GRIGORE T. POPA UNIVERSITY OF MEDICINE AND PHARMACY IASI

PEDIATRIC NUTRITIONAL PATHOLOGY: FROM MALABSORPTION SYNDROME TO OBESITY

HABILITATION THESIS

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GRIGORE T. POPA UNIVERSITY OF
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Contents

LIST OF ABBREVIATIONS

AAP – American Academy of Pediatrics
ADIPOQ – Adiponectin
AEA – Anti-endomisium antibodies
AGA – Anti-gliadin antibodies
AHA – American Heart Association
Alg – Alginate
aP2 – Adipocyte protein 2
BCP-ALL – precursor B-cell acute lymphoblastic leukemia
BM – Bone marrow
BMI – Body mass index
BP – Blood pressure
CBL – Case-Based Learning
CD – Celiac disease
CF – Cystic fibrosis
CFLD – Cystic fibrosis related liver disease
Chol – Cholesterol
CNS – Central nervous system
COSI – Childhood Obesity Surveillance Initiative
CS – Chitosan
CVD – Cardiovascular disease
DC – Dimethylaminoethane-carbamoyl
DOPE – Dioleoyl-phosphati-dylethanolamine;
DOTAP – 1,2-dioleoyloxy-3-trimethylammoniumpropane
DODAG – N′, N′, -dioctadecyl-N-4,8-diaza-10-aminodecanoylglycine amide
DMPA – Depot-medroxyprogesterone acetate
DMPE – Dimyristoyl phosphatidyethanolamine.
DM – diabetes mellitus
DSPC – Disteraoyl phosphatidylcholine
DMPG – Dimyristoyl phosphatidylglycerol
DNA – Deoxyribonucleic acid
DPPC – Dipalmitoyl phosphatidylcholine
DSPE – 1,2-Distearoyl-sn-glycero-3-phosphorylethanolamine
ESPGHAN – European Society of Pediatric Gastroenterology, Hepatology and Nutrition
FAP-B – Fibronectin receptor
FDA – Food and Drug Administration
GDM – Gestational diabetes mellitus
HA – hyaluronic acid
HbA1c – hemoglobin A1c
HBV – hepatitis B virus
HCV – hepatitis C virus

HSPC – Hydrogenated soy phosphatidylcholine
ICAM – Intercellular Adhesion Molecules
IGF-2 – Insulin-like growth factor 2
IL6 – Interleukine 6
IPF – Idiopathic pulmonary fibrosis
IRS-1 – Insulin receptor substrate 1
IR – Insulin resistance
IMT – Intima media thickness
IUDs – Intrauterine devices
LC – Lung cancer
LEP – Leptin
LY86 – Lymphocyte antigen 86
MS – Metabolic syndrome
NCDs – Non-communicable diseases
NAFLD – Non-alcoholic fatty liver disease
NHANES – National Health and Nutrition Examination Survey
NRS – Numerical Rating Scale
NSAID – Nonsteroidal anti-inflammatory drugs
OC – Oral contraceptive
OGTT – Oral glucose tolerance test
POMC – Pro-opiomelanocortin
PGC1α – Peroxisome proliferator-activated receptor coactivator 1 α
POCs – Progestin-only contraceptives
Pref-1 – Pre-adipocyte factor-1
PC – Phosphatidylcholine
PS – Polystyrene
PEG – Polyethylene glycol
PSA – Poly (sebacic acid)
PLGA – Poly (lactic-co-glycolic acid)
PGR – Prednisone good response
PPR – Prednisone poor response
PVA – Poly (vinyl alcohol)
PEI – Polyethyleneimine
RNA – Ribonucleic acid
SCFAs – Short chain fatty acids
SD – Standard deviation
STIs – Sexually transmitted infections
T-ALL – T-cell acute lymphoblastic leukemia
VAS – Visual Analog Scale
VRS – Verbal Rating Scale
VPCs – Virtual patient cases
WBC – White blood cells
WC – Waist circumference
WHO – World Health Organization

REZUMATUL TEZEI

Profesia de cadru didactic presupune o achiziție continuă de cunoștințe, abilități și competențe specifice în trei domenii esențiale: educație, sănătate, cercetare. Astfel, chiar dacă satisfacțiile profesionale sunt numeroase, acestea necesită o formare continuă multidisciplinară pentru a putea îndeplini cerințele, exigențele sau concurența existente în fiecare dintre aceste domenii. Lucrarea de față reprezintă o descriere a activităților desfășurate, a competenţelor profesionale atinse în perioada post-doctorală (2005-2021) precum și direcțiile viitoare de cercetare.

Teza de abilitare este structurată în trei părți în conformitate cu criteriile recomandate și aprobate de către Consiliului Național de Atestare a Titlurilor, Diplomelor și Certificatelor Universitare (CNATDCU). Lucrarea reflectă preocupările mele în domeniul nutriției pediatrice, cu evidențierea rezultatelor în domeniul malnutriției datorate sindromului de malabsorbție din timpul efectuării doctoratului și completate cu rezultatele preocupărilor în domeniul extrem de actual al obezității infantile, ambele teme fiind cercetate în cadrul Disciplinei de Pediatrie a Universității de Medicină și Farmacie" Grigore T. Popa" Iași. Teza este structurată astfel:

Secțiunea A: cuprinde prezentarea realizărilor științifice, academice și profesionale din perioada postdoctorală;

Secțiunea B: prezintă direcțiile viitoare de cercetare;

Secțiunea C: este alcătuită de lista de referințe bibliografice.

Plecând de la preocupările mele de cercetare, prima secțiune este împărțită la rândul ei în două părți:

Pe de o parte cuprinde rezultatele cercetărilor din domeniul sindromului de malabsorbție care reprezintă o cauză importantă de deficite nutriționale severe în copilărie care duc la afectarea creșterii și greutății, dar și la afectarea dezvoltării neuro-cognitive atât pe termen scurt cât și pe termen lung. Tulburările frecvente care determină malabsorbția intestinală și maldigestia la copii sunt reprezentate de o multitudine de afecțiuni, dintre care fibroza chistică și boala celiacă, afecțiuni cronice care afectează atât calitatea vieții pacientului cât și a familiei. Activitatea științifică inclusă în această primă parte a tezei de abilitare se axează în primul rînd pe cercetările efectuate în domeniul malabsorbției intestinale cercetate în teza de doctorat intitulată „Considerații clinico-evolutive și terapeutice în sindromul de malabsorbție la copil", susținută în anul 2004. Astfel, prin colaborarea cu colegii mei din Clinica III Pediatrie a Spitalului Clinic de Urgență pentru Copii "Sfânta Maria" din Iași dar și prin colaborările multidisciplinare a fost posibilă elaborarea de lucrări științifice care au fost publicate în reviste de specialitate. În prima parte, care include realizările științifice din perioada doctorală și postdoctorală se evidențiază cele două direcții majore de cercetare din cadrul malabsorbției intestinale, respectiv fibroza chistică și boala celiacă la copil.

A doua direcție de cercetare este reprezentată de o altă față a malnutriției, respectiv obezitatea copilului și toate consecințele sale asupra stării de sănătate. În prezent asistăm și în România la o creștere continuă a prevalenței supraponderii și obezității la copil. Excesul de greutate și obezitatea sunt probleme complexe prin multiplele comorbidități pe care le generează. Excesul de greutate și obezitatea copilului cresc riscul de apariție a bolilor cronice netransmisibile, deces prematur și dizabilitățile la vârsta adultă. Preocuparea mea pentru

obezitatea copilului s-a finalizat prin crearea Centrului regional de Diagnostic, Consiliere și monitorizare a copiilor cu suprapondere si obezitate din regiunea Moldovei, în care printr-o echipă multidisciplinără a fost posibilă atât efectuarea unei activități de cercetare cât şi realizarea şedintelor de consiliere nutrițională și psihologică a copiilor cu exces ponderal. Capitolul privind obezitatea copilului începe cu un review referitor la etilogia și factorii predispozanți și continuă cu rezultatele studiilor privind abordarea practică a obezității copilului, multiplele comorbidități și, nu în ultimul rând, strategiile terapeutice necesare pentru combaterea obezității și patologiilor asociate.

Secțiunea B are ca obiectiv prezentarea viitoarelor direcții personale de cercetare. În această secțiune sunt incluse o serie de strategii specifice fiecăruia dintre cele trei domenii importante: activitatea didactică, activitatea de cercetare științifică și activitatea clinică. În subcapitolul dedicat activității didactice am prezentat direcțiile de evoluție privind parteneriatul cu studenții și medicii rezidenți. În cercetarea științifică mă voi concentra pe continuarea cercetărilor atât în domeniul obezității cât și a fibrozei chistice la copil. În ceea ce privește obezitatea copilului doresc să continui studiile în prevenția obezității, pe de o parte, dar și în ceea ce privește complicațiile și comorbiditățile sale, pe de altă parte. Fibroza chistică, boala pe care am început să o studiez în urmă cu douăzeci de ani în cadrul tezei de doctorat, va continua să reprezinte o prioritate în activitatea mea de cercetare. Astfel, voi cerceta o serie de biomarkeri precoce cu rol predictiv în evoluția bolii și răspunsul la tratament. De asemenea, un subiect extrem de actual pe care doresc să-l cercetez se referă la rolul microbiotei intestinale în patologia nutrițională pediatrică, plecând de la malabsorbția din fibroza chistică și ajungând la obezitate. În ceea ce privește activitatea clinică viitoare, acesta se va focusa pe acumularea de noi cunoștințe, tehnici și dezvoltarea de noi competențe.

Secțiunea C cuprinde bibliografia care însoțește teza de abilitare.

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SUMMARY OF THE THESIS

The teaching profession involves a continuous acquisition of specific knowledge and skills competencies in three essential areas: education, health, research. Thus, even if the professional satisfactions are plentiful, they require continuous multidisciplinary training in order to be able to meet the existing requirements and competition in each of these fields. This paper is a description of the activities I have carried out, the professional competencies I have achieved in the post-doctoral period (2005-2021) as well as the future research directions I intend to follow.

The habilitation thesis is structured in three parts that respect the criteria recommended and approved by the National Council for Attestation of University Degrees, Diplomas and Certificates (CNATDCU). The paper reflects upon my interests in the field of pediatric nutrition, while also highlighting the results in the field of malnutrition due to malabsorption syndrome which were obtained during my doctoral studies; these are supplemented with results in the area of childhood obesity. Both topics are being researched in the Department of Pediatrics which belongs to the University of Medicine and Pharmacy "Grigore T. Popa" Iasi. The thesis is structured as follows:

Section A: includes the presentation of scientific, academic, and professional achievements from the postdoctoral period;

Section B: presents future research directions;

Section C: comprises the list of bibliographical references.

Based on my research concerns, the first section is in turn divided into two parts:

On the one hand, it comprises the results of research in the field of malabsorption syndrome which is an important cause of severe nutritional deficiencies in childhood that lead to growth and weight impairment, but also to scanty neuro-cognitive development in both the short and long term. Frequent disorders that cause intestinal malabsorption and maldigestion in children are represented by a multitude of conditions, including cystic fibrosis and celiac disease, chronic conditions that affect the quality of life of both the patient and family. The scientific activity included in this first part of the habilitation thesis focuses primarily on the research carried out in the field of intestinal malabsorption. This was the topic of my doctoral thesis entitled Clinical-evolutionary and therapeutic considerations in child malabsorption syndrome, defended in 2004. Through collaboration with my colleagues from the IIIrd Pediatric Clinic of “Saint Mary” Emergency Clinical Hospital for Children from Iaşi but also through multidisciplinary ones, it was possible to develop scientific papers that were published in specialized journals. The first part, which includes the scientific achievements of the doctoral and postdoctoral period, emphasizes the two major directions of research in intestinal malabsorption, namely cystic fibrosis and celiac disease in children.

The second direction of research is represented by another aspect of malnutrition, namely childhood obesity and its impact on health. Currently, in Romania, we are witnessing continuous growth in the prevalence of overweight and childhood obesity. Overweight and obesity are complex problems due to the multiple comorbidities they generate. Overweight and childhood obesity increase the risk of developing chronic non-communicable diseases, premature death, and disability in adulthood. My concern for childhood obesity materialized

with the creation of the Regional Center for Diagnosis, Counseling and Monitoring of Overweight and Obese Children in the region of Moldova. Through a multidisciplinary team, it was possible to conduct research activity and simultaneously get involved in nutritional and psychological counseling of these overweight children. The chapter on childhood obesity begins with a review of ethylology and predisposing factors and continues with the results of studies on the practical approach to childhood obesity and its multiple comorbidities. Last but not least, it focuses on the therapeutic strategies needed to combat obesity and associated pathologies.

Section B aims to present future personal research directions. This section includes a series of strategies specific to each of the three important areas mentioned: teaching, scientific research and clinical work. In the subchapter dedicated to teaching, we presented the directions of evolution regarding the partnership with students and resident doctors. In scientific research I will focus on continuing research in both obesity and cystic fibrosis in children. Regarding the child's obesity, I want to continue my studies in the field of obesity prevention, on the one hand, but also I will research its complications and comorbidities, on the other hand. Cystic fibrosis, the disease I started studying twenty years ago in my doctoral thesis, will continue to be a priority in my research. Thus, I will research a series of early biomarkers with a predictive role in the disease evolution and in the treatment response, too. Also, an extremely current topic that I want to research refers to the role of the intestinal microbiota in pediatric nutritional pathology, starting from cystic fibrosis malabsorption and reaching to obesity.

Section C is set to include the bibliography that accompanies the habilitation thesis.

SECTION A. BACKGROUND OF PERSONAL, PROFESSIONAL, ACADEMIC AND SCIENTIFIC ACCOMPLISHMENTS

The implementation of professional standards in the career of a university teacher is essential for increasing the quality of the educational system. The teaching profession requires the continuous acquisition of specific knowledge, skills, and competencies in three essential areas: education, health, research. Thus, even if the professional satisfactions are numerous, constant multidisciplinary training is required in order to be able to meet the requirements or competition existing in each of these fields. The university environment offers both a multitude of opportunities, but also a multitude of duties. In recent years, I have had the responsability to pass over to my students, in addition to actual medical notions, passion, and respect for the profession of doctor, respect for the patients and their suffering, and, last but not least, interest in scientific research as a source of progress and innovation. Support provided by the university is fundamental for both the professional and personal development, as well as for achieving one's own and community objectives.

I look at my work over the last twenty years as a sum of pieces of knowledge gained in the medical, educational and research fields. It is natural to try to go back around and to recall the important stages that represented the basis of my professional training in all the fields in which I worked.

