaining the informed consent in pediatric oncology; this doctrine seems to apply particularly in case of children and teenagers, who lack the decisional capacity or the legal empowerment to give their informed consent.

The informed consent principles presents particular differences and questions in comparison with the adult oncology (Meisel et al., 1977). The great importance of informed consent is to be recognized as a continuous process in which obtaining a signature represents only a stage and not the purpose (Farnell, 2003).

Debates related to the rights of the child have lately taken a different direction with the spreading acceptance by national and international law of the fact that children also benefit from most of human rights.

A modification of the traditional status of the child in family and society represents an acknowledgement of the respect for the child's personality. With regard to the child with no

capacity of consenting, we have to take into account that any intervention must be done in its benefit and with the agreement of his/her parent, tutor or any other legally designated authority. The child's opinion must be therefore taken into consideration as an increasingly important factor, according to the age and the degree of maturity of this one. This authorization can be withdrawn anytime by another competent body in the interest of the child (Leikin, 1993). It is not also very clear the way in which one could establish the competence of a child and his capacity of understanding the nature of the disease and of the treatment. Every particular case should be considered in its own context. The patient's and the families' uncertainty and anxiety leads some specialists towards further caution when involving children in decision-making, even if they seem to accept this concept (Thomson, 2001).

Many times medical knowledge does not solve the whole complexity of a case, given the multiple interference with social, legal and moral aspects. The ability of modern medicine of treating, healing and prolonging life are so advanced that an end without resuscitation and intensive care manoeuvres would seem inconceivable, regardless of the prejudices it generates. The highest ethical dilemmas in pediatric practice come from clinical situations that lead to "life and death" decisions or the decision with future long-term repercussions or with great impact both on the patient and his/her family, physician and / or medical staff. There should be certain limits imposed by the promotion of the best interests of the patient and the respect of his/her dignity, the medical act being considered based on the notions of "beneficence" and "futility". A family oriented approach respects the complex nature of a parent – child relationship. Medical decision making process, especially for terminal patients, must be balanced and relevant from the medical, ethical, psychological, social, cultural, religious and legal points of view.

- ***Cancer Care in Romania***

Communication about diagnosis and medical treatment for children suffering from life-threatening illnesses is complex. It is a primary step in involving underage patients and families in care and lays the foundation for obtaining parental permission and patient assent for treatment. In practice, child participation in care is often difficult to obtain due to patients' different and sometimes fluctuating preferences, but also parents' protective strategies. Physicians may be susceptible to parental wishes to limit information and feel uncomfortable discussing issues related to uncertainty of cure with patients.

Provision of information is a fundamental step towards shared decision making. It enables minor patients and families to frame personal values when making decisions (Committee on bioethics, 1995). Communication is a complex process and studies report that children wish to know what is happening to them and for clinicians to listen to their preferences (Coyne et al., 2014; Young et al., 2003; Olsson et al., 2015). Some patients prefer to know only certain aspects, to receive information from their parents or to leave decisions up to adults (Young et al., 2003). The process is further complicated by parents' reaction to diagnosis and inclination to protect children from distressing news (Bluebond et al., 2010; Stewart, 2003). Most physicians consider it disrespectful to withhold information, but may comply with parental restrictions (Bartholdson et al., 2015).

4.2.2. Aim

Our article "***Ethical particularities and dilemmas of informed consent in pediatric oncology"*** aim was to draw attention on the need to customize the informed consent in pediatrics, to discuss the differences between adult and pediatric oncological informed consent and to highlight the key role of ethics in this direction.

In the articles "***End of life in children non medical decisions in a medical case"*** and ***"Implications Of The Lack Of Romanian Legal Framework On The Medical Attitude Toward Terminal Paediatric Patients-Case Study"*** our aim was to analyze some of the main moral issue involved by the end of life in pediatric oncology based on two suggestive cases. Our goal was to not provide a moral recommendation, but to show that the medical decision has a strong ethical backup and there may always be several solutions, depending on the physician's and patient's perspective, the hosting institution.

The study published in 2015, "***Cancer care in Romania: challenges and pitfalls of children's and adolescents' multifaceted involvement***" explores the complex process of involving patients with cancer in their care from the perspectives of oncologists and parents in Romania. It analyses how adults act either to limit or support patient participation by evaluating the impact of these actions on children.

4.2.3. Material and Methods

- ***Cancer Care in Romania***

The study published in 2015 was based on semistructured interviews conducted with proxies and treating physicians of patients aged 8–17 receiving treatment at collaborating centres in Romania. Interviews were recorded, transcribed and thematic analysis was applied for all datasets by identifying codes that formed a pattern and organising them in major themes synthesising participants' experiences. We employed purposive sampling to enrol 28 nonrepresentative participants, eighteen proxies and 10 physicians of 18 patients, between May and October 2013.

4.2.4. Results

- ***Ethical Considerations in Pediatric Oncology – From Informed Consent Particularities to End of Life Decisions***

Our paper "***End of life in children non medical decisions in a medical case***" presented the case of a 10 year –old girl, admitted in the pediatric oncology department and diagnosed with end stage of liver cancer with extensive lung metastases. The girl was accompanied by an aunt, her mother working abroad, in Italy. Little was known abou her father or other relatives. Seven days after chemotherapy, the girl's status deteriorate, with generalized pain and she could only be fed by parenteral solutions. Blood tests were modified, showing that any attempt to continue the chemoterapic treatment led to a reduced defense capacity of the body and to the enhancement of bleeding in different organs. At this point she was treated by palliative means, focusing primarily on pain control and support of vital functions. Three days later, the patient became confused, with

a poor response to verbal stimulas and became oxygen dependent. Both physician and aunt understood and accepted that these were the last days of her life. However, her mother was on her way back home. The mother kept in touch with the medical staff, insisting that every effort be made to keep her child alive until her return and to have the chance to say her good -bye and to ask for forgiveness for being away from her.