Teaching activity

The teacher, educator, trainer of the 21st century has expertise and skills in two major areas: on the one hand the technical area, which requires analysis, design, structuring, development of teaching materials and events (courses, seminars, laboratories and practical assignments, course materials, presentations, guides, books, etc.), in which the communication and teaching skills must be constantly improved and refined, and on the other hand a non-technical area, governed by leadership and emotional intelligence, the area where the teacher empathizes with students (individually or at group or series level) or the one in which the teacher can manifest creativity, can innovate.

I started my teaching career in 2005 as a university teaching assistant in Pediatrics under the guidance of Professor PhD Dan Moraru. Later I passed all the didactic qualifications (obtained through a competition): University Assistant, Senior Lecturer, and Associate Professor.

Throughout this period, one of my continuous concerns has been related to the accumulation of as much quality and cutting-edge information as possible in the field of pedagogy, communication, and the most efficient transmission of medical information. Thus, I participated and graduated: the psychopedagogy course for the training of the teaching staff from "Grigore T. Popa" University of Medicine and Pharmacy Iaşi and the life-long training course entitled *Narrative communication skills. Narrative medicine.* I put into practice the information I accumulated and I have permanently improved my teaching skills by constantly interacting with students in many contexts (apart from those imposed by the teaching standard of the university qualification held at a given time), such as: I guided 5th year students to

develop, communicate and publish scientific papers on paediatrics, I coordinated annually bachelor's degree papers of the graduates of the Faculty of Medicine and General Health Care "Grigore T. Popa" University of Medicine and Pharmacy Iasi, I organized together with 5th year students the Student Clinical Evening. As secretary of the Pediatrics department within the Society of Physicians and Naturalists Iasi I contributed to the organization of clinical case presentation sessions that took place in the Pediatrics amphitheatre of "Saint Mary" Emergency Clinical Hospital for Children Iasi. At the same time, as a tutor for 5th year students, I participated in meetings with students and I got involved in training and professional counselling issues. As a member of the Senate of the University of Medicine and Pharmacy, I am part of the Commission for student issues, which contributes to the improvement of the teaching act in accordance with the students' requests. Besides all these, I am currently the coordinator for the discipline of Pediatrics within the Simulation Centre – "Grigore T. Popa" University of Medicine and Pharmacy Iasi and I participated in the course *Instructor Course for Medical Simulation* - Human Simulation Centre, Munich since 2019. I also applied my teaching skills and in the field of postgraduate courses, where from the position of coordinator of my own courses or as a lecturer (in various areas of pathology) I interacted with dozens of resident doctors, specialists and pediatrician consultants, family doctors, nutritionists, physiotherapists.

In addition to the aspects related to the teaching act, the didactic activity requires also operational or organizational abilities. Throughout time, I was a member of different examination committees, for example, one for obtaining the title of specialist doctor and primary care physician and another for filling available positions in the health network of Moldova for pediatricians. In the past five years, I have participated, once again as a member, in committees for occupying positions of university assistants, foremen as well as committees assisting doctoral dissertations. I was part of the commission for defending the bachelor's degree papers in the discipline of Pediatrics, of the commission for drafting the grids for the bachelor's degree exam for the students of the Faculty of Medicine. Also, in the last two years I have been involved in the simulation of residency exam and in the residency contest.

Last but not least, the involvement in two interactive simulation projects in the medical field for the training of students and residents contributed to the improvement of my teaching and pedagogical activity: the internal grant conducted between 2013-2015 and entitled *Creating and implementing an IT platform for assisted learning and computerized assessment based on the concept of virtual patient and the Erasmus+ project entitled Case-based learning and virtual cases to foster critical thinking skills of students (CLEVER),* which is currently taking place in our university. By creating virtual pediatric patients, we participated in the development of a modern and effective method of computer-assisted learning. This software is increasingly used because it facilitates the training of students and resident physicians by increasing the variability of clinical cases to which students can have access in a controlled environment that does not endanger the life of the real patient.

My preoccupations for the continuous improvement of the didactic act within the university were acknowledged by means of the confidence my colleagues had in me when they voted me in the university Senate, which will enable a deeper involvement in the process of organizing the didactic activity.

Medical activity

My entire teaching activity is supported and always improved by the medical career, the reciprocal being also applicable. The significant stages from the point of view of the medical career were of trainee doctor, later resident in the pediatric specialty. In 2005 I took the exam for becoming a specialist and later I became a pediatrician consultant. Due to my passion for gastroenterology and initially by working in a pediatric clinic with a gastroenterology profile, I completed my second specialty in gastroenterology, becoming a resident of gastroenterology and starting with 2019, I am a senior pediatric gastroenterologist.

I have been, I am and will always be concerned with my professional development as a pediatrician and pediatric gastroenterologist and for this reason I have acquired many skills over time: general ultrasonography, pediatric endocrinology, phytotherapy and practical phytopharmacology. At the same time, I graduated the Master of Nutrition and Prophylactic and Curative Dietetics in 2017. I participated in numerous workshops, trainings, training courses, which allowed me to continuously update my knowledge in the field of interest and to constantly improve my high training level: CD Medics Road Course - show Training Seminar (2010), Summer School of Pediatric Nutrition of the European Society of Gastroenterology and Pediatric Nutrition (2013), Holistic Approach to the Obese Patient (2015), Workshop on Obesity Prevention in children organized by the WHO Office in Romania (2016), Post Graduate Program in Pediatric Nutrition from Boston University School of Medicine, EASO Summer School -Training the Trainers in the Prevention and Management of Obesity (2018).

My concerns were also aimed at the administrative aspects of the medical act by obtaining the certificate in Health Services Management awarded by the Romanian Ministry of Health in 2013.

I am also involved in other activities designed to increase my development in the medical and administrative medical field. Thus, since 2016 I have been part of the Jurisdiction Commission of the College of Physicians - Iasi, between 2017-2019 I was a member of the disciplinary committee of "Saint Mary" Emergency Clinical Hospital for Children in Iaşi, and manager of fixed assets and inventories of the 3rd Pediatric Clinics. I am coordinator within the hospital where I carry out the activity of the *National Programme for evaluating the status of vitamin D by determining the serum level of 25-OH of vitamin D in people in risk groups* from 2019.

Starting from the strategic principles of "Grigore. T. Popa" University of Medicine and Pharmacy from Iasi, which are the educational ones, those of advanced research and social mission, we set up the *Regional Center for Diagnosis, Counselling and Monitoring of Children with Excess Weight and Obesity*. Between 2016 and 2020 I was the coordinator of this center. Given that obesity is a chronic condition with multiple associated metabolic pathologies, I consider it extremely necessary to prevent it in the overweight stage or if it has set in, an early diagnosis, a treatment and multidisciplinary counselling ever since childhood in order to prevent long-term disabling complications specific to adulthood are required. Thus, within the centre I carried out several projects destined to identify overweight and obese children in the region of Moldova, but also for the prevention and treatment through specific nutritional counselling: *Healthy eating in childhood - a chance for a healthy future adult.*

I also organized a series of scientific events, round tables, workshops attended by registrars and residents, students, psychologists, teachers, parents and children. I developed a series of teaching and educational materials for the public and I published the book *Multidisciplinary aspects of childhood obesity* – "Gr. T. Popa" UMF Iasi publishing house in 2019. I was also a member of the AUF project team- ECO/2017 entitled *Création d'un réseau universitaire régional dans le domaine de la santé, la nutrition et la sécurité alimentaire* (conducted in 2017-2019, coordinator Agence Universitaire de la Francophonie in partnership with "Grigore T. Popa" University of Medicine and Pharmacy Iasi, project manager: Associate Professor PhD Mihai Bogdan Mircea. Other projects I was part of: COST Action CA15135 entitled Multi-target paradigm for innovative ligand identification in the drug discovery process (MuTaLig), (carried out between 2016-2020), management committee member: Prof. PhD Toma Vasilica and the private project funded by *Progress through Education Foundation entitled Development of an innovative online service aimed at streamlining the system of screening, medical counselling, registration, monitoring and research on overweight in children and adolescents* (carried out during 2016-2017), Project Manager: Assoc. Prof. PhD Moscalu Mihaela. As a partner of *Save the Children* association I contributed to the development of the project *Eat healthy* developed in kindergartens and schools in Iași County since 2012 on promoting a healthy lifestyle. These projects involved students, resident physicians and specialists in paediatrics, diabetes and nutrition, endocrinology, epidemiology, family medicine and school medicine.

I was also an expert in curriculum and development of training programmes within the project *HOSPITAL - COMMUNITY, flow of continuous care of the newborn and infant at high risk of illness and death* developed within the Human Capital Operational Programme and coordinated by the Alessandrescu-Rusescu National Institute for Maternal and Child Health - Bucharest (project code: POCU/91/4/8/109586). Within this project I was a curriculum expert and I participated as a lecturer in the courses held for family doctors and pediatricians in the hospitals from the counties of Moldova. I was also co-author of the two books published in the project: *Diagnosis and modern therapy in the main diseases of the newborn and infant* and *Newborn and infant care in the practice of family doctor in the community*, Amalthea publishing house, 2019.

Scientific research activity

The scientific research is a basic component of the educational-formative development that any university assumes, a duty and, at the same time, a privilege of any teacher. My research activity began with the admission to doctorate under the supervision of PhD Professor Ioan Tansanu, school trainer and outstanding personality of Romanian paediatrics. The doctoral thesis entitled Clinical-evolutionary and therapeutic considerations in malabsorption syndrome in children was completed in 2004 and comprised an extensive study that included 586 patients with malabsorption syndrome monitored in the 3rd Paediatric Department of "Saint Mary" Emergency Clinical Hospital for Children from Iași. Thus, the concerns for the nutritional pathology of children originate from that period because the malabsorption syndrome diagnosed at the pediatric age, in its many clinical, biological and nutritional forms, is a long-term nutrition problem until adulthood.

The main consequence of the malabsorption syndrome is malnutrition, and malnutrition refers to poor, incorrect nutrition, which will lead to a wide range of manifestations, from classic malnutrition to obesity. Thus, improper nutrition applied to infants and underweight children will invariably result on the long run in the onset of obesity in older children, adolescents or adults. I have developed my research skills since my doctorate and I have continuously improved it by participating in various courses and workshops on medical research technique, such as life-long education courses: Clinical epidemiology. Medical research methodology (Iași, 2002), How to write a paper, How to do a systematic review and Developing your academic career within ESPGHAN Summer School (Prague, 2013), The ESPGHAN Eastern Europe School (Sinaia, 2017), Evidence Based Medicine in 10th Pediatric Nutrition Course (Switzerland, 2017), Statistical analysis techniques in medical studies and research (Iași, 2019).

The entire research activity of the last 20 years has materialized in papers communicated at various scientific events, articles published in journals in the country and abroad ISI (25 articles) or BDI (54 articles) indexed. As I have always liked teamwork, I constantly promoted transdisciplinarity by creating a functional framework of interdisciplinary interaction, the consequence being the multidisciplinary approach to the nutritional pathology of children and adolescents and the subsequent dissemination of research results.

The acknowledgment of my performance in scientific research was confirmed by the two awards granted in 2017 and 2019 by UEFISCDI for two articles published in specialized journals with an important impact factor. I also won nine other awards and medals at various congresses, scientific events and book fairs for both the research and journalism activity.

Of all the six grants and projects I participated in, I was a manager of an international grant won through a competition at the *European Society for Contraception and Reproductive Health entitled Perceptions and attitudes on reproductive health and contraception in obese teenagers* (conducted in 2017 -2019). The aim of this project was to achieve a reproductive health education intervention programme for improving the knowledge of obese adolescent girls aged 14-18 years old. Because the Romanian curriculum is not providing sexual education classes, this project was very necessary. Obesity is an important risk factor associated with adolescent sexual behaviour because teen pregnancy is associated with increased risk of developing complications (gestational diabetes, arterial hypertension, hypercoagulability, dead fetus in utero). Obese teenagers show a lower efficacy in case of combined oral contraceptives due to a higher basal metabolic rate, increased metabolism of liver enzymes, higher storage of drugs in the adipose tissue. Therefore, between November 2017 and September 2019, the mother-daughter couples, teenager girls with overweight and obesity, aged between 12 and 18 years old were counselled in the Regional Centre for Diagnosis and Monitoring of Obese Children. All couples received two questionnaires about sexual education and contraception. The results indicated an increased frequency of mothers who showed deficits regarding information in the field of reproductive health and contraception, an aspect that significantly influenced the level of correct knowledge in case of obese teenager girls. The analysis of the questionnaire revealed that the information held by obese teenager girls comes from non-specialized sources (colleagues, friends, media, family members) and not from qualified personnel (teachers, medical staff). The teenager girls do not have sufficient knowledge about contraception methods and their effects on obesity that further creates major implications for the quality of life.

We organised a workshop in the *Regional Center for Diagnosis and Monitoring of Obese Children* for health professionals working with obese teenagers during which we offered information about reproductive health and contraceptive methods through a social media page dedicated to obese teenagers (https://ReprodObeseTeen.grant.umfiasi.ro/en). The results obtained from the statistical analysis helped us to create a contraception guideline. We identified specific problems of the obese teenager and we developed medical and psychological counselling programmes in order to improve their quality of life.

I am a founding member of Center for Diagnosis, Counseling and Monitoring of Obese Children of University of Medicine and Pharmacy "Grigore T. Popa" from Iasi of the cross-border project *entitled Integrated cross-border network for advanced health services in the field of Obesity, Diabetes and other metabolic disorders - OBDIA - NET* of "Grigore T. Popa" University of Medicine and Pharmacy Iasi in partnership with Nicolae Testemitanu State University of Medicine and Pharmacy from Chisinau, project ID 1HARD/4.1/93. This project will provide the medical infrastructure necessary to study obesity and its cardiovascular, metabolic and hepatic complications in children and adolescents in Moldova. As childhood and adolescent obesity is a significant predictor of obesity in adulthood and a risk factor for the many complications it generates, the project will allow a multidisciplinary approach to obesity by involving teams of specialists in various specialties: paediatrics, nutrition, cardiology, nephrology and, last but not least, surgery. It is an extremely important project in which paediatric patients will be fully investigated with state-of-the-art equipment that will be purchased, will be monitored in the short and long term, and finally will be taken over by the adult network.

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CHAPTER I. MALABSORPTION SYNDROM AND CHILDHOOD MALNUTRITION

Introduction

Nutritional disorders in children are an important public health problem with major psycho-socio-economic impact. Nutritional disorders have multiple causes, including insufficient intake of nutrients (improper diet, malabsorption syndromes, etc.) or, conversely, increased calorie intake but with micronutrient deficiency. Thus, malnutrition can take many forms in children: infants and children with failure to thrive, children who do not grow properly or who suffer because their diets are imbalanced as well as children and adolescents who are obese or suffer from nutrition-related non-communicable diseases (such as cardiovascular disease, diabetes mellitus, etc.). Malnutrition affects all countries and one in three people on the planet. Nearly half of all countries face multiple serious burdens of malnutrition such as poor child growth or obese children and adolescents (Global Nutrition Report, 2015).

Severe nutritional deficiencies in childhood are an important cause of weight and/or short stature. The accompanying immune deficiencies are also the cause of repeated infections and/or chronic diseases. From the first year of life, insufficient protein and energy intake due to intestinal malabsorption has significant repercussions on children's psychomotor and neurocognitive development. The common clinical characteristic of malabsorption syndrome, which includes many disorders, is malnutrition due to intestinal malabsorption of one or more nutritional principles. The frequent disorders that determine intestinal malabsorption and maldigestion in children are represented by cystic fibrosis (CF), celiac disease (CD), cow's milk protein intolerance.

The malabsorption syndrome study began in the doctoral thesis entitled "Clinical-evolutionary and therapeutic considerations in the malabsorption syndrome in children". Research on CF and CD continued later. The results were presented at national and international conferences, and they were also published as articles in journals that were further indexed in databases. The fact that I have the chance to carry out my activity in the III rd Pediatric Clinics, which is the Regional Center for Diagnosis and Monitoring of Children with CF from the region of Moldova, allowed me to follow the evolution of CF in children from the diagnosis until they turned 18 years old. Afterwards the patients are transferred to the corresponding adult network.

I.1. RELATIONSHIP BETWEEN CYSTIC FIBROSIS AND CHILD MALNUTRITION

I.1.1. Background

CF is the most common genetically lethal disease in Caucasian people, caused by mutation in the gene encoding the CF transmembrane conductance regulator (CFTR) protein (Panagopoulou P., Fotoulaki M., Nikolaou A. et al., 2014). Located on chromosome 7 in the

region of 7q31.2, CFTR predominantly functions as a chloride channel, which affects sodium and water movement across the cell membrane. The mutated chloride channel leads to thick mucus secretions in various glands and organs. The viscous mucus secretions in the lungs lead to airway obstruction increasing the risk of infection, pulmonary exacerbation and respiratory failure (Grasemann H. and Ratjen F., 2013). In addition, people with CF commonly develop endocrine, gastrointestinal, pancreatic, liver, and reproductive disorders (Miller A.C., Comellas A.P., Hornick D.B. et al., 2020).

According to World Health Organization (WHO) and to available CF registers, there are approximately 80.000 CF patients worldwide, with prevalence varying according to ethnicity (WHO, 2018). WHO presents in the gene diseases global prevalence map that in Europe, 1 in 2000-3000 new borns were found to be affected by CF with discrepancies among the different countries, while in America the incidence is reported to be 1 in every 3500 births. On the contrary, the existing evidence indicates that CF prevalence in Africa and Asia is rare, due to the severely under diagnosed patients. The Cystic Fibrosis Foundation (CFF) data shows that the probability for people with two defective CF genes, one from each parent (named carriers), is 25% of having a child with CF, 50% that the child will be a carrier but will not have CF, and 25% that the child will be a non-carrier and will not have CF (https://www.cff.org/What-is-CF/Testing/Carrier-Testing-for-Cystic-Fibrosis/).

CF is an inherited disorder manifested by predominantly digestive and respiratory signs and symptoms but with significant impairment of nutrition. Digestive signs and symptoms vary, depending on the severity of the disease and include especially chronic diarrhea with greasy stools or constipation, poor weight gain and growth and many other symptoms: meconial ileus, vomiting, rectal prolapse. In children with CF, liver disease is early and frequent complication. According to the prospective study done by Lindblad et al., 25% of the children over 4 years old presented biochemical markers of liver disease, with an evolution to multilobular cirrhosis in their first life decade in 5 to 10% of the cases (Lindblad A., Glaumann H., Strandvik B., 1999, Colombo C., Battezzati P.M., Crosignani A. et al., 2002). In a study by Lamireau et al. in which 241 patients with CF were included, the authors observed that cystic fibrosis related liver disease (CFLD) developed especially in the first ten years of life, with a prevalence of 41% in 12-year-olds: 7.8% of patients have been diagnosed with liver cirrhosis and 5 patients benefitted from liver transplant (Lamireau T., Monnereau S., Martin S. et al., 2004).

Malnutrition is both a frequent feature and a comorbidity of CF and nutritional status is strongly associated with pulmonary function and survival (Turck D., Braegger C.P., Colombo C. et al., 2016). In CF patients, malnutrition is mainly caused by a combination of nutrient malabsorption secondary to exocrine pancreatic insufficiency and increased energy expenditure mainly related to breathing efforts (Bell S.C., Saunders M.J., Elborn J.S. et al., 1996). Impaired nutritional status in children with CF has been a matter of concern since the first description of this disease because malnutrition was the most common presenting symptom in early infancy and is associated with lower pulmonary function and increased early mortality (Dodge J.A. and Turck D., 2006). Over the years, efforts focused on advances in pancreatic enzyme technology, development of new-generation antibiotic preparations, and acceptance of intense nutritional support with high-energy high-fat diets led to improvement both life expectancy and quality of life increased. Prevention and early detection of growth failure is the key to successful nutritional intervention. Therefore, follow the recommendations of the current guidelines on

continuous nutritional status monitoring with regular instruction of parents regarding food and dietary supplement requirements is essential for maintaining normal growth and preventing nutritional deficiencies and increased life expectancy in CF patients (Kalnins D. and Wilschanski M., 2012). But the relation between malnutrition and pulmonary death in patients with CF has resulted in intensive nutritional intervention over the last few decades, leading to a significant decline in underweight and the emergence of overweight/obesity as a potential new problem (Hanna R.M. and Weiner D.J., 2015).

Currently, many children with CF now reach adulthood, at which time the risk of cancer is increased. CF patients have an increased risk of digestive tract cancer, especially after transplantation, but they also have increased risk of lymphoid leukemia and decreased risk of melanoma.

Supportive treatments have significantly increased the prognosis of patients with CF. Current efforts in CF research aim at repairing the basic defect, using improvement in pulmonary function as an endpoint. The US Food and Drug Administration has recently approved small molecules that are able to modulate the channel function.

Ivacaftor (VX770), a corrector of the gating defect of CFTR, was approved for patients with G551D, a class III mutation, and has shown promising improvements in outcomes, such as sweat chloride concentration, forced expiratory volume in 1 second, body mass index, and exacerbation rate.

Lumacaftor (VX-809), a channel corrector that allows the ΔF508 CFTR to bypass proteomic degradation and increases trafficking of the protein to the plasma membrane, has been very recently approved in combination with Ivacaftor (Orkambi) in patients homozygous for F508Δ. Unfortunately, the efficacy is not impressive mainly because of the instability and reduced half-life of the rescued CFTR at the plasma membrane. This is due in part to the inflammatory status of the airway's epithelia and in part to more profound targeted defects. Thus far, no data have been reported on the effects of these molecules in patients with CFLD. However, recent discoveries in the field of CFLD pathophysiology may open new therapeutic paradigms (Fiorotto R. and Strazzabosco M., 2016).

The combination therapy of Ivacaftor and Lumacaftor has produced considerable improvements in lung function, weight gain, and reduced exacerbations of pulmonary symptoms. The weight increase observed under this combination is speculated to be due to both improvement in pulmonary function and improvements in GI physiology (Elborn J.S., Ramsey B.W., Boyle M.P. et al., 2016).

It is believed that the effective treatment of CFLD consists of an adjuvant approach that repairs the primary defect and decreases inflammation. Nuclear receptors that modulate inflammation could be a new target in controlling inflammation observed in CF biliary epithelium (Fiorotto R. and Strazzabosco M., 2016, Al Sinani S., Al-Mulaabed S., Al Naamani K. et al., 2019).

Several other new CF medications are in clinical trials. Ataluren (PTC124), an orally bioavailable small molecule, designed to induce ribosomes to selectively read through premature stop codons during mRNA translation to produce functional CFTR. As nonsense mutations account for 10% of CFTR mutations, this modality can be an example of personalized medicine, with which patients with specific gene mutations are targeted with a specific modality of therapy (Peltz S.W., Morsy M., Welch E.M. et al., 2013).

Currently, the CF patients' life expectancy increased worldwide as explained in a 35-year observational study in France (Scotet V., Duguépéroux I., Saliou P. et al., 2012), by early diagnosis, routine screening and improvements in disease management, adequate nutritional support and new treatments. However, this drop has a drawback, namely adult CF patients tend to experience additional health challenges (diabetes, osteoporosis, hearing loss, arthropathies, musculo-skeletal problems, infertility, etc.) that increased the number of inpatient hospitalizations related to CF along with an increase in the associated costs to about 138.31% according to Agrawal et al. (Agrawal A., Agarwal A., Mehta D. et al., 2017).

Personal contribution related to comorbidity and therapeutic strategies in cystic fibrosis in children was synthesized in the following papers:

Articles

1. **Trandafir LM**, Leon MM, Frasinariu O, Baciu G, Dodi G, Cojocaru E. Current Practices and Potential Nanotechnology Perspectives for Pain Related to Cystic Fibrosis. *J. Clin. Med*. 2019; 8: 1023. (IF =3.03)
2. Cojocaru FD, Botezat D, Gardikiotis I, Uritu CM, Dodi G, **Trandafir L**, Rezus C, Rezus E, Tamba BI, Mihai CT. Nanomaterials Designed for Antiviral Drug Delivery Transport across Biological Barriers. *Pharmaceutics*. 2020; 12(2): 171. (IF = 4.421)
3. Alecsa MS, Moscalu M, **Trandafir LM**, Ivanov AV, Rusu C, Miron IC. Outcomes in Pediatric Acute Lymphoblastic Leukemia—A Single-Center Romanian Experience. *J. Clin. Med*. 2020; 9(12):4052. (IF = 3.303)
4. Miron O, Afrasanie VA, Paduraru MI, **Trandafir LM**, Miron L The relationship between chronic lung diseases and lung cancer-a narrative review. *J BUON*. 2020 Jul-Aug; 25(4):1687-1692. (IF = 1.695)
5. **Trandafir LM**, Moscalu M, Diaconu G, Cîrdeiu E, Tudose AA, Coman G, Păduraru DT. The impact of respiratory tract infections on the nutritional state of children with cystic fibrosis. *The Medical-Surgical Journal*. 2013; 117(4):863-869. PMID: 24502062
6. **Trandafir LM**, Straticiuc Ciongradi I, Baciu G, Teodora D, Păduraru A. Liver disease in children with cystic fibrosis. *Roumanian Journal of Pediatrics*. 2014; LXIII (1): 22-29.
7. Anton-Paduraru DT, Drochioi AS, Teslariu O, Murgu AM, Cernescu I, Ifrim AD, **Trandafir LM** Osteoarticular disorder in children with cystic fibrosis. *Romanian Journal of Pediatrics*. 2016; 65(1): 40-44. (EBSCO)
8. Anton-Paduraru DT, Moscalu M, Coman G, Florescu L, **Trandafir LM.** The impact of the staphylococcus aureus infection on the evolution of children with cystic fibrosis from a regional centre in North-Eastern Romania. *Romanian Journal of Pediatrics*. 2014; 63(2): 128-134. (EBSCO)
9. Anton-Paduraru DT, Oltean C, Iliescu ML, Baciu G, **Trandafir LM.** Cystic fibrosis-related diabetes. *Romanian Journal of Pediatrics*. 2014; 63(1): 7-12. (EBSCO)

ISI Proceedings

1. Trandafir LM, Teslariu O, Anton-Paduraru DT. Gastrointestinal Manifestations in Children with Cystic Fibrosis, NEUROGASTRO 2017 - Meeting of the Romanian Society of Neurogastroenterology with Rome IV Regional Central East European Meeting, 2017, 147-150 (Web of Science Core Collection) ISI Proceeding

Book chapter

1. Fibroza chistică - Dana Teodora Anton Păduraru, **Laura Mihaela Trandafir**, în Pediatrie (editor Ingrid Miron). Ed. "Gr. T. Popa", UMF Iasi, 2016, ISBN 978-606-544-429-4, pg. 300-308.
2. Sindromul de malabsorbție - Dan Moraru, **Laura Mihaela Trandafir** in Pediatrie – patologie digestivă, nutrițională și neurologică la copil (editor Dan Moraru, Marin Burlea, Evelina Moraru, Eugen Cirdei, Georgeta Diaconu), Ed. Fundaţiei Academice AXIS, 2008, ISBN 978-973-7742-64-3, pg. 38-44.

I.1.2. Respiratory and gastrointestinal manifestations and nutritional status in cystic fibrosis patients

I.1.2.1. Introduction

During infancy and childhood, many conditions are associated with poor weight and height gain, and one of these is CF. The clinical course of CF and the quality of life of the patients are directly affected by their nutritional status, and malnutrition is one of the most serious and difficult challenges in CF treatment. Malnutrition results from a discrepancy between energy/nutrient requirements and food intake, which can be caused by malabsorption secondary to exocrine pancreatic insufficiency and increased energy expenditure (Bell S.C., Saunders M.J., Elborn J.S. et al., 1996). It is known that the occurrence of malnutrition is associated with lung function decreasing and survival.

Chronic pulmonary infections and decreased lung function result in increasing calorie needs and reduced appetite, which worsen the nutritional status of CF patients (Barni G.C., Forte G.C., Forgiarini L.F. et al., 2017). On the other hand, gastrointestinal symptoms of CF including distal intestinal obstruction syndrome, meconium ileus, intussusception, and constipation, focal biliary cirrhosis and cholangiectasis are another prognostic factor for malnutrition (Kelly T. and Buxbaum J., 2015). The relation between malnutrition and pulmonary death in patients with CF has resulted in intensive nutritional intervention over the last few decades, leading to a significant decline in underweight and the emergence of overweight/obesity as a potential new problem.

Part of the preoccupations related to respiratory and gastrointestinal manifestations in cystic fibrosis associated with malnutrition were synthesized in the following articles:

1. **Trandafir LM**, Moscalu M, Diaconu G, Cîrdeiu E, Tudose AA, Coman G, Păduraru DT. The impact of respiratory tract infections on the nutritional state of children with cystic fibrosis. *The Medical-Surgical Journal*. 2013;117(4):863-869. PMID: 24502062.
2. **Trandadfir LM**, Teslariu O, Anton-Paduraru DT. Gastrointestinal Manifestations in Children with Cystic Fibrosis, NEUROGASTRO 2017 - Meeting of the Romanian Society of Neuro-gastroenterology with Rome IV Regional Central East European Meeting, 2017, 147-150 (Web of Science Core Collection) ISI Proceeding

Using the theoretical data mentioned above, the two articles aimed to:

- ***Recurrent Pulmonary Infections in Children with Cystic Fibrosis***

Our study evaluated the relationship between recurrent pulmonary infections and nutritional status in children with CF.