Unfortunately, the risk of death from respiratory failure was very high, blood oxygen saturation was decreasing progressively. The only method to prolong life for a short period of time was the intubation and the administration of a supportive pharmacological treatment. The family reconciled with the idea of death and refused to prolong her life by extraordinary measures. They wanted a "short" prolongation, until her mother reached the hospital. Therefore, we contacted the intensive care department in order to perform the intubation, while explaining the „non— medical" reasons for that request. The anesthetist accepted, but he draw the attention that this procedure was permanent, and that he would not agree with any subsequent extubation that may allow the girl to die (the family wanted just a short-time intubation, to give time to the mother to come and, after that, to let her die). In this situation, thinking that intubation and supportive measures should be an extended ordeal, her aunt, who had become a second mother over the last years, requested no intubation. The girl died shortly after, before the arrival of her mother.

- ***Implications Of The Lack Of Romanian Legal Framework On The Medical Attitude Toward Terminal Paediatric Patients-Case Study***

Another case of a terminal paediatric patient diagnosed with a hematological malignancy was discussed in this paper. A 12 year old female diagnosed with acute lymphoblastic leukaemia presented numerous meningo-cerebral relapses. The unfavorable evolution of the condition generated at last relapse an episode of status epileptic, which required major anticonvulsant therapy, tracheal intubation and respiratory support. The parents were informed about the inevitable death of their child even in conditions of overcoming the critical situation. However, neither family nor medical team could not make the decision to discontinue life support. After a week, the patient was extubated, with spontaneous breathing, but with significant neurological impairment. The progression of the haematological malignancy determined the patient's death five months later.

- ***Cancer Care in Romania***

The study cohort was represented by eighteen proxies and 10 physicians of 18 patients. Parents and physicians' attitudes contributed to the pace of involvement which can be construed by four themes: restricting, unintentional, widening and enhancing involvement. The unintentional category applied to all patients. Children either passed through all involvement stages, from restricting to enhancing, remained in the restricted and unintentional categories or started from limited involvement at diagnosis and leapt into the widening or enhancing phase.

Restricting involvement Many physicians reported that at diagnosis time they often refrain from disclosing the exact nature of the illness to patients due to uncertainty about parents' preferences on how much children should know. Clinicians found it challenging disrespecting parents' attitudes on information provision and accepted to restrict involvement at parents' request.

This impacted the timing and depth of some patients' involvement and limited information provided at diagnosis and shortly afterwards (quotes 1 and 2 in Table 4.1).

Parents requested partial disclosure because of fear that the word 'cancer', usually associated with dying, would have a negative impact on their child's willingness to fight (quote 3 in Table 4.1.). Retrospectively, parents regretted and recognised that they cannot shield children from all distressing information and the steps they took may not entirely control what children, particularly teenagers learnt about the illness (quote 4 in Table 4.1).

Table 4.1. Restricting child and adolescent involvement

Participant	Quote
1 John's physician (patient age 16)	"[…] during the first discussion, we do not know exactly what the parents want us to do, if they want the child to find out about it [diagnosis] and how they want the child to find out. And so we respect the parents' decision. […] If the parent tells us from the beginning: 'He does not know and we do not want him to know …' in that case, we also have to be careful what we talk with the parents when the child is present."
2 James' father (patient age 11)	"I said…that I don't want the boy to think that this is an illness (without hope) […] I said: 'It is a tumor, sweetie.' […] He (the physician) did not tell him about the illness, because I told him (James) that he has a tumor and it was not necessary for the physician to tell him too."
3 Cody Ray's mother (patient age 10)	"[…] I hope that he will not make the connection (that leukemia is cancer of the blood). […] I saw that he was scared of this word 'cancer'. You know what I mean? That is why I am scared. I am not scared about something else. So he knows that you die from cancer."
4 Melissa's mother (patient age 10)	"You do not have time to think about these things before. They happen to you and you act instinctively as a result and you just go ahead. She asked me if leukemia means cancer and I told her that is does not. I lied to her about this. […] I really regret that I lied to her but to be honest, how can you tell this to your child?"
Names of participants have been changed to protect the identity of responders.	

Unintentional involvement Despite desires to control the pace of information provision, parents could not buffer the outside world or prevent children from seeking information from other sources, such as conversations on the ward and the Internet. Both parents and physicians were aware that parental boundaries on children's knowledge about their illness were fragile and could easily be transgressed (quotes 5–7 in Table 4.2.).

Involvement was in many ways unavoidable in the context of hospitalisation. Physicians mentioned that children were exposed to observation during medical rounds, other patients' experiences and medical procedures. This also implied that patients were confronted with the possibility of treatment failure and of dying by witnessing other patients' evolution (quotes 8–10 in Table 4.2). Some physicians mentioned that they did not want to take the first steps towards revealing the exact diagnosis. This way those children who wanted to know may decide whether to seek more information, how much and when (quote 11 in Table 4.2.).

Table 4.2. Unintentional child and adolescent involvement

Participant	Quote
Cody Ray's mother (patient age 10)	"[…] he also knows he has leukemia. He found out […] From parents who have younger children and their children do not understand what it is happening and they talk about it without thinking about it."
Lisa's grandmother (patient age 17)	"But after they [physicians] gave us all (reports) from the biopsy, she then took the paper and looked it up (on the Internet). Well, she found it (medical forms). I could not hide it from her anymore. […] And so she started to look it up online. How it manifests, how it can be treated and all sort of…all these things, other cases."
John's physician (patient age 16)	"[…] in the end, they find out. It is impossible that they do not get their hands on the discharge forms, even if it does not happen after the first hospital admission. And after that they look it up online and they know."
Melissa's physician (patient age 10)	"I think that nevertheless, they know …. They hear about it. It is impossible for them not to hear about it. They hear what treatment they are getting and they kind of know the name of those substances."
John's physician (patient age 16)	"But in general, the children find out. And I think it is better that they find out from the healthcare workers, best suited for this is the treating physician, so better from him than from (reading it up on) the Internet or from other children."
Desiree's mother (patient age 11)	"She also sees, Desiree, that around her children go disappearing (dying)."
Cody Ray's physician (patient age 10)	"[…] after two-three rounds when obviously they found out about it. They know because they come for the treatment, their hair falls out. […] [To do it like this] For them to find out on their own and afterwards for them to learn more from me. For me to see how much they want to know from me."
Names of participants have been changed to protect the identity of responders.	

Widening involvement As parents and patients became more acculturated with hospitalisations and had the time to come to terms with their new situation, many parents slowly abandoned efforts to shield children. While some parents involved their children concurrently or immediately after they were told the diagnosis, for most parents the gradual awareness that involvement was somehow inevitable made them change their attitude and support widening child participation. Over time they provided more information in steps. Parental avoidance of full diagnosis disclosure persisted only for few patients after treatment started.

For many patients, widening involvement mainly consisted of information on treatment and possible side effects. Parents and physicians considered these discussions necessary to prepare children and ascertain their collaboration for painful procedures. Physicians often reported that they could not force children to undergo treatment and some parents acknowledged child benefits from participation. Typical of this 'practical' involvement is that when parents resisted open communication about the diagnosis it did not always lead to full disclosure. This resulted in physicians and parents walking a fine line between providing and withholding information.

Enhancing involvement Physicians described children's involvement in care in relation to the information they provided and to actions taken to elicit preferences and give children a voice. Enhancing involvement was grounded in assessing patient perceptions and tailoring information to expressed needs. According to physicians, some children limited themselves to asking more daily-life-affecting questions. Other patients were curious and took on a more active role regarding the course of the illness, recommendations for treatment and dietary regimens (Table 4.3.).

Table 4.3. Enhancing child and adolescent involvement

Participant	Quote
18 Harry's physician (patient age 11)	"I encourage them and try to, so to say 'make them', I insist that they ask me absolutely everything that they want to."
19 Lisa's physician (patient age 17)	"Basically I talk with the patient every day. And when we talk every day, some questions that might interest them about various things, like the evolution and so forth, come up."
20 Bruce's physician (patient age 17)	"He is not a very, very curious adolescent. […] In general he wants to know how many days is the treatment session, if it is with methotrexate or without, because he knows he will get mouth sores."
21 Lisa's physician (patient age 17)	"The girl is also present for the discussions because she is older, she is open, she is smart. She knows what she has. And she also has a lot of questions to ask. So she also wants to get explanations."
22 Tyler's mother (patient age 17)	"When we got discharged […] The physician asked us to give him an email address. And then, I sent Tyler (to talk to the physician and give the e-mail address). […] I said to him: 'The physician said that you should go to him to give him your email address so that he can communicate with you directly.'"
23 Desiree's mother (patient age 11)	"But she even goes and asks about the test results. She learned which are the white cells, what do they mean, the neutrophils, she gets involved and she is calm if she knows. […] She already knows …. I have low neutrophiles I am not allowed outside, I have no immunity."

Parents also mentioned discussing with physicians test results and treatment uncertainties in children's presence. This tactic allowed patients to participate in the sharing of information and created opportunities for them to react. Proactive parental approaches helped support and encourage some children to ask questions and engage with them regarding what patients learned from observation or other sources of information (Table 4.3.).

4.2.5. Discussion

- ***Ethical Considerations in Pediatric Oncology – From Informed Consent Particularities to End of Life Decisions***

All three reviwed articles, "***Ethical particularities and dilemmas of informed consent in pediatric oncology",*** "***End of life in children non medical decisions in a medical case" "*** and ***"Implications Of The Lack Of Romanian Legal Framework On The Medical Attitude Toward Terminal Paediatric Patients-Case Study"*** discuss the ethical dilemma concept as a frequent issue within the practice of physicians dealing in pediatric oncology. Few medical specialties encounter so many ethical challenges as pediatric oncology does (Mitchell, 1990). Most of oncologists share a personal experience in communicating with patients suffering from cancer. At the same time patients claim more and more their rights to be actively involved in the process of therapeutical decision making (Nelson, 2007).

The informed consent concept becomes one of the main pillars of the relationship between the patient and the doctor and it represents the clearest expression of the respect for the individual autonomy that is the individual's right of free choice, of living according to one's own principles and values. The doctrine of the informed consent apply particularly in case of children and teenagers, who lack the decisional capacity or the legal empowerment to express their consent (Astarastoae et al., 1998). From the medical point of view, the competence and the mental capacity bear different meanings, although they are perceived as synonyms.

Competence is the legal term which designates the legal authority wihin the management of peculiar situations, in a particular field of life.

On the other side, *the mental capacity* designates the cognitive, perceptive and communicative capacity of fulfilling a particular task. The latter reflects a clinical rather than a legal determination, and this is why it implies a field of clinical experience. For example, a 17-year old teenager may have the capacity of understanding medical decisions, but is considered to be legally incompetent in most of jurisdictions (Drane, 1985).

Obtaining the informed consent involves integrating the understanding and the communicative capacities, reasoning and deliberation in analyzing possibly conflictual choices by using a set of personal values.

The parental right to decide on the child's behalf relies on several arguments: parents are strongly motivated in taking decisions in the best interest of their child; it is supposed that, when growing up, children will borrow and express the same values as their parents, so that parental decision has good chances to be similar to theirs, when these ones will become legally entitled; parents are the ones that will have to live with the consequences of the decisions made on the children's behalf (including emotional and financial consequences); parents usually make most of non-medical decisions (i.e. school), so they should also be responsible for medical decisions (Emanuel, 2008; Joffe et al., 2006).

The concept of parental agreement (more appropriate than consent), represents that kind of reassigned decision which particularize the ethical issues in pediatrics. The respect for the child's

interest supposes sometimes ignoring the child's disagreement when for example, a surgical act is essential for saving his life (Thomson, 2001).

Children of older age and teenagers have the emotional and cognitive capacity of fully participating in decisions regarding their situation, especially when speaking of a chronical evolution of a cancer. For this reason, the teenager will cautionally receive the same information as adults do. Parents' role is limited to one of guidance, counseling and protection. In cases supposing high risks, the best interest of the child prevails, and not the parent's right to decide (Committee on Bioethics, 1995). Whenever a child or a teenager refuses the treatment, this refusal must be analyzed and not absolutized. A legal dispute may occur in these situation, when the under-aged person (or teenager) refuses treatment and this action is not in its best interest, as pediatric cancers have a better prognosis than adults'. In such a situation disregarding the child's decision is considered to be justified. Efforts are to be made in order to understand the causes of the refusal.