- ***Gastrointestinal Symptoms in Cystic Fibrosis***

The objective of this study was to evaluate the contribution of the gastrointestinal symptoms to early diagnosis of CF in Romanian's children in the absence of neonatal screening.

I.1.2.2. Material and Methods

- ***Recurrent Pulmonary Infections in Children with Cystic Fibrosis***

We realised a retrospective study included 27 child patients diagnosed with CF between 1994 – 2011 in the IIIrd Pediatric Clinics of "Saint Mary" Children's Hospital from Iasi. Ponderal index (PI), BMI, Z score for weight and waist were used to evaluate the children nutritional status. The microbacteriologic exam of hypopharyngeal aspirate was performed. In patients with pulmonary infections the authors established statistical correlations between the age of symptoms onset and diagnosis, frequency of acute infectious episodes, bacterial agents identified and nutritional status.

- ***Gastrointestinal Symptoms in Cystic Fibrosis***

Our study group included 40 children diagnosed with CF in the IIIrd Pediatric Clinic of The Children's Hospital "Saint Mary" from Iasi between 2011-2015 who were evaluated. The diagnostic of CF was established using two positive values of sweat test (chloride level >60 mmol/ml). The algorithm of monitoring included weight, height, BMI, at the time of diagnose and 6 months after the treatment beginning. We noted the respiratory and gastrointestinal symptoms, age at the onset of symptoms, age at time of diagnosis, the evolution of nutritional status. The nutritional therapy protocol included pancreatic enzymes, fat-soluble vitamins and hypercaloric nutritional support.

I.1.2.3. Results

- ***Recurrent Pulmonary Infections in Children with Cystic Fibrosis***

In our study the patient's age ranged from 3 months to 17 years (4.1 years mean age). The sex repartition showed that 17 patients were male (62.96%) and 10 females (37.04%), sex ratio: 1,7:1.

Although the onset of CF was in the first year of life in 77.77% of cases, however, the final diagnosis was established later in 70.37% of cases (Fig. 1).

The average of patients age at the disease's onset was 7.23months±4.32DS (between birth to thirteen-month-old). We noted that early disease onset in the first 3 months of life was found in 22.22% of cases (Fig.2.).

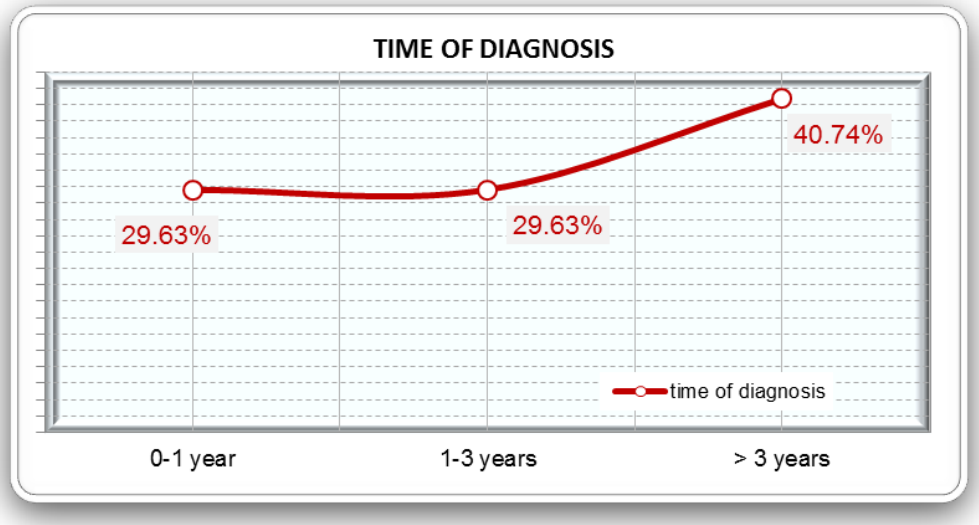

Figure 1. *The patient's distribution by diagnosis age*

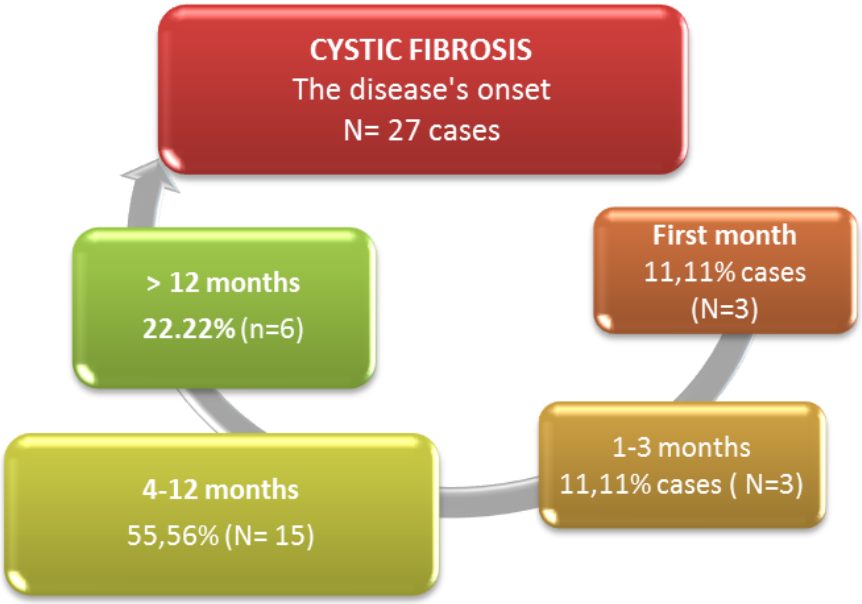

Figure 2. *Distribution of patients by age of onset disease*

The clinical forms of CF encountered in our study group were predominantly respiratory features in 48,14% of cases, predominantly digestive symptoms in 33.33% of cases and the mixt type, with both respiratory and digestive symptoms, in 18,52% of cases (Fig.3.).

Figure 3. *Clinical forms of CF in children*

In figure 4 we observed the frequent lung features: bronchopneumonia (27% of cases) and recurrent wheezing and chronic cough (20% of cases).

Patients diagnosed with both pulmonary and digestive disease displayed severe exocrine pancreatic insufficiency and recurrent respiratory infections. We analysed the relation between the onset age and the severity of pancreatic insufficiency and respiratory features. So, we noted that the early onset is correlated with severe types of diseases (r=0.696, p=0.418, 95% CI) (Fig. 5).

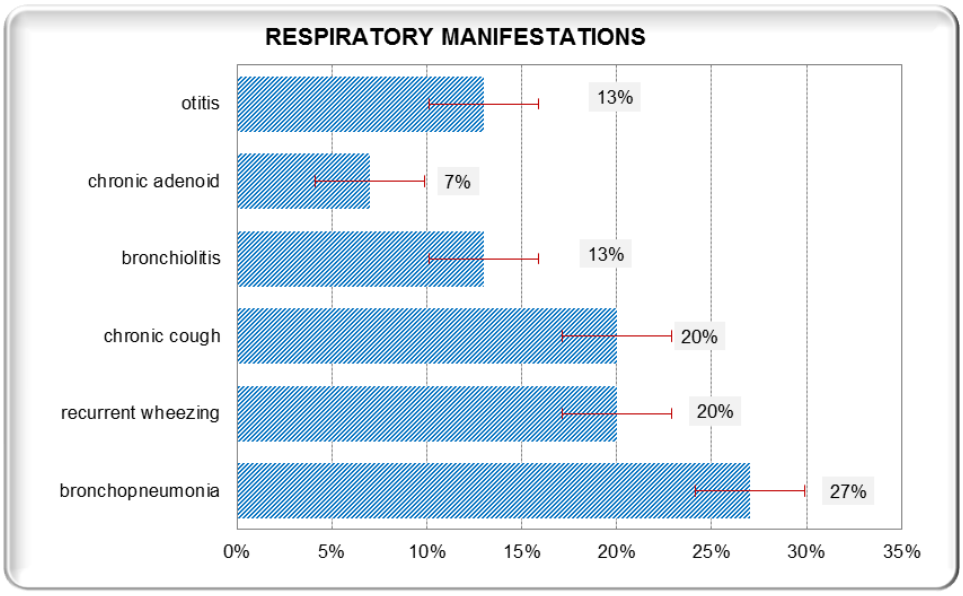

Figure 4. *Respiratory manifestations in children with CF*

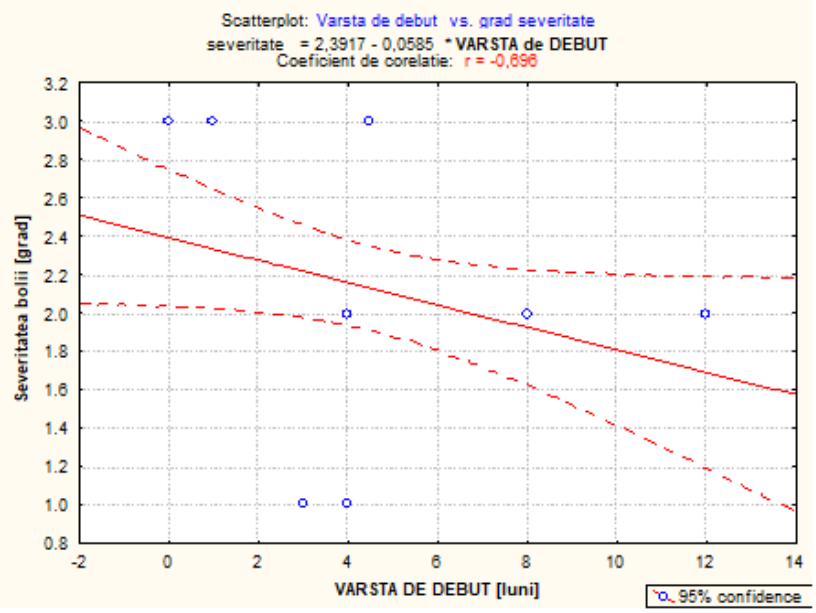

***Figure 5**. Correlation between the age of onset and the disease severity*

In our group study only 14.81% patients presented normal weight. The delayed growth for weight and/or height was observed in 85.19% of cases, with three stages of low body weight according to Z score: 37.04% of cases – stage I dystrophy, 18.52% of cases – stage II dystrophy and 7.41% - stage III dystrophy (Fig.6).

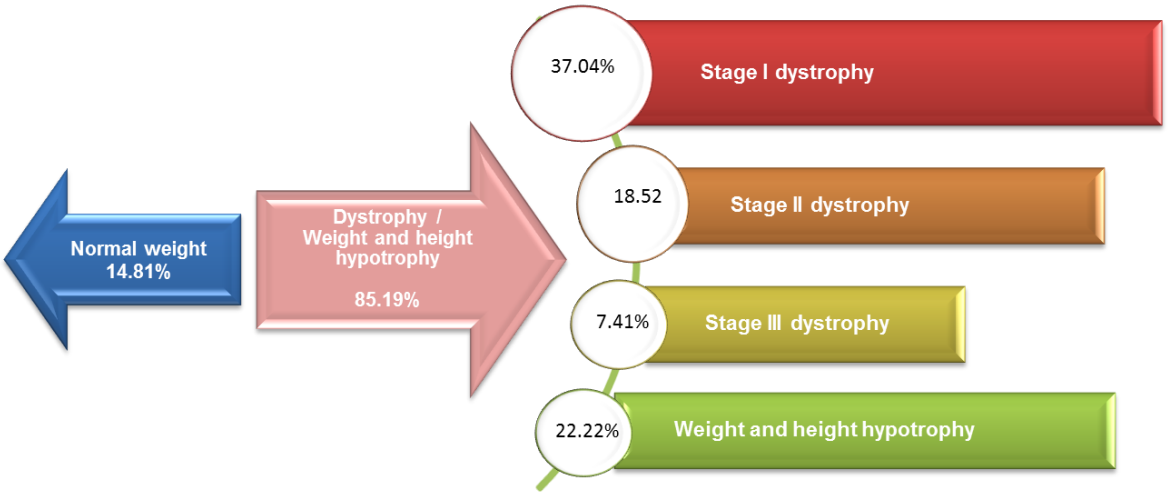

***Figure 6.** The nutritional status in CF patients*

In the sample we studied we observed that early onset and recurrent pulmonary infections are greatly linked to abnormal nutritional state, aspect revealed by multivariate analysis. Partial correlations from the multivariate analysis between the age at the debut, the presence of recurrent infections ($r=-0.434$, $p=0.0045$) and nutritional state impairment ($r=-0.517$, $p<<0.05$) were statistically significant.

The logistic regression was used to perform the multivariate analysis between disease onset and severity of recurrent infections. Table I shows predictive factors for recurrent

infections incidence and patients' nutritional status. The results showed that early onset determines both the appearing of recurrent infections (b=-0.434-partial correlation indices) and nutritional state abnormalities (b=-0.517) (Fig. 7).

Table I. Multiple correlation between CF onset vs. impaired nutritional status and recurrent infections

Multivariate correlation	Value
Multiple R	0.88694
Multiple R^2	0.78667
F	44.24963
***p* – value**	0.00000
Std.Err. of Estimate	2.07712

Multiple linear regression CF onset vs.	Beta	Std.Err. (Beta)	B	Std.Err. B	t	*p*-value
Intercept			10.64286	0.555133	19.17172	<0.001
recurrent infections	-0.434356	0.241544	-3.90667	1.248193	-3.12986	0.004549
Impaired nutritional status	-0.517684	0.138778	-4.39286	1.177615	-3.73030	0.001039

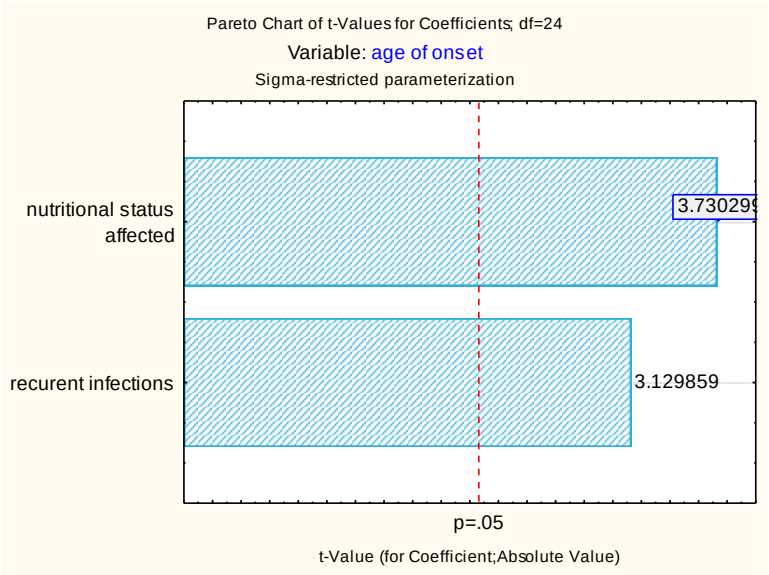

Figure 7. *Absolute value of the "t" statistics in the evaluation of multivariate analysis (onset vs. impaired nutritional status, recurrent infections)*

The etiologic agents involved in pulmonary infections were *Staphylococcus aureus* (*S. aureus*) (48,14%), *Pseudomonas aeruginosa* (33,33%), *Stenotrophomonas maltophilia* (18,51%), *Haemophilus influenzae* (14,8%), *Klebsiella pneumoniae* (11,10%), *Moraxella catarrhalis* (7,40%), *Streptococcus pneumoniae* (7,40%), *Neisseria sica* (7,40%) (Fig. 8).