Consent process can be corrupted if parents limit the information provided to the children (Joffe et al., 2006). That is why counseling the parents or the tutors regarding the child's disease in a sensitive and individualized manner seems to be reasonable. It is also useful to ask parents to sincerely answer to the children's questions, in order for the therapeut not to be forced to deliberately distort truth in front of them (Nelson, 2007). Also, it is worth considering thatnot all children want to take part in that kind of decisions regarding their treatment (Bourne, 1995).

There is an important difference between the informed consent for purposes of general, „standard" treatment, and that for clinical study participation (experimental drugs). Informed consent for treatment develops as a process, that the patients must follow both for acknowledging the potential risks and benefits implied by the active oncological treatment and for protection agains malpraxis. Unlike the clinical situations, the informed consent for research clinical trial participation is rather seen as a crucial element of the deontology of clinical research; it is the contract by which patient authorizes participation in the research on the basis of information provided by the physician or by the researcher. Proper information and obtaining the informed consent of potential subjects for clinical trials represent an ethical guarantee in the frame of the research process concerning cancer therapeutical improvements (Sussman et al., 1992).

At least two factors augment the complexity of the achievement of the informed consent in the pediatric oncology. First, most discussions take place between parents and professionals or other decision factors, being directly influenced by the problem of autonomy. The second aspect is that an increasing number of patients included in clinical studies require information on both details of protocols and the results of the research (Sussman et al., 1992).

If the aims of informed consent are similar in pediatric and adult oncology, in clinical studies some additional deontological constraints are added. In both cases, child and adult informed consent will include information on: aims of treatment, randomisation procedures, anticipated risks and benefits, other anticipated procedures, therapies as well as their facultative character (Sussman et al., 1992).

A randomised study analyzed the problem of informed consent in children's clinical trials, as compared to adults in terms of information. The goal was to identify significant differences

between the two situations, with the purpose of optimizing the information process in randomized clinical studies (Simon et al., 2004). Study data identified significant differences between informed consent in adults and children, recognizing that, on average, adults have been better informed and were more actively involved by the healthcare provider. On the other side, pediatric oncologists provided however more information on the protocol types, map roads, survival, randomization, facultative feature. Difficulties of understanding were more frequent amongst decision factors in pediatrics.

The path for obtaining informed consent in adults is less influenced by lack of autonomy, by cohercision of the family or by other factors linked to ethics of decision. Adult medical oncology has a more immediate and less ambiguous link between patient and doctor, and probably this is one of the reasons that makes decision to enroll in clinical studies much easier than in pediatrics. In general, adults develop more rapidly a relation of trust with their attending physician, and less frequently regret participation in clinical studies. When it comes to decision-makers, children and adolescents need the most often support in understanding the treatment within the clinical trial and the possible benefits of study treatment, as compared to standard treatment. The elements of informed consent are usually less well understood by pediatrical decision-makers, if no detailed explanations of the differences between therapy options were previously offered. Pediatrics staff has often to deal with a straight discussion on therapy options on standard classic procedures and on specific trials procedures. Sentences like „this combination of drugs is not applicable in conventional standard treatment" or „this new treatment will be done only if you decide to participate in the study, otherwise we will respect your choice" are very important, and must be repeated all along the process of obtaining informed consent (Sussman et al., 1992).

On the other side, in situations like disease relapse or unfavorable course of disease, or treatment with a less clear risk/benefit ratio, the ethical part is very important (Committee on Bioethics, 1995). In the **"*End of life in children non medical decisions in a medical case "*** and ***in "Implications Of The Lack Of Romanian Legal Framework On The Medical Attitude Toward Terminal Paediatric Patients-Case Study"*** we refer to the end of life that comes to the beginning of life, trying to analyze some of the major moral issues based on suggestive cases. Roger Bone affirmed "Dying can be a peaceful event or a great agony when it is inaapropriately sustained by life suport" (Bone, 1997). Decisions such as the avoidance of the initiation or discontinuation of supportive treatment in terminal patients does not means their abandonment, but initiation of palliative care for the purpose of alleviating pain and suffering and respect patient dignity.

In **"*End of life in children non medical decisions in a medical case"*** we tried to avoid the ethical deconstruction using the classical ethical theories and principles and to make an appeal to four arguments: quality of life, life expectancy, (f)utility, the sanctity of life. In the presented oncological case, if any of the physicians would have requested an ethical opinion to clarify their own moral decision, there would have been two alternatives:

1. The child should have been intubated before the arrival of her mother, given the terrible suffering of a mother with a dying child, and her wish to be beside her daughter in the last moments of life. The contact with her child before death, a final farewell and forgiveness would

ease the suffering of separation. Having no chances to survive, the prolongation of her suffering and that of her family did not justify the use of any extraordinay means for further maintenance of the vital support (i.e. after her mother arrival). The extraordinary procedures of life maintenance must be a priority for those with high chances of survival, aiming at the improvement of their health condition and not to postponing death

2. The child should not have been intubated and then extubated on request, even if the disease is in terminal stage, with lethal prognostic and an irreversible progress towards death. It is against the natural laws, the sanctity of life and all medical codes of ethics.

Quality of life. The child presented cannot take her own life and can not express any wish at the end of life, even if she would have had the legal right to do so. We do not say this to indirectly emphasize the potential benefit of adults' autonomy and their greater capacity to reason but to highlight a similar argument for both adults and children, used in life end decisions: the quality of life. This is a new attribute raised by modern medicine (Carr and Higginson, 2001).

There is a tendency for physicians to define this argument in relation with clinical and laboratory data, which are actually the result of physical functions during normal daily activities. However, the physician tends to ignore the personal and social functions, the values attached to patients' quality of life. Thus, any discussion on quality of life, involves not only the biological body functions but also the patient's or the family's preferences. If we agree with that last statement, our case is edifying for the fact that this argument cannot be taken into consideration: the child is cared for by her aunt and not by her parents, but her aunt cannot and does not want to take a long term decision, but only a temporary one, until the girl's mother comes back and takes full responsibility (Muirhead, 2004).