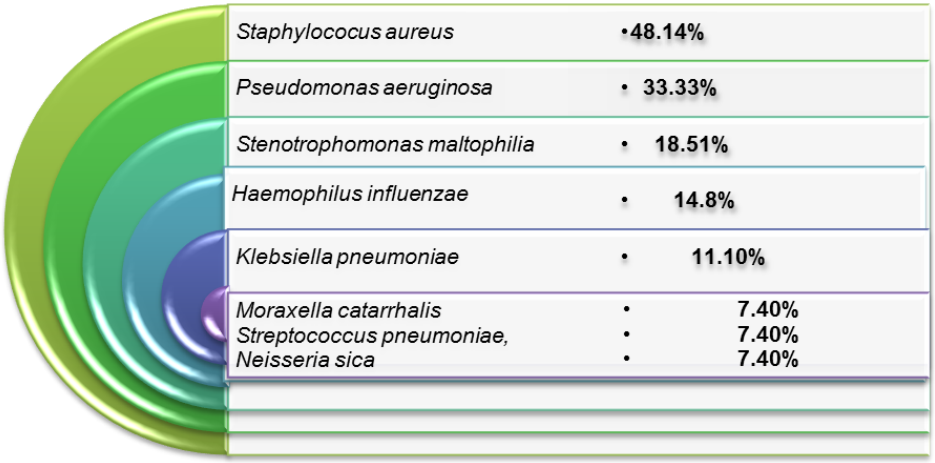

Figure 8. *The etiologic spectrum of respiratory infections*

We noted that *Staphylococus aureus*, *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia* respiratory infections were correlated with failure to thrive.

- ***Gastrointestinal Symptoms in Cystic Fibrosis***

In our study, only 17 patients presented gastro-intestinal features, such as chronic diarrhea (7 cases), failure to thrive (4 cases), meconium ileus (3 cases), intestinal, intussusception (1 case), peritonitis (1 case), ano-rectal prolapse (1 case) (Fig. 9).

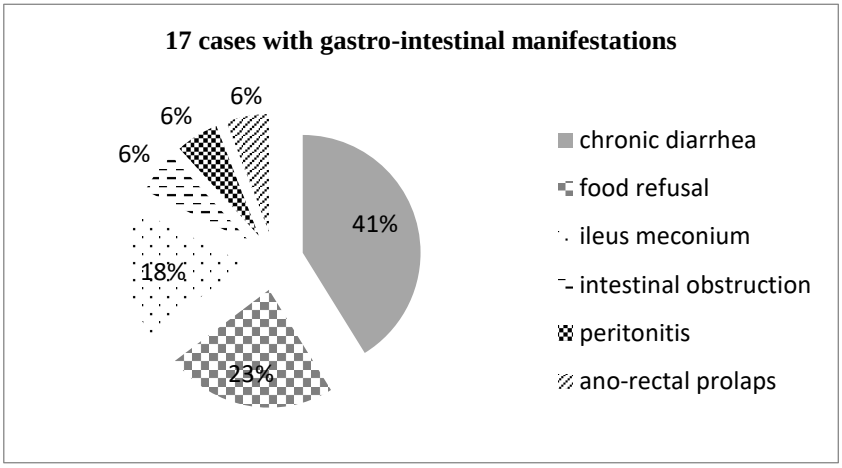

Figure 9*. Distribution of gastrointestinal symptoms of the selected patients from the study group*

The patient's age at onset of symptoms ranged from 2 days to 9 months. 59% of patients developed early digestive symptoms during infancy and young childhood. 35% of patients were identified in neonatal period, while in only one case the disease began later than 3 years old (Table II).

Table II. Time of CF onset

No. cases	Age of onset of digestive symptoms
6 (35%)	Neonatal period
10 (59%)	Toddler and infant (< 3 years)
1 (6%)	Child (> 3 years)

I.1.2.4. Discussions

- ***Recurrent Pulmonary Infections in Children with Cystic Fibrosis***

During infancy and childhood, many conditions are associated with poor weight and height gain, and one of these is CF. Without the possibility of performing neonatal screening at birth, in our study 88.88% of CF cases were diagnosed lately. The average age of diagnosis was 3.4 years compared with the mean age of symptoms onset which was 7 months.

Delayed weight and/or height gain was found in 85.19% of cases. In the study group was observed that the disease onset in the first years of life and recurrent respiratory infections were significantly correlated (r = 0.94, CI = 95%) with impaired nutritional status. All 6 patients with weight and height hypotrophy presented chronic lung infections: three patients with methicillin-resistant *S. aureus*, a patient with *Pseudomonas aeruginosa*, and two patients with co-infection with methicillin-resistant *Staphylococus aureus* and *Pseudomonas aeruginosa*. So, the earlier disease onset associated with the late diagnosis is correlated with early respiratory infections and severe impaired nutritional status. The main pathogen of the CF lung infections is *Staphylococus aureus* (Hoiby N. and Frederiksen B., 2000, Cocchi P., Cariani L., Favari F. et al., 2011, Stone A. and Saiman L., 2007). The study by Beringer et al. highlighted that about 50% of children with CF until the age of ten presented chronic pulmonary infections with *Staphylococus aureus* (Beringer P.M. and Appleman M.D., 2000) In patients with CF, *Staphylococus aureus* itself is common cause of poor growth and mortality, but by progressive destruction of lung tissue and worsening pulmonary obstruction allows co-infection with *Pseudomonas aeruginosa* (Rosenbluth D.B., Wilson K., Ferkol T. et al., 2004, Vu-Thien H., Hormigos K., Corbineau G., 2010, Goss C.H. and Muhlebach M.S, 2011, Hart C.A. and Winstanley C., 2002). In our study pulmonary infections with *Staphylococcus aureus, Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia* were more associated with impaired nutritional status.

Despite correct pancreatic enzyme replacement and nutritional supplements received by patients with CF, these patients did not grow properly in weight and height due to loss of appetite, the catabolic status during respiratory exacerbations who requiring repeated hospitalizations. On the other hand, poor nutritional status has a significant negative effect on lung disease progression and survival rate in patients with CF (Konstan M.W., Butler S.M., Wohl M.E. et al., 2003). So, recurrent respiratory infections, inappropriate nutritional intake, low compliance for substitutive treatment led to poor weight and waist gain. Increased incidence of yearly pulmonary infections is associated with impairment of nutritional state in children with CF.

- ***Gastrointestinal Symptoms in Cystic Fibrosis***

The earliest digestive features of CF were meconium ileus (Agrons G.A., Corse W.R., Markowitz R.I. et al., 1996). In our study five of all patients were diagnosed with meconium ileus, and two of them presented complications such as intestinal obstruction and peritonitis. In these cases, the diagnosis has been established immediately after birth.

Another intestinal symptom, chronic diarrhea, due to insufficiency of pancreatic enzymes and malabsorption (Haak A., Aragao G.G., Carvahlo Garbi Novaes M.R., 2013) was the most frequent digestive manifestation in our study group, being present in 41% of the patients, six cases during infancy and young childhood and one case later than the age of three

years. The ano-rectal prolapse should be considered an alarming sign for establishing the CF diagnose (Hubbard V.S., 1985). In our study group, ano-rectal prolapse was diagnosed in one patient at the age of 2 years (Table III). Our results showed that the age at diagnosis ranged between 4 days and 3 years and 6 months, in the absence of neonatal screening for CF in Romania. Unfortunately, late diagnosis after the age of two is associated with higher rate of morbidity and unfavourable prognosis (De Monestrol I., Klint A., Sparen P. et al, 2011, Sims E.J., Clark A., Mehta G. et al, 2007).

Table III. Distribution of digestive symptoms according to the onset age

Age of onset of digestive symptoms	Types of symptoms
Neonatal period	Intestinal obstruction
	Meconium ileus x 2
	Chronic diarrhea x 2
	Perforation of transverse colon with peritonitis
Toddler and young infant (<3 years)	Meconium ileus
	Failure to thrive x 4
	Chronic diarrhea x 4
	Ano-rectal prolapse
Child (>3 years)	Chronic diarrhea

Periodic monitoring of nutritional status in order to early detection and treat the failure to thrive in CF children is essential to successful nutritional therapy. A consensus report of the North American Cystic Fibrosis Foundation recommends the daily caloric requirement of 100-150% compared to diet of a healthy child and 35-40% daily calories to be provided by fat, in order to ensure the energy for normal development of the patients (Kalnins D. and Wilschanski M., 2012, Hortencio T.D.R., Nogueira R.J.N., de Lima Marson F.A., 2015).

The evaluation of the weight curve in the dynamics showed a statistically significant increase between the time of diagnosis and at six months after initiating specific therapy. Periodic evaluation to improve nutrient intake (diet high in energy and fat) and absorption (pancreatic enzyme replacement therapy) are vital for patient care and have contributed to observed increases in pulmonary function and lifespan.

I.1.2.5. Conclusions

- ***Recurrent Pulmonary Infections in Children with Cystic Fibrosis***

The respiratory infections with aggressive etiological agents in the first years of life determine poor growth in both weight and height. The early diagnose is important to prevent the colonisation of the airways with multidrug resistant agents and to a proper nutritional management. The proper nutrition and the optimal nutritional status strongly associated with pulmonary function and long-term prognosis and survival rate.

- ***Gastrointestinal Symptoms in Cystic Fibrosis***

Our study supports the idea that CF gastrointestinal features often develop before the respiratory symptoms. It is obvious that the early diagnosis in the absence of neonatal screening contribute to better therapeutic management and improves the quality of life in CF patients.

I.1.3. Pain related to Cystic Fibrosis

I.1.3.1. Introduction

In the last years, pain is also recognized as a potential health challenge in CF, reported to affect 75% of children and 89% of adults (Blackwell L.S. and Quittner A.L., 2015, Kelemen L., Lee A.L., Button B.M. et al. 2012). Pain was significantly more intense and lasted notably longer among adults, but its rate and recurrence did not differ and was not related to the severity of CF, a comparative study for the prevalence of pain symptoms, characteristics and occurrence on children and adults with CF stated (Sermet-Gaudelus I., De Villartay P., de Dreuzy P. et al., 2009). Many sources of pain are recognized: appears as a complication of the CF disease; accompanies pulmonary, digestive and articular manifestations; may be secondary to the diagnosis clinical tests (hypopharyngeal aspiration, bronchoalveolar lavage, functional respiratory tests, pleural puncture) or to the administrated chronic treatment (postural drainage, respiratory physiotherapy, evacuation enema) (Koh J.L., Harrison D., Palermo T.M. et al., 2005). The most prevalent locations of pain were the back, head, and chest for adults and the abdomen for children. The particularities of visceral, gastrointestinal, abdominal, musculo-scheletal, pulmonary and chest pain, and pain associated with medical procedures should investigated in patients with CF. Pain of various causes is common in patients with CF. Therefore, the Clinical Guidelines for CF patients care should include recommendations related to the complex management of pain in these patients.

Part of the preoccupations related to pain in cystic fibrosis was synthesized in the following article:

Trandafir LM, Leon MM, Frasinariu O, Baciu G, Dodi G, Cojocaru E. Current Practices and Potential Nanotechnology Perspectives for Pain Related to Cystic Fibrosis. *J. Clin. Med*. 2019; 8: 1023. (IF =3.303)

Using the theoretical data mentioned above, the article aimed to:

To highlight some data regarding the pain characteristics and clinical association in children and adults with CF, the relationship between pain symptoms and disease severity and its impact on life quality and prospects for advanced nanotechnology or other therapeutic strategies.

I.1.3.2. Material and methods

We revised the current literature concerning pain as a potential health challenge in CF patients. The dynamic character of the field is reflected in the increasing number of recent publications. A recent query on the search engine ScienceDirect returned 32,470 hits for the keywords “cystic fibrosis pain”, representing a massive increase from 1996 with 429 publications to 1519 in 2018, 869 in 2019 and 44 in 2020. An analysis of the literature data using “nanotechnology in CF pain” keywords on Science direct engine, found 76 reviews and research articles but all related-on nanotechnology approach for CF treatment. Although this

search may not be entirely representative, to our knowledge there is no data available for the use of nanomaterials in CF pain. Instead, the combination of "nanotechnology in pain" revealed about 7,975 publications from 1996.

We researched the multiple causes of pain (complication of the disease, secondary to diagnostic tests or therapy), assessment tools pain, pain prevalence, characteristics and clinical association in children and adults with CF, impact on life quality and prospects for advanced nanotechnology or other strategies.

I.1.3.3. Results

The first identified pain assessment tool was Medical Chart Review, used to examine the incidence and therapy of chronic pain in a group of older patients with CF. The Multidimensional Pain Inventory was another pain assessment tool, utilized in a sample of adult outpatients with CF to assess their perception of pain and its psychosocial consequences (Ravilly S., Robinson W., Suresh S. et al., 1996, Epker J., Maddrey A.M., Rosenblatt R., 1999).

In 2011 Kelemen et al. described the intensity and location of pain and its relationship with health-related quality of life and pain catastrophizing in 73 adults with CF using three questionnaires, the Brief Pain Inventory, Pain Catastrophizing Scale and the CF-Quality of Life as pain assessment tools (Kelemen L., Lee A.L., Button B.M. et al., 2012). They suggested that Pain Catastrophizing Scale could be used by clinician and physiotherapists to identify patients who shoud be targeted for pain management.

In 2018, Tomaszek et al. (Tomaszek L., Dębska G., Cepuch G. et al., 2018) used NRS tool for pain intensity evaluation of 95 polish patients aged between 14 and 25 years reported at least one source of pain over the prior 2 weeks. Zero value score meant no pain, 1-3 mild, 4-6 moderate and 7-10 was given for severe pain. Mild pain was described by 20% of the subject patients, moderate pain by 45% and 35% of patients reported severe pain perception. The obtained results correlated the pain intensity with poor quality of life. In 2019, a personalized pain subscale, CFSS was used to assess pain by Maras et al. (Maras D., Balfour L., Tasca G.A. et al., 2019).

This tool is comprised by a 41-item questionnaire that evaluates the frequency of pain signs in the past 7 days using a 5-point Likert scale from 0 to 4 (4-all the time) and 17 pain subscales from 0 to 16 (16-the highest number of indicated pain symptoms). The results from this study suggest that pain significantly contributes to poor quality of life in patients with CF.

Location and prevalence of pain in CF

The variety of pain locations and the wide range in prevalence of CF patients reporting pain are provided in Table IV.

As we can observe in the table IV, the most common location of pain is at thoracic and spinal levels. Although the studies highlighted a large individual variety of pain localization in patients and a wide range of prevalence between studies, the most common location of the pain varied from the abdomen in pediatric studies to the back, head and chest in adolescent and adult populations with CF.

Also, it can be observed that the pain was not correlated with the severity of CF. Moreover, the frequency of painful episodes of the children varies between one day and less

than once per month, with no significantly differences than adults, and the duration of painful episodes ranged from less than 30 minutes to a few days.

Table IV. Pain regions and prevalence on children/adolescents and adults

Pain location	**Prevalence on children/adolescents**	**Prevalence on adults**
Abdominal pain	42-100% (Koh J.L., Harrison D., Palermo T.M., 2005, Palermo T., Harrison D., Koh J., 2006, Lechtzin N., Allgood S., Hong G., 2016)	19-50% (Hayes M., Yaster M., Haythorthwaite J., 2011, Festini F., Ballarin S., Codamo T., 2004, Michel-Cherqui M., Ley L., Szekely B., 2015, Rose J., Gamble J., Schultz A., 1987)
Gastrointestinal pain	10% (stomach) (Sermet-Gaudelus I., De Villartay P., de Dreuzy P., 2009)	10%–51% (Sermet-Gaudelus I., De Villartay P., de Dreuzy P., 2009)
Chest pain	10-38% (Koh J.L., Harrison D., Palermo T.M., 2005, Blackwell L.S. and Quittner A.L., 2015, Sermet-Gaudelus I., De Villartay P., de Dreuzy P., 2009, Munck A., Pesle A., Cunin-Roy C., 2012, Lechtzin N., Allgood S., Hong G.,2016)	9%–72% (Kelemen L., Lee A.L., Button B.M., 2012, Sermet-Gaudelus I., De Villartay P., de Dreuzy P., 2009, Hubbard P.A., Broome M.E., Antia L.A., 2005, Flume P.A., Ciolino J., Gray S., 2009, Hayes M., Yaster M., Haythorthwaite J., 2011, Ravilly S., Robinson W., Suresh S. et al., 1996, Festini F., Ballarin S., Codamo T., 2004)
Back pain (mid-back, lower back and/or both)	6-16% (Sermet-Gaudelus I., De Villartay P., de Dreuzy P., 2009, Lechtzin N., Allgood S., Hong G., 2016)	19%–70% 9%–72% (Kelemen L., Lee A.L., Button B.M., 2012, Sermet-Gaudelus I., De Villartay P., de Dreuzy P., 2009, Flume P.A., Ciolino J., Gray S., 2009, Hayes M., Yaster M., Haythorthwaite J., 2011, Ravilly S., Robinson W., Suresh S. et al., 1996, Rose J., Gamble J., Schultz A., 1987)
Head and neck pain	13-42% (Koh J.L., Harrison D., Palermo T.M., 2005, Munck A., Pesle A., Cunin-Roy C., 2012, Blackwell L.S. and Quittner A.L., 2015, Lechtzin N., Allgood S., Hong G., 2016)	6%–64% (Kelemen L., Lee A.L., Button B.M., 2012, Sermet-Gaudelus I., De Villartay P., de Dreuzy P., 2009, Hayes M., Yaster M., Haythorthwaite J., 2011, Flume P.A., Ciolino J., Gray S., 2009, Ravilly S., Robinson W., Suresh S. et al., 1996, Michel-Cherqui M., Ley L., Szekely B., 2015)
Cervical	3% (Sermet-Gaudelus I., De Villartay P., de Dreuzy P., 2009)	10-28% (Hayes M., Yaster M., Haythorthwaite J., 2011, Sermet-Gaudelus I., De Villartay P., de Dreuzy P., 2009)
Limbs (upper and /or lower limp pain)	11-19% (Sermet-Gaudelus I., Lechtzin N., Allgood S., Hong G., 2016)	6-27% (6%–64%) (Kelemen L., Lee A.L., Button B.M., 2012, Hayes M., Yaster M., Haythorthwaite J., 2011, Flume P.A., Ciolino J., Gray S., 2009, Michel-Cherqui M., Ley L., Szekely B., 2015)
Musculoskeletal pain	3-19% (Sermet-Gaudelus I., Lechtzin N., Allgood S., Hong G., 2016)	6%–44% (Hayes M., Yaster M., Haythorthwaite J., 2011, Sermet-Gaudelus I., De Villartay P., de Dreuzy P., 2009, Michel-Cherqui M., Ley L., Szekely B., 2015)

Inflammation and pain aspects

The specific alteration in CF is a defect in CFTR protein that leads to impaired secretion of chloride and an increase in sodium absorption, causing airway surface liquid depletion, defective mucociliary clearance and reduced mucus clearance. The consequences is that secretory cell products have low water content, adhere to the epithelium and are difficult to remove, therefore, encourage for bacterial colonization, recurrent infections, chronic inflammation and irreversible damage to the airway epithelium (Bilton D., 2008). Lungs, pancreas, liver, and intestine are the organs affected by this vicious cycle of inflammation and infection.

CF lung disease is characterized by early colonization and airway infection associated with chronic inflammation, which leads to permanent structural damage to the CF airways and impaired lung function. The mechanisms of CF airway inflammation regulation are not fully understood, but epithelial dysfunction plays a vital role in the development of specific inflammatory responses. Neutrophils (or polymorphonuclear leukocytes, PMN) are essential in the process of CF airway disease, being present in massive amounts due to a combination of increased influx and low clearance. Neutrophils are the first cells to migrate into the lung compartment to fight bacterial and fungal pathogens, but their role in the pathophysiology of respiratory damage in CF is harmful by releasing oxidants and proteases (elastase) that interfere with CFTR expression and / or function.

Although the implication of the CFTR gene is unclear, neutrophil elastase stimulates the inflammatory response of the airways by administering serine proteases and matrix metalloprostheses that cause a variety of harmful effects and correlate with impaired lung function and respiratory exacerbations. Cantin et al. illustrated some of the potential links between CFTR deficiency and innate and acquired immune dysregulation, cell membrane lipid abnormalities, various transcription factor signaling defects, and altered kinase and receptor response with charge and intense airway inflammation, which links the underlying CF defect to inflammation (Cantin A.M., Hartl D., Konstan M.W. et al., 2015, Palaniyar N., Mall M.A., Taube C. et al., 2015).

The CF patients' airway includes numerous pro-inflammatory mediators in different concentrations, including TNF-α (Tumor necrosis factor- α), IL(Interleukin)-1β, IL-6, IL-8, IL-17, IL-33, GM-CSF (Granulocyte-macrophage colony-stimulating factor), G-CSF (Granulocyte-colony stimulating factor), and HMGB-1 (High-mobility group protein 1) (Bonfield T.L., Panuska J.R., Konstan M.W et al., 1995, Roussel L., Farias R., Rousseau S., 2013).

IL-1β encourages acute phase responses, fever and muscle protein catabolism. TNF-α stimulates neutrophil oxidative activity and secretory responses associated with cachexia. Also, the most relevant pro-inflammatory action of GM-CSF in CF is represented by its ability to delay neutrophil apoptosis and inhibit their clearance from the lungs. Moreover, Nichols et al. highlighted the CF airways deficiency in several counter-regulatory molecules including IL-10, nitric oxide (NO), and lipoxin-A4 (LXA4) (Nichols D.P. and Chmiel J.F., 2015). Decreased NO synthesis could promote inflammation, that intensifies abnormal airway surface liquid height and impairs the ability of airway smooth muscle to relax. IL-10 molecule ends the acute inflammatory response, decreases the chemokines production, hinders pro-inflammatory transcription factors and induces neutrophil apoptosis. The LXA4 lipid mediator stimulates the

resolution of inflammation and reduces bronchoalveolar damage (Iannitti R.G., Napolioni V., Oikonomou V. et al., 2016, Nichols D.P. and Chmiel J.F., 2015, Mhanna M.J., Ferkol T., Martin R.J. et al., 2001, Karp C.L., Flick L.M., Park K.W. et al., 2004).

Intestinal inflammation in CF has been extensively studied in the last years. The inflammatory biomarkers measurements on 21 children with pancreatic insufficient CF and 12 controls demonstrated increased values of albumin, IgG, IgM, IL-1β, IL-8, neutrophil, elastase, and eosinophilic cationic protein, suggesting that there is immune activation in the gastrointestinal mucosa of children with CF, which may result from the basic cellular defect. In a separate study that compared duodenal mucosal specimens from 14 pancreatic insufficiency CF patients, 20 healthy controls, and 4 non-CF patients with chronic pancreatitis, an increased mononuclear cell infiltrate expressing the intercellular adhesion molecule (ICAM -1), the IL-2 receptor α, IL-2 and interferon- γ has been observed at the lamina propria level of the duodenal mucosa. These results suggest also that the perturbation of local mucosal immune response may contribute to the overall clinical picture in CF patients (Lee J.M., Leach S.T., Katz T. et al, 2012, Smyth R.L., Croft N.M., O'Hea U. et al., 2000, Raia V., Maiuri L., de Ritis G. et al., 2000).

The incidence of intestinal inflammation in children with CF was assessed by Bruzzese et al. (Bruzzese E., Raia V., Gaudiello G. et al., 2004) on 30 pancreatic insufficiency CF children compared to 30 healthy controls using faecal calprotectin and rectal NO production. The obtained data suggested that intestinal inflammation is a virtually constant feature in patients with CF and faecal calprotectin concentration and rectal NO production are two non-invasive reliable markers of intestinal inflammation.

CF pain management

Current practices in CF related pain

Traditional pharmacotherapy for CF pain includes the use of analgesics: acetaminophen, nonsteroidal anti-inflammatory drugs (NSAID), aspirin, antispasmodic treatments and opioids. Sermet-Gaudelus et al. found that self-medication for the pain is a common practice among CF patients and almost 40% of the children and 50% of the adults used over the counter analgesics. Acetaminophen was one of the most frequently used (59%) followed by other NSAIDs (10%) and Aspirin (5%). Abdominal pain was treated mainly with antispasmodic drugs and by increased dosages of pancreatic enzymes. Up to 60% of CF children and adults reported improvement after treatment, whereas 25% of the children and 10% of the adults presented lack of any aid.

Physiotherapy and massage are also used to treat pain. Homeopathic products and non-pharmacological remedies such as acupuncture, physical activities, rest, heat or cold, distracting activities, yoga, meditation, self-hypnosis or osteopathy, have gained an impressive attention as methods to relieve the pain.

In 2016, McNamara et al. (McNamara C., Johnson M., Read L. et al., 2016) conducted a phase II study on the treatment of anxiety/depression and pain through relaxation yoga for patient with CF. The clinical trial enrolled 20 participants, 12 females and 8 males within the ages of 7 and 20, that participated in six one-on-one sessions over a 10-week period with a certified instructor who designed each yoga program specifically for the patient with CF. The results demonstrated a significant decrease in immediate anxiety and in reported joint pain from before and just after yoga therapy sessions.

Nanotechnology potential in CF related pain management

One of the primary objectives of the clinician during the CF period should include an effective pain relief. Analgesic drug-delivery nanosystems have been applied in pain therapy due to their remarkable properties: enhanced drug delivery profile, increased drug action and bioavailability, targeted/sustained or prolonged drug release profile, stability in biological fluids, nontoxicity, carried drug protection up to the target cell population, and reduced side effects of the incorporated analgesic drugs (Moradkhani M.R., Karimi A., Negahdari B., 2018, Koning G.A., Schiffelers R.M., Storm G., 2002).

Table V gives a brief overview of several current strategies used in pain therapy with the help of the nanotechnology science: liposomes, nanoparticles, nanoplates, nanocapsules, nanofibers, nanotubes, micelles and dendrimers were developed for delivering analgesics, local anaesthetics, NSAIDs or opioids compounds intended for pain therapies (Chakravarthy K.V., Boehm F.J., Christo P.J., 2018).

Table V. Nanomaterials for pain therapy

Formulation type	Materials + drug	Key summary
Liposomes	PEG+ Methylprednisolone hemisuccinate;	80 nm sterically stabilized drug loaded nanoliposomes were used to treat Lewis rats with adjuvant-induced arthritis (Avnir Y., Ulmansky R., Wasserman V. et al., 2008)
	Shea butter lipid nanoparticles+ Nimesulide	90 nm polydisperse loaded lipid nanoparticles presented significant *in vivo* antinociceptive activity compared with free nimesulide (Raffin R.P., Lima A., Lorenzoni R. et al., 2012)
	Liposomes + Celecoxib+ embedded in hyaluronic acid gel	Celecoxib loaded liposomes showed high efficiency in pain control and cartilage protection on in a rabbit knee osteoarthritis model after intra-articular injection (Dong J., Jiang D., Wang Z. et al., 2013)
	Lipids+ Bupivacaine	Single dose of the liposomal formulation reduced the pain through 72 hours and decreased opioid requirements in 184 patients undergoing haemorrhoidectomy (Gorfine S.R., Onel E., Patou G. et al., 2011)
Nanoparticles	Anti-ICAM-1 (Intercellular Adhesion Molecule 1) + Loperamide HCl	Administration of targeted nanoparticles exerted analgesic and anti-inflammatory effects in peripheral painful inflamed tissue on adult male Wistar rats (Hua S. and Cabot P.J., 2013)
	Poly(amidoamine) (PAMAM) dendrimer + esterase activated morphine prodrugs	Esterase-sensitive prodrugs administration enhanced the sustained release of morphine which extended the action of morphine-induced analgesia in an animal pain model from 2 to 6 hours (Ward B.B., Huang B., Desai A. et al., 2013)
	Butylcyanoacrylate nanoparticles+ polysorbate 80+Endomorphin-1	Intravenously administered nanoparticles act as an analgesic agent to target the brain (Liu H., Ni J., Wang R., 2006)
Nanofibers	PLGA nanofibers + Lidocaine	The nanofibers introduced into the epidural space of rats after laminectomy provided a sustained release of lidocaine for more than 2 weeks (Tseng Y.Y., Liao J.Y., Chen W.A. et al., 2014)
	PVA+ Meloxicam	Nanofiber mats loaded with meloxicam as a transdermal analgesic drug delivery system (Ngawhirunpat T., Opanasopit P., Rojanarata T. et al., 2009)

CF treatment perspectives

Recent advances in the treatment of CF have focused on correcting the defective CFTR protein. The new Ivacaftor personalized or genomically guided therapy for CF, a potent CFTR modulator in patients carrying G551D mutation, has shown an improvement of gastrointestinal symptomatology and relieving pain. According to Accurso et al. around 5% of the total CF population with CF are eligible for treatment with ivacaftor worldwide (Accurso F.J., Van Goor F., Zha J. et al., 2014).