Life expectancy is not a concept of moral nature, but its significance becomes ethical, if differences in life expectancy between individuals or groups of individuals constitute a determinant factor between choices that affect life in many ways (Small R., 2002). We have not considered this criterion when presenting this case because life expectancy of a young child actually means the entire life. Life expectancy, in medical terms, is defined as an accumulation of information to be processed and properly understood. Thus, life expectancy differs depending not only on the disease per se, but also on sex, ethnicity, etc (Kodish et al., 1998).

Naturally, the death is considered a tragedy and in addition, the death of a young person generates greater suffering than one of an adult or elderly person. However, a premature death involves a greater, which is translated by the fact that a lower life expectancy is associated with a disaster. On the other hand, everyone agrees that the life of a person is as valuable as it is the life of any other person, and this suggests that the misfortune of a death could have the same implications for everyone.

We can define *(f)utility* as a probabilistic concept, being difficult enough to assess it accurately. It still remains in question the *(f)utility* argument for the use of extraordinary rescue means for treatment or life prolongation (when it seems that life is at its end) (Burns and Truog, 2007).

The sanctity of life in general, and religious beliefs in particular may take advantage over any medical proofs that might clearly demonstrate the futility of a medical treatment. If in adult patients with end-stage incurable disease, the decision of withholding or withdrawing treatment is accepted both morally and legally, in children at the end of their life, the moral aspect weighs more, its sanctity shadowing any notion of futility of medical treatment.

It is noticed that the Romanian legislation includes mainly regulations of principles. In the approach of the development of a legislation regarding the decision at the end of life is, however, required the articulation of the legal normative aspects or of procedure with cultural and religious peculiarities, as well as with the social reality from our country (Petris et al., 2011; Toader and Toader, 2012).

- ***Cancer Care in Romania***

Communication about diagnosis and medical treatment for children suffering from life-threatening illnesses is complex. It is is shaped by societal views of illness and breaking bad news practices (Surbone et al., 2004; Young et al., 2002). Through this study, ***"Cancer care in Romania: challenges and pitfalls of children's and adolescents' multifaceted involvement"*** we were trying to add knowledge on the challenges and risks of involving children in care when they are not immediately or fully informed about their cancer diagnosis.

Patient participation was described as a step-by-step, intermittent process resulting in various types of involvement. Provision of information and explanations regarding care are the main tools to foster participation in paediatric oncology (Clemente, 2007; Steele et al., 2014). In our study, participation was dominated by parental and physician uncertainties regarding how and to what extent they can and should involve their children (Young et al., 2003; Barthoklson et al., 2015). Uncertainty was linked to fears of children's emotional reaction to the cancer diagnosis. For parents themselves, the diagnosis evoked a powerful threat, of dying, which accounts for the withholding techniques (Semple and McCance, 2010).

Physicians often complied with parents' requests to withhold the exact diagnosis in view of legal parental rights, but most saw it merely as delaying patients from finding out on their own (Young et al., 2003). This results in an ethical conundrum: limiting truth telling poses moral internal dilemmas for physicians, leading to conflict with some parents (Bartholdson et al., 2015). Despite uneasiness, physicians in this study complied with parental wishes. This does not reflect a denial of patients' right to information, as patients were gradually involved in care, even in the absence of full disclosure for some. This finding highlights the grey zone of how to professionally and empathically disclose diagnosis to families and children (Young et al., 2013). The degree of involvement desired by patients is difficult to assess especially when parents and oncologists take an adultcentric view and exclude child participation from what they consider difficult situations (Coyne et al., 2014; Kars et al., 2015).

Participation and information sharing related to treatment occurred even as physicians accepted parental restrictions. Physicians considered discussing likely side effects and explaining treatment procedures an imperative as these often result in pain and considerable physical changes. This communication was a way of soliciting and obtaining assent. As such, children's involvement

was seen as valuable in and of itself, rather than just a foreseeable manoeuvre to achieve patient compliance and collaboration. In other studies, clinicians held the same view that causing harm to children is ethically charged (Bartholdson et al., 2015; Solomon et al., 2005).

For some patients, communication became more open between parties and information flow was less and less inhibited by parental or physicians' attitudes. Oncologists allocated time for children to ask questions and reassured them with each medical visit that they could voice concerns. These actions aimed to build patient confidence to engage in care and facilitate a two-way communication. Similarly, parents supported children by including them in discussions with physicians and in some cases encourage them to have direct contact with clinicians. These attitudes may be highly supportive of adolescents' needs during cancer care (Young et al., 2003; Olsson et al., 2015). In early diagnosis stages, children may prefer parents to act as buffers and messengers of medical communication (Young et al., 2003) but they also need to have access to physician time without parental involvement (Olsson et al., 2015). Physicians' and parents' accounts in our study are aligned with such adolescent wishes as participants were aware of differences in patient preferences. When parents agreed to open communication, they were guided in their supportive actions by teenagers' own behaviour and wishes.

Besides parental and physician sources of information, children were reported to acculturate to the hospital setting and therefore knowing more about diagnosis, treatments and illness consequences. Participants mentioned that some children manifested a wish to know more detailed information about their illness and likely outcomes. These patients also went searching online (Stinson et al., 2011; Zebrack et al., 2013) or when parents opposed absolute disclosure some teenagers researched their symptoms on the web to arrive at a concrete diagnosis on their own. This scenario exposed children to abundant information (poor prognosis, end-of-life issues and long-term effects) that they may not be able to filter or structure in the same way as when informed by clinicians. Patients may think their situation is direr than it is in reality (Bluebond-Langner et al., 2010). This shows how ineffective restricting child involvement for protection can be: it can trigger patient curiosity and exacerbate fears.