According to Fajac and De Boeck review there are currently several new potentiators being evaluated in clinical trials, such as QBW251 from Novartis (phase II), GLPG1837 from Galapagos (phase II) or CTP-656 from Concert Pharmaceuticals (phase I), but their actual relative high costs will limit the access in lower income countries (Fajac I. and De Boeck K., 2017).

Other pharmacological strategies under investigation involve the development of various agents that:

- ✓ increase the amount of CFTR, namely the amplifier PTI-428 from Proteostasis Therapeutics 218;
- ✓ stabilize CFTR at the cell membrane, as the stabilizer Cavosonstat from Nivalis that inhibits the S-nitrosoglutathione reductase; this stabilizer is currently under investigation in Phase II in combination with Orkambi® and Kalydeco®;
- ✓ over-read the premature stop codons: Ataluren agent, tested in a new phase III trial in patients with CF bearing a nonsense mutation that do not receive inhaled Tobramycin 218;
- ✓ by-pass the CFTR channel by identification of alternative chloride channels that should facilitate the development of specific activators (Mall M.A. and Galietta L.J., 2015).
- ✓

Nanotechnology perspectives on CF

A recent review argument the multidisciplinary approach comprised of physicians, psychologists, pharmacists, chemists, material science specialists, engineers, therapists, that is required to address the optimal care of CF patient (King C.S., Brown A.W., Aryal S. et al., 2019).

Based on specific small size, large surface area-to-volume ratios, improved pharmacokinetics and minimal side effects, nano-sized materials have shown potential as carriers for targeted drug delivery in pharmacotherapy (Yhee J.Y., Im J., Nho R.S., 2016). An important advantage of drug delivery-based nanotechnology is the improved diffusion and degradation characteristics of the encapsulated nanomaterial, allowing the drug to be protected during its transit to the target, while maintaining the biological and chemicals properties and be released at an appropriate effective rate. Therefore, the main question among scientists and clinicians is if nanotechnology will bring new hope for CF patients and its related pain.

In the specific area of CF treatment, nanomedicine occupies an important position in the identification, design and development of new molecular targets. Gene therapy and nano-selective and sustained drug delivery systems can improve drug therapies systemically and locally (Ferreira A.J., Cemlyn-Jones J., Robalo Cordeiro C., 2013).

The success of CF inhalation therapies, according to d'Angelo et al. review is related to the penetration of anatomical/biological barriers imposed by CF lung such as: drug deposition and lungs distribution, CF mucus, cellular barriers (bacteria biofilm and airways epithelial cells)

and phagocytosis. In the Era of designing new inhalable drug delivery strategies for CF patients, researchers must consider several mandatory parameters, namely engineered size, density and shape for lung deposition, drug incorporation, delivery and macrophage clearance escape.

Theoretically, nanoparticles hold great promises to improve drug delivery limitations in the lungs, since their surface, structure and composition can be tailored to reach the right target at the right time by providing accurate and controlled drug delivery (Da Silva A.L., Santos R.S., Xisto D.G. et al., 2013). Still, the success of nanotherapy depends on several factors, such as nanoparticle characteristics and toxicity, route of administration and physiological aspects of the lung in the presence of respiratory disease (Weers J., 2015).

Table VI. Presents the drug-loaded delivery engineered systems under development in the last decade for overcoming "biobarriers" enforced by CF lung.

Formulation type	Materials + drug	Key summary
Liposome-encapsulated drugs (aqueous dispersions)	DSPC/DMPG + Tobramycin;	Significant increase of drug residence time within rat lungs infected with *Pseudomonas aeruginosa.* (Omri A., Beaulac C., Bouhajib M. et al., 1994)
	DPPC/DMPG + Tobramycin;	Efficient bactericidal activity on chronic pulmonary infection caused by mucoid *Pseudomonas aeruginosa.* *(*Beaulac C., Clément-Major S., Hawari J. et al., 1996*)*
	DPPC/DMPG + Gentamycin+ Ga (III) nitrate	Optimized and efficient co-delivery reduced Ga toxicity, antimicrobial activity and complete eradication of antibiotic-resistant clinical isolates of *Pseudomonas aeruginosa.* (Halwani M., Yebio B., Suntres Z.E. et al., 2008)
	DSPC/Chol+ Tobramycin+ bismuth-ethanedithiol;	Non-toxic and stable formulations that penetrate sputum, reduce quorum sensing molecule and virulence factors production and inhibit the growth of biofilm-forming clinical strains of *Pseudomonas aeruginosa*. (Halwani M., Hebert S., Suntres Z.E. et al., 2009, Alipour M., Suntres Z.E., Lafrenie R.M. et al., 2010)
	DPPC/Chol+ Amikacin	Sustained and targeted release, biofilm and infected *Pseudomonas aeruginosa* mucus penetration, superior efficacy on both *in vivo* and phase II clinical trials of inhaled liposomal Amikacin. (Meers P., Neville M., Malinin V. et al., 2008, Okusanya O.O., Bhavnani S.M., Hammel J. et al., 2009, Okusanya O.O., Bhavnani S.M., Hammel J.P. et al., 2014)
	DC-Chol/DOPE+ CFTR cDNA	*In vivo* administration of CFTR cDNA transfection of cationic liposomes corrected the ion transport defect in the airways of the mouse model of CF created by insertional mutagenesis. (Middleton P.G., Caplen N.J., Gao X. et al., 1994)
	DOTAP+ pCMV-CFTR expression vector	Pilot randomised, double-blinded study of cationic liposome complex single dose-administration to the nasal epithelium of 8 CF patients; Significant gene transfection, no adverse effect and no evidence of inflammation. (Porteous D.J., Dorin J.R., McLachlan G. et al., 1997)
	DODAG/DOPE/PEG4600-Chol+ Plasmid DNA encoding luciferase	New cationic liposome systems mediated efficient transfection of healthy murine lung tissue *in vivo* without significant inflammation; the obtained carriers could form the basis for nucleic acid therapeutic strategies for CF gene therapy. (Aissaoui A., Chami M., Hussein M. et al., 2011)

Formulation type	Materials + drug	Key summary
	GL67A/pGM169 (GL67/DOPE/DMPE-PEG5000+ Plasmid DNA)	Novel cationic lipid-PEG formulation (GL67A) associated with plasmid DNA (pGM169); reduced inflammation and sustained pulmonary gene expression after *in vivo* aerosol delivery; single-dose phase I and IIa safety and gene expression study of pGM169 or GL67A administered to the nose and lungs on CF patients (ClinicalTrials.gov number: NCT00789867); randomised, double-blind, placebo-controlled, phase IIb clinical trial (ClinicalTrials.gov number NCT01621867). (Eastman S.J., Lukason M.J., Tousignant J.D. et al., 1997, Hyde S.C., Pringle I.A., Abdullah S. et al., 2008, Alton E.W., Boyd A.C., Cheng S.H. et al., 2013, Alton E.W., Boyd A.C., Porteous D.J. et al., 2015, Alton E.W., Armstrong D.K., Ashby D. et al., 2015)
Solid lipid particles (saline solutions or dry powders)	Stearic acid/PC+ Myriocin	Good uptake and delivery of myriocin-loaded nanocarrier, significant reduction of lung infection and reduced inflammation on CF mice. (Caretti A., Bragonzi A., Facchini M. et al., 2014)
	Chol/lecithin+ Tobramycin	Formulation and *in vitro* evaluation: large surface area, low bulk density, good flowability, size and shape suitable for use in carrier-free dry powder inhalers. Pilot study on CF patients: high lung deposition and reduced systemic bioavailability determined by a pharmacoscintigraphic method. (Pilcer G., Sebti T., Amighi K., 2006, Pilcer G., Goole J., Van Gansbeke B. et al., 2008)
	DSPC+ Tobramycin	PulmoSphere™ porous particles are obtained by spray-drying method in 4 steps: emulsion-based feedstock preparation by high-pressure homogenization of perfluorooctyl bromide, DSPC, tobramycin and calcium chloride in water; atomization with a twin fluid nozzle into a hot air stream; drying of the emulsion droplets and collection of resulting dry powder comprising porous spheroidal particles. (Geller D.E., Weers J., Heuerding S., 2011)
	DSPC+ Ciprofloxacin	Phase I, randomized, single-blind, placebo-controlled, dose-escalation study in patients with CF; the formulation was well tolerated, has targeted and sustained release, minimal systemic exposure and no apparent accumulation of ciprofloxacin over 7-day treatment period. (Stass H., Weimann B., Nagelschmitz J. et al., 2013)
Polymeric nanoparticles (aqueous dispersion or dry powders)	PS-PEG	200 and 500 nm nanoparticles; the dense surface coating of the 200 nm non-mucoadhesive nanoparticles helped the penetration of CF sputum. (Suk J.S., Lai S.K., Wang Y.-Y. et al., 2009)
	PSA-PEG	Biodegradable nanoparticles with an average hydrodynamic diameter of 173 nm, prepared using a conventional solvent diffusion method rapidly penetrated sputum expectorated from the lungs of patients with CF due to a dense surface coating of low PEG. (Tang B.C., Dawson M., Lai S.K., et al., 2009)
	PVA-Alg/PLGA+Tobramycin and CS-Alg/PLGA+Tobramycin	2 types of PLGA nanoparticles were prepared by a modified emulsion/solvent diffusion technique for the production of dry powders for antibiotic inhalation; the formulations displayed good *in vitro* antimicrobial activity against *Pseudomonas aeruginosa* planktonic cells, and differentiated *in vivo* biodistribution and deposition pattern, dependent on the nanoparticle composition. (Ungaro F., d'Angelo I., Coletta C. et al., 2012)
	PLGA+ Pirfenidone	Intratracheal administration of biodegradable pirfenidone nanoparticles in bleomycin-induced pulmonary fibrosis in mice, determined sustained lung delivery and anti-fibrotic enhanced efficacy.

Formulation type	Materials + drug	Key summary
		(Trivedi R., Redente E.F., Thakur A., et al., **2012**)
	PLGA-PEG+ PS-341	Mono-dispersed and spherical in shape loaded nanoparticles were synthesized using non-polar core of oil-in-water microemulsion technique with PEGylated phospholipid DSPE-mPEG^{2000} as the emulsifier; the drug delivery system provided controlled and sustained PS-341 delivery for selective inhibition of proteostasis. (Vij N., Min T., Marasigan R. et al., 2010)
	Transferrin-gelatin/chloroquine/calcium + DNA	A plasmid DNA encoding CFTR and gelatin nanoparticle coacervate transfected in the presence of calcium and transferrin, resulted in CFTR expression in over 50% of the cells; effective transport activity. (Truong-Le V.L., Walsh S.M., Schweibert E. et al., 1999)
	CS/FAP-B+ DNA encoding luciferase	250 nm nanoparticles nebulized to mice lungs determined 16-fold increase of gene expression compared with CS-DNA NPs without FAP-B receptors. (Mohammadi Z., Dorkoosh F.A., Hosseinkhani S. et al., 2011)
	PEI+ miRNA and CS+ miRNA	Non-toxic miRNA-PEI nanoparticles (300 nm) facilitated greater uptake into CFBE41o- cells, and efficient delivery than miRNA-CS nanoparticles (115 nm). (McKiernan P.J., Cunningham O., Greene C.M. et al., 2013)
	PEI+HA+ plasmid DNA encoding luciferase	Inhalable dry microparticle form of mannitol, encapsulating ternary complex composed of plasmid DNA, disulfide-crosslinked low molecular weight linear PEI and HA as a gene carrier, improved CF artificial sputum penetration and transport but with gene transfer agent's aggregation. (Ibrahim B.M., Park S., Han B. et al., 2011)
	Poly-l-lysine-PEG+DNA encoding CFTR	Rod-shaped DNA nanoparticles with different PEG molecular weights (2, 5 or 10 kDa) provided partial protection against DNase I digestion and exhibited the highest gene transfer to lung airways following inhalation in BALB/c mice but were immobilized in freshly expectorated human CF sputum due to inadequate PEG surface coverage. (Boylan N.J., Suk J.S., Lai S.K. et al., 2012)
	PEI-PEG+DNA encoding CFTR	Synthetic gene carrier platform composed of PEG (5 kDa) and branched PEI (25 kDa) penetrated human CF mucus due to highly dense PEG coating, achieved uniform airway distribution and prolonged lung retention, and enhanced gene transfer to mouse lung with no inflammatory responses. (Suk J.S., Kim A.J., Trehan K. et al., 2014)

One promising and innovative strategy is to develop a treatment based on gene therapy, where both viral and non-viral delivery systems were investigated, and nano-selective and sustained delivery of proteasome inhibitor drugs, one of the future trends in nanomedicine. Particularly, in the CF treatment region, nanoparticulate gene delivery vectors are considered as a promising option due to their non-immunogenicity and feasibility for repetitive doses, but still with low efficiency (Roy I. and Vij N., 2010).

The ideal properties of nanomaterials used in gene therapy according to a recent review of Wong et al. (Wong J.K.L., Mohseni R., Hamidieh A.A. et al., 2017) are nontoxicity, biodegradability, biocompatibility, stability in biological fluids, non-immunogenicity, personalized size for cellular barriers penetration (1-100 nm), nuclease breakdown protection, high transfection efficiency, induced sustained gene expression and cost-efficient. Although

different multifunctional gene vectors were developed in the last years, there are several practical challenges to successful gene delivery, such as nucleic acids delivery into cells, possibility to conjugate molecules at intracellular levels, safety and efficacy concerns. Also, the penetration of gene carriers to the highly adhesive and hyperviscoelastic mucus gel in the airways of CF patients encourages further development.