We relied on treating physicians' judgement in approaching parents for interviewing. This may have led to selecting less difficult patient cases or families that physicians had a good relationship with. The study has the strength of capturing both parental and physician perspectives on child involvement and juxtaposing their views to identify challenges to patient participation and risks when restricting involvement. By having the double perspective parent oncologists and in light of reports of limiting involvement, we believe that social desirability tendencies were minimised. Recall bias may have also played a role in interviewees' accounts. Interviewing allowed in depth probing of parental and physician actions and attitudes towards child involvement. This qualitative method may limit to some extent the results' generalisability to other paediatric oncology contexts. However, the issues identified may be relevant for similar crowded oncology treating centres with limited psychosocial services.

4.2.6. Conclusions

- ***Ethical Considerations in Pediatric Oncology – From Informed Consent Particularities to End of Life Decisions***

Ethical dilemmas are frequent in pediatric oncology from every stage of obtaining the informed consent to futher decisions. The competence and mental capacity issues are less made clear and they often represent dilemmas.

In pediatric oncology in particular, where patients and families have to deal with several decisions all along the course of the illness the importance of the informed consent is being recognized as a continuous process. Two factors at least particularize the informed consent in pediatrics. First, many conversations and decisions take place primarily between clinicians and parents, with an increasing involvement of children according to their development capabilities. Secondly, patients cared for within the context of clinical trials is increasing. The differences between the informed consent in adult and pediatric oncology reffer to the level of information, the active involvement, the awareness on major risks and of randomization are concerned. The understanding and emotional difficulties are more frequent in pediatric oncology.

On the other hand, we consider that in situations as complex as the ones presented in this article, namely children who reaches the end of life, where the question is whether to apply or not extraordinary resuscitation measures to prolong the life, the physician will make appeal to his right to consciousness. The parents' autonomy regarding the withdrawal of the medical treatment, as well as the responsibility of the medical teams in situations where non-treatment represents the only alternative in the best interest of the patient are aspects that require legal analysis and regulations. In the absence of a legal framework and clear protocols in paediatrics, the entire responsibility for the medical decisions concerning the end of life for terminal paediatric patients is transferred to parents and the medical team.

- ***Cancer care in Romania***

Parents’ and physicians’ accounts paint an image of children’s involvement in cancer care that has different facets. Some parents who adopted restricting techniques, particularly concerning diagnosis disclosure, viewed involvement rather as a practical step or as being unavoidable given the long treatment and different sources of information to which patients were exposed to. Parental explenations further emphasise that child involvement in care is indisputable, despite the many grey areas in allowing or ensuring patient participation in care. High parental uncertainty and fears related to cancer diagnosis suggest the need for research on how clinicians can support parental communication with patients. Physicians should aid patients in their involvement, separate from actions to soften parents’ boundaries. Oncologists and parents should be aware of the hazards of leaving patients with unanswered questions about diagnosis for a long time. Patients may resort to external sources of knowledge and face information overload, no longer being able to pace its rhythm. Professional support is essential in untangling information relevant to patients’ situation from worst case scenarios.

Section B. Further Academic, Professional and Scientific Development

Over the course of three decades, my defining goals for my personal and professional growth have been clinical practice and continuous experiential learning of pediatrics. the particularly challenging specialization of pediatric hematology andoncology has demanded great commitment to clinical precision, scientific research, and didactic mentorship. Preserving the children, teaching the students, training the residents, continous research work in certain areas and implementing the innovations have kept me busy and focused on my mission despite the inherent difficulties of the journey.

Academic Perspectives

Knowledge in pediatric oncology and hematology field is advancing at a rapid pace and clinical applications for new scientific insights are being implemented with increased promptitude such as in the form of innovative therapies. Concurrently, fundamental research is inviting paradigmatic shifts in how we approach pediatric solid tumors, benign and malign hematology, which adds to the importance of continuously updating the professional competences of all medical staff, as well as the curriculum for medical students and residents. In pediatric hematology and oncology, for instance, some information about the mechanisms of cancer development is already obsolete. One of my academic goals is to stimulate interest in pediatric oncological diseases whose tragic natural course has been altered for the better by scientific research, e.g. leukemia (overall survival rate of 80% in children) and Hodgkin's lymphomas (over 90% survival). Knowledge and training are key factors in order to achieve the best survival rates possible, and both can now be made more widely and readily available via digital pedagogies and technologies.

The higher education curriculum is not particularly generous with compulsory contents and classes focused on adult and pediatric hematology and oncology, so my intention is to provide additional, elective courses on hematological problems in pediatrics. Such learning opportunities are already available for the 5th year students from the French study program, and similar courses would be necessary for their colleagues studying medicine in Romanian and in English. The final examination may consist of a combination of multiple choice questions and practical assessment by the patient's bedside.

Pediatric Hematology and Oncology is a newly established specialty. It was certified by the Ministry of Health less than 5years ago and, since then, more and more graduates have chosen to work as residents in the field. At the same time, residents in General Pediatrics are spending several months in these specialized hospital units and wards.

As didactic coordinator of the Pediatrics discipline, also responsible for our residents in Pediatrics and Pediatric Hematology and Oncology, I plan to use all the means available to cultivate their professional interest and enthusiasm. Young doctors may be further encouraged to appreciate the importance of working in this field and to get involved by attending conferences,

congresses, and the meetings of the Romanian Society of Pediatric Hematology and Oncology, of which I am a founding member and an executive committee member.

I am the coordinator of the pediatric branch of the Iasi Society of Doctors and Naturalists, organizing numerous professional meetings over the years. The inclusion and enrolment of residents in our branch and Society is an opportunity for professional development for them occasioning their participation in meetings, conferences, case presentations etc. Residents and young doctors will be more directly involved in brainstorming by multidisciplinary assemblies and encouraged to present case reports, even study cohorts or organize debates themselves. With the support of the University managerial team and International Relations Department, I plan to identify national/international exchange opportunities and organize exchanges between young professionals with similar interests.