Another therapeutic CF challenge includes selective and sustained delivery of small molecules, namely the use of the nano-based selective proteasome inhibitor drug, bortezomib with controlled and sustained drug delivery at lower doses (Vij N., Fang S., Zeitlin P.L., 2006) but with potential risks. In this context, the design of novel nano-based biodegradable therapeutic vehicles for CF pathophysiology without any side effects remains the main challenges for the coming years.

The complex CF management should include a combination of pharmacologic treatment based on new drug developments including CFTR protein modulators, personalised biomarkers, gene therapy and nanoparticulate drug delivery along with psychological interventions to create hope for treatments of the CFTR defect.

I.1.3.4. Discussions

Pain represents a subjective symptom and consequently cannot be measured by objective methods. The clinician must depend on the patient to supply key information on the localization, quality, and severity of the pain. Pain scores have gained acceptance as the most accurate and reliable measure of assessing a patient's pain and response to pain treatment There are multiple possibilities to assess and monitor pain, of which the most popular is the Visual Analog Scale (VAS), followed by Verbal Rating Scale (VRS) and the Numerical Rating Scale (NRS), evaluated as unidimensional procedures. Many studies published over the past decade describe a high prevalence of pain in patients with CF rating it as being severe, but its impact on the clinical outcomes and quality of life of these people is often underestimated. Being considered as a multidimensional phenomenon, no single pain assessment tool is available to measure all aspects of pain in CF.

Pain control in CF patients is an important link in the complex therapy of this multisystemic disease. The proper treatment of pain is still a major medical challenge. In addition to pharmacological therapy for CF pain (Acetaminophen, NSAID), Aspirin, antispasmodic treatments and opioids), physiotherapy and massage are also used to treat pain. Different approaches using various nano-formulated materials have been studied in the last decade (Beiranvand S. and Sorori M.M., 2019).

Although NSAIDs are a relatively good choice for pain management for CF patients during remission of chronic lung infection, their usage during the exacerbation period with intravenous aminoglycoside treatment can increase the nephrotoxicity risk. However, long-term use of NSAIDs may be unacceptable (Alexa T., Marza A., Voloseniuc T. et al., 2015) due to high risk of gastrointestinal hemorrhage. Opioids are also not ideal alternative for refractory pain treatment in CF population for many reasons, as they can cause constipation and thus increase the risk of distal intestinal obstruction syndrome, and can lead to cough suppression, which could reduce airway clearance results (Havermans T., Colpaert K., De Boeck K. et al., 2013).

Nanotechnology, one of the most innovative tools of the century, will transform the biomedical field by improving prevention, diagnosis and diseases therapy. Indirectly, the nano-personalized strategy facilitates a better quality of life and life expectancy for patients with CF.

Nevertheless, nanotechnology received remarkable attention because of its future prospective that can literally revolutionize medicine. Nanomedicine is referred to as the field of medicine that deals with the application of nanotechnology to address medical problems. Despite the nanotechnology impact and progress, discussion of the potential effects on humans is just initiated. Since over the next couple of years it is widely predicted that nanotechnology will be applied in medical sciences, is mandatory to mention an important challenge for researchers, namely the approval from Food and Drug Administration (FDA) for their use into the human body.

I.1.3.5. Conclusions

Maintenance of adequate treatment status is essential to CF care. In this sense, an attractive perspective on pain management that must be considered is the efficient CF treatment, since pain is a potential complication of the disease. Currently, CF treatment that includes antibiotics, anti-inflammatory drugs, bronchodilators, mucolytics and osmotic agents aims to attenuate disease progression and to delay the beginning of irreversible lung damage, but unfortunately has complications on daily regimens. Nanotechnology, one of the most innovative tools of the century, will transform the biomedical field by improving prevention, diagnosis and diseases therapy. Indirectly, the nano-personalized strategy facilitates a better quality of life and life expectancy for patients with CF.

I.1.4. Cystic Fibrosis-related liver disease

I.1.4.1. Introduction

Since the advances in specialized patient care have led to increasing the life expectancy of patients with CF, CFLD has increasingly been reported. CFLD is a chronic inflammatory sclerosing cholangiopathy commonly presenting before the age of 20 years and may evolve into advanced liver disease, biliary cirrhosis, portal hypertension, and liver decompensation (Fiorotto R. and Strazzabosco M., 2016). The CFLD is the third cause of mortality after lung disease and post-transplant complications (Parisi G.F., Di Dio G., Franzonello C., 2013). Without specific diagnosis markers, the true prevalence of CFLD is unknown. The literature data show that children's CFLD prevalence varies between 27 and 35%, decreasing after 18 years old (Debray D., Deirdre K., Roderick H. et al., 2011). Because CFLD might be the first occurrence of CF in children, the complete diagnostic evaluation of the liver disease should include a sweat test (Lindblad A., Glaumann H., Strandvik B., 1999).

In children, liver steatosis is a common liver lesion associated with CF, which has been frequently associated with selective nutritional deficits, especially in essential fatty acids. Still, another cause of liver steatosis can be chronic infections with hepatitis B virus – HBV and hepatitis C virus – HCV. The increased drug resistance and constant viral replication have been the trigger for important studies regarding nanotechnology in antiviral therapies.

Part of the preoccupations related to CFLD was synthesized in the following articles:

1. Cojocaru FD, Botezat D, Gardikiotis I, Uritu CM, Dodi G, **Trandafir L**, Rezus C, Rezus E, Tamba BI, Mihai CT Nanomaterials Designed for Antiviral Drug Delivery Transport across Biological Barriers. *Pharmaceutics*. 2020; 12(2): 171. (IF = 4.421)

2.**Trandafir LM**, Straticiuc Ciongradi I, Baciu G, Păduraru ADT Liver disease in children with cystic fibrosis. *Roumanian Journal of Pediatrics*. 2014; LXIII (1): 22-29.

Using the theoretical data mentioned above, the two articles aimed to:

- ***Cystic Fibrosis-Related Liver Disease***

  The objective of this study was to highlight the pathophysiology, clinical and paraclinical features, and the role of the interprofessional team in managing patients with CFLD.

- ***Chronic Infections with Hepatitis B and C Viruses***

  The scope of this review is to highlight the drug delivery nanosystems incorporating the major antiviral classes and their transport across specific barriers at cellular and intracellular level.

I.1.4.2. Material and Methods

- ***Cystic* Fibrosis-Related Liver Disease**

  In this article we researched current literature data on the pathophysiology, diagnostic and the complex algorithm of treatment and monitoring of CFLD.

- ***Chronic Infections with Hepatitis B and C Viruses***

  The search strategy employed in this review includes internationally accepted databases, using specific keywords of both whole plant products and plant extracts, pain, and analgesic and antinociceptive effects. Essential aspects on nanomedicines currently approved or undergoing investigations for the treatment of viral infections are presented in this review.

I.1.4.3. Results

- ***Cystic Fibrosis-related liver disease***

  According to literature data CFLD developed especially in the first ten years of life, and some patients with cirrhosis requiring liver transplantation since childhood (Lindblad A., Glaumann H., Strandvik B., 1999, Lamireau T., Monnereau S., Martin S. et al., 2004).

  Physiopathology of the CF-associated liver disease

  Many hepatobiliary features that characterize CFTL are represented by neonatal cholestasis, sclerosing cholangitis, microlithiasis, gallstones, focal or multilobular biliary cirrhosis, portal hypertension are the consequence of dysfunction of CFTR chlorine channel, post-drug hepatotoxicity or extrahepatic causes (heart failure, common bile duct stenosis, chronic infection, and malnutrition). It has been shown that liver inflammation in susceptible

individuals can be caused by activation of innate immune responses to bacterial products from gut microbiota. Once in the liver, gut-derived endotoxins or pathogen-associated molecular pattern is normally cleared by the hepatocytes that take up endotoxins and discharge them partly unmodified into the bile via vesicular transport (Szabo G., Dolganiuc A., Mandrekar P., 2006). However, the pathophysiology of the gut microbiota, implicated in CFTL, is not well understood.

Clinical manifestations of CFLD

CFLD features ranging from mild asymptomatic elevation in liver function tests to liver cirrhosis (Table VII). The most common presentation of CFLD is an incidental finding of hepatomegaly with or without splenomegaly, jaundice, or abnormal liver function tests. Other manifestations of CFLD may include micro-gallbladder, cholelithiasis, and sclerosing cholangitis (Debray D., Deirdre K., Roderick H. et al., 2011).

The clinical picture of progressive CFLD include jaundice, coagulopathy, ascites, and portal hypertension signs. Biochemical indicators of liver failure such as increased level of bilirubin, lower albumin level, and prolonged prothrombin time appear late in advanced CFLD (Kobelska-Dubiel N., Klincewicz B., Cichy W., 2014). Liver steatosis is the most frequent liver injury described in up to 67% of CF patients, especially for those with pancreatic insufficiency and severe malnutrition (Colombo C., Battezzati P.M., Crosignani A. et al., 2002).

Table VII. Clinical manifestations of CF liver disease

Clinical manifestation	**Estimated frequency, %**
Asymptomatic elevation of liver enzymes	Common
Hepatic steatosis	25-60
Focal biliary cirrhosis	20-30
Multilobular biliary cirrhosis	10
Neonatal cholestasis	<10
Cholelithiasis and cholecystitis	15
Micro-gallbladder	30
Portal hypertension	2-5
Sclerosing cholangitis	Often silent

Paraclinical investigations in CFLD

Stages of the liver disease in CF:

- Stage I – biochemical changes – change in the liver enzymes
- Stage II – liver steatosis revealed by the ultrasound exam
- Stage III – decompensated liver disease – portal hypertension, hypoalbuminemia, ascites, coagulation disorders.

Diagnostic criteria of CFLD

According to the current diagnosis and management CF guidelines, CFLD diagnosis involve at least two of the following 4 diagnosis criteria (Debray D., Deirdre K., Roderick H. et al., 2011):

- clinical manifestations: hepatomegaly associated or not with splenomegaly
- dysfunctions of liver function test: elevated transaminase levels and GGT value (at least three consecutive determinations during twelve months with excluding other causes of hepatopathy
- ultrasound changes of the liver, signs of portal hypertension or anomalies of the bile ducts

- anatomo-pathologic lessions in liver biopsy.

Management of CFLD

CFLD require annual follow-up by multidisciplinary team including primary care providers, gastroenterologists, hepatologists, pulmonologists, nutritionists, radiologists and surgeons (Fig.10). The follow-up would necessitate screening for the development of portal hypertension and other complications via annual physical examinations, liver function tests, and abdominal ultrasound +/- CT or MRI (Kamal N., Surana P., Koh C., 2018). There are no existing evidence-based guidelines or specific recommendations for the prevention or management of CFLD. Therefore, once CFLD is diagnosed, therapy is directed towards lowering the impact of complications (Palaniappan S.K., Than N.N., Thein A.W., 2017).

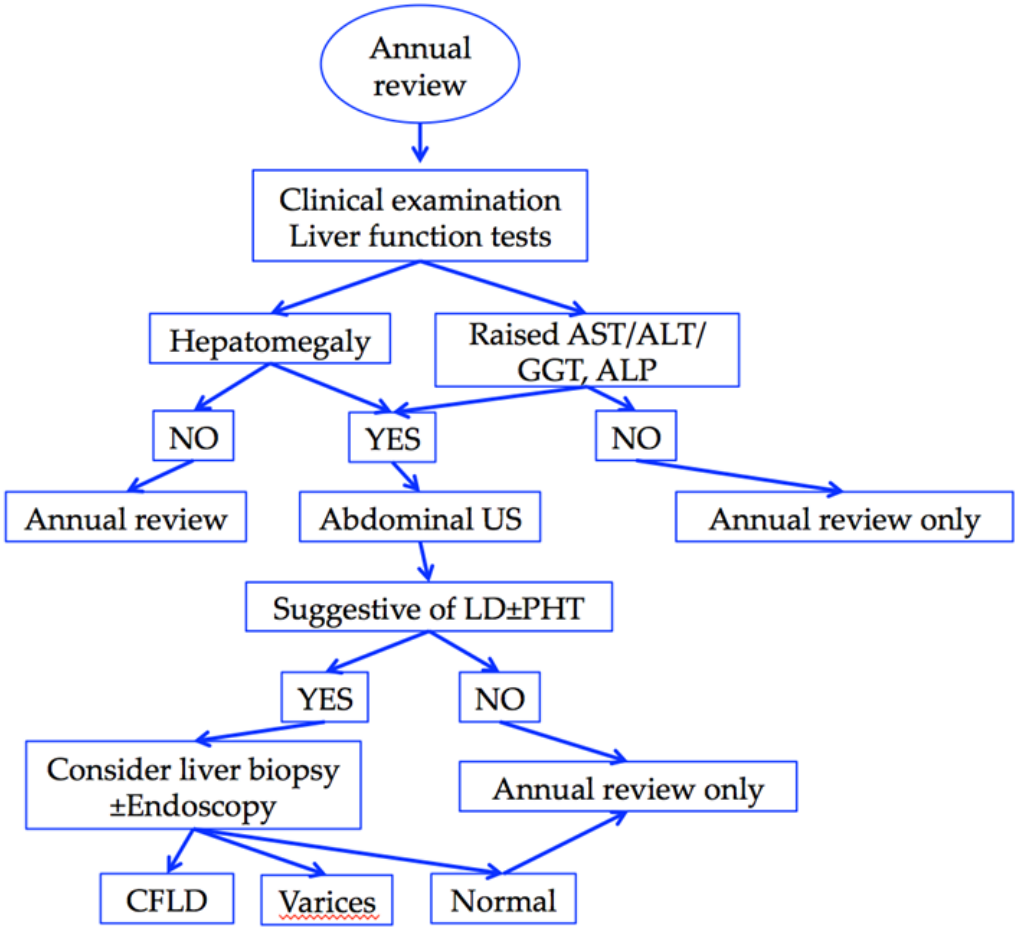

Figure 10. *Flow chart for the investigation and management of CFLD (Debray D., Deirdre K., Roderick H., et al., 2011)*

Treatment for CF - associated liver disease

Nutritional recommendations

CFLD associated malnutrition due to lipid malabsorption and hypoproteinemia. Nutritional recommendations in patients with CFLD include increased the caloric intake to 150%, the lipid intake by 40-50% to the daily caloric intake and supplementing the protein intake to ensure 3g/kg/day in patients with no signs of liver failure. Is recommended optimum dose of pancreatic enzymes to ensure optimum absorption on long-chain triglycerides and

essential fatty acids. Administrating liposoluble vitamins is essential in these patients: vitamin A (5000-15000 UI/day per os), vitamin E (alpha tocopherol