Also, a very effective way of engaging residents and young doctors is to invite them to collaborate with senior specialists in writing medical papers. The young doctors most substantially involved will be encouraged to pursue master's and doctoral studies. Promising young holders of PhD degrees can be invited to consider post-doctoral research topics unpacking the broader "Diagnosis and Treatment of Chronic Neutropenias", which is related to one of our ongoing projects. Other topics of great interest are "Molecular-based therapy for the subsets of acute lymphoblastic leukemia resistant to current therapy" and "Characteristics of Viral Infections in Immunocompromised Pediatric Patients with Malignancies". Our Pediatrics handbook was published 5 years ago and I will once again coordinate the team of pediatricians in order to update and adapt the handbook for students and residents in accordance to the latest scientific achievements and requirements.

Professional Perspectives

Improving the severe or life-threatening condition of a child suffering from illness requires commitment to an advanced level of knowledge and competence. In pediatric hematology and oncology, this includes acquiring theoretical notions and clinical practices produced by fundamental research and practical experience nationally as well as internationally. Also, the complexity of the pathology and of the medical care requires close multidisciplinary teamwork manifested as daily meetings in which cases are discussed with pediatric surgeons, anesthetists, pathologists, pediatric nephrologists, pediatric cardiologists, epidemiologists, laboratory doctors.

As Head of the Oncology and Hematology Pediatric Unit, I will further expand the collaboration with genetic clinical researchers, neurosurgeons with expertise in pediatrics, specialists in nuclear medicine, radiotherapists etc. in order to raise the standard of diagnosis and treatment. For example, our current work with a laboratory unit of medicine and molecular genetics from the Regional Institute of Oncology could be developed to include the genetic profiling of tumors from the Ewing Family. This would be of great help, because Ewing sarcoma is a highly malignant bone / soft tissue pediatric solid tumor with early metastases and poor outcome. The identification of alternative gene fusions involving EWSR1 (fusion product FLI1-EWSR1) will

provide valuable information in order to introduce a targeted, adapted therapy. Also, establishing the presence of the N-myc proto-oncogene in neuroblastoma can justify a certain therapy from the beginning and bring immediate benefits to the patient.

Scientific Perspectives

Cancer is the second leading cause of death in children between the ages of 0 and 14, after trauma (Siegel et al., 2019). Compared to adult tumors, in whose genesis environmental factors such as smoking or alcohol consumption are known contributors, external factors are less significantly involved in pediatric cancers.

An important direction for research moving forward will be to participate in a nation-wide epidemiological study on child's cancer in Romania. The 5 centers in the country which provide specialized care for neoplastic children have long operated without a national registry of cases. The National Cancer Registry for Children was established relatively recently with the help of a Non-Governmental Organization, awarded with the European Citizen's Award in 2020. The registry is an essential tool and source of information in order to plan and optimize healthcare services for patients suffering from these diseases, to correctly analyze the epidemiology of various cancers by regions of Romania, and to assess our situation compared to elsewhere in Eastern or Western Europe. In this general context, my future research projects and aims are summarized below.

- ***Pediatric cancer prone syndromes: focus on Li-Fraumeni Syndrome***

The only known relevant cause of childhood cancer is a hereditary cancer predisposition (HCP). Childhood cancers mostly arise from mutations in some major driver genes such as CDKN2A, KRAS, NOTCH1, NRAS or TP53, and pathways disrupted by driver alterations (Ma et al., 2018). Regarding HCP, genome-wide studies identified pathogenic germline variants in 8–10% of the affected children and adolescents, which had previously been underestimated (Grobner et al., 2018; Scotting et al., 2005). At present, over 100 cancer predisposition genes have been described (Parsons et al., 2016). In childhood cancer, some genetic conditions may have an active role in tumorigenesis: neurofibromatosis type 1, tuberous sclerosis, Beckwith-Wiedemann syndrome, Proteus syndromes or adult cancer syndromes with an increased risk of cancer at pediatric age, such as Li-Fraumeni syndrome (LFS) (Teplick et al., 2013).

Li-Fraumeni syndrome (LFS; OMIM# 151623) is an autosomal dominant hereditary cancer predisposition associated with a high risk of a broad spectrum of tumors such as soft tissue and bone sarcomas, breast cancer, brain tumors, pancreatic cancer, adrenocortical carcinoma, germ cell tumors, melanoma, acute leukemia. Also, an increased risk of developing second and third malignancy or multiple synchronous primary tumors was noticed in patients diagnosed with Li-Fraumeni syndrome. TP53 is the only gene that has been associated with LFS and is caused by germline mutations of the TP53 protein. TP53 is a transcript factor which functions as a tumor suppressor regulating cell-cycle arrest, cellular apoptosis, and DNA-repair (Nelson et al., 1990).

A reduced transcriptional activity decreases cellular growth, resulting in a high cancer risk (Malkin, 2011).

Several childhood cancers are strongly associated with LFS. Osteosarcoma and rhabdomyosarcoma of the diffuse anaplasia subtype are the most common tumor types diagnosed in chidren affected by LFS, followed by adrenocortical carcinoma (ACC), medulloblastoma, soft tissue sarcoma, acute lymphoblastic leukemia (Lalloo et al., 2003; Holmfeldt et al., 2013).

According to our understanding, a significant number of patients affected by LFS are not diagnosed during childhood or adolescence because the testing for TP53 mutations is not routinely performed.

The main purpose of my future research project will be to assess the TP53 germline mutation in pediatric patients that fulfill at least one of the Chompret updated criteria (Bougeard et al., 2015):

1. Familial presentation: proband with a LFS spectrum tumor (e.g. premenopausal breast cancer, soft tissue sarcoma, brain tumor, ACC) prior to age 46 AND at least one first- or second-degree relative with a LFS tumor (except breast cancer, if the proband has breast cancer) before the age of 56, or with multiple tumors.

2. Multiple tumors: proband with multiple malignancies (except recurring breast cancer), of which at least two belong to the LFS spectrum before the age of 46.

3. Rare tumors: patients with ACC, choroid plexus carcinoma, or embryonal anaplastic subtype rhabdomyosarcoma independent of family history.

4. Early onset of breast cancer (before the age of 31).

Study groups will be represented by pediatric patients with a highly suspected Li-Fraumeni syndrome according to the Chompret criteria and their family histories. Beside the identification of TP53 germline mutation, the research also aims to develop surveillance strategies or adaptative treatment approaches (e.g. avoiding radiotherapy when possible).

- ***Molecular studies of prognostic factors in childhood acute lymphoblastic leukemia – IKZF1 deletions***

Acute lymphoblastic leukemia is the most common cancer in childhood. It is caused by the somatic acquisition of genetic abnormalities and malignant transformation of immature lymphocytes in the bone marrow, most commonly of B-cell lineage (Pui et al., 2017). Nowadays, more than 80% of pediatric patients diagnosed with acute lymphoblastic leukemia are cured with modern treatment protocols. Although the treatment success rate is high, in order to avoid relapses and treatment-related toxicities, we need to identify and to understand the genetic aberrations that are implied in leukemic disease.

IKZF1 has been in the spotlight of leukemia-related research since 2008 (Mullinghan et al., 2009). The *IKZF1* gene is located on chromosome band 7p12.2 and codes for the transcription factor IKAROS with key regulatory functions in lymphopoiesis. The heterozygous deletion of *IKZF1* has been associated with high risk of disease and poor treatment outcomes in BCP ALL (Mullinghan and Willman, 2011).

Deletions of *IKZF1* are observed in 10–15% of pediatric ALLs (Mullinghan, 2013; Dorge et al., 2013; van der Veer et al., 2014). Churchman et al., characterized *IKZF1* as a leukemia predisposition gene by reporting mostly adverse germline *IKZF1* variation in familial pediatric ALL (Churchman et al., 2018)

Several studies demonstrated that *IKZF1* has a direct link to glucocorticoid response in BCP ALL (Dorge et al., 2013; van der Veer et al., 2014). This is an important step towards the development of new specific therapeutic strategies targeting *IKZF1*-altered signaling networks in BCP ALL. ALL-BFM protocols add IKZFI status as a prognostic factor for the intermediate risk group and highlight the importance of vincristine-dexamethasone pulses in conventional maintenance for this group (Dorge et al., 2013; Marke et al., 2017; Chan et al., 2017).

The aim of my research in this field is to establish the frequency and prognostic impact associated with the *IKZF1* in childhood B-cell precursor ALL in Romanian children using multiplex ligation probe-dependent amplification (MLPA) analysis. As such, the goal is to improve diagnosis and risk-stratification in the context of existing treatment protocols, while occasioning new directions of therapy for *IKZF1*-positive pediatric ALL patients according to the latest international protocols.

The field of Pediatric Hematology and Oncology also covers a wide spectrum of benign blood disorders, among which bone marrow failure disorders (e.g. aplastic anemia), red cell, white cell, and platelets disorders, as well as coagulation and thrombotic disorders. My scientific interests in this field are mainly oriented towards identifying ways to improve the standards of care for the patients suffering from these disorders.

- ***Fc-gamma receptors gene alterations in pediatric immune thrombocytopenia***

Immune thrombocytopenia (ITP) is an acquired hematological disorder with autoimmune underpinnings and which may cause thrombocytopenia-related bleeding. The disease is characterized by a low platelet count in the peripheral blood (less than 100 × 109/L) manifested in the absence of other causative systemic disorders (Zafar H et al, 2018, Nomura S, 2016). ITP can be classified clinically as newly diagnosed/acute (under 3 months), persistent (3 to 12 months), and chronic (lasting for more than 12 months) (Rodeghiero F et al, 2008, Schoettler ML, 2018). Despite the progress made in the diagnosis of ITP, it is still an exclusion diagnosis (Pell J et al, 2018).

Different mechanisms are involved in the immune dysregulation causing ITP: the formation of antiplatelet antibodies of the immunoglobulin G (IgG) type against several platelet surface antigens, complement fixation, and the dysfunction of T lymphocytes (Grodzielski M, 2018). However, the pathophysiology of ITP is incompletely understood: initially, it was thought to be related to the reduced lifespan of platelets as the result of thrombocytes binding pathogenic autoantibodies against platelet-specific antigens such as glycoprotein Ib/IX and IIb/IIIa (Zafar H et al, 2018).

The majority of current protocols use corticotherapy as the first line of treatment. Importantly, apart from the fact that this approach has certain known side effects, some patients are non-responders or suffer frequent relapses especially in dosage tapering (Neunert CE, 2018).

The susceptibility to develop ITP, as well as the self-limited course and response to IV Immunoglobulin were associated with the genetic polymorphisms of the Fcγ receptor of phagocytes FCGR2C*ORF and FCGR2A*27W and the FCGR2B promoter variant 2B.4 (Schmidt DE et al., 2019). Receptors for the Fc portion of IgG play an essential role in the protection of the body by removing antigen-antibody complexes from the circulation. They are present on monocytes, macrophages, neutrophils, natural killer (NK) cells, and T and B lymphocytes. The receptors participate in a variety of functions, such as the phagocytosis of immune complexes and the modulation of antibody production by B cells. Genes for several low-affinity FcG receptors are clustered on chromosome 1q23-24 (Hargreaves CE, 2015). Fcγ receptors (FcγR) display polymorphisms that are often observed in ITP cohorts, or specifically during the chronic course of the disease (Audia et al, 2017). Chronic ITP was associated with a deletion of FCGR2C/FCGR3B. Taken together, susceptibility to transient and chronic ITP is distinctly affected by polymorphic variants of FCGR2/3 genes (Schmidt DE et al, 2019).

These polymorphisms were associated with the prognosis of the disease and several studies concluded that they may be useful as predictive factors for the outcome of ITP and response to different choices of treatment. However, the decision whether or not to treat is not taken considering gene polymorphisms (Audia S et al, 2017). Better understanding of the mechanisms of this bleeding disorder can lead to a more personalized approach of the patients suffering from the condition. As such, I find it of clinical and scientific value to identify patients positive for genetic polymorphisms of FcγR by detecting abnormal copy numbers of the FCGR2A, 2B, 2C, 3A and 3B genes in DNA samples via the MLPA technique. The study group for such a research endeavor includes pediatric patients diagnosed with ITP and with a platelet count <100x109/L.

Additionally, I am interested in investigating hypothesized correlations between the type of polymorphism, clinical course and response to treatment.

Section C – References

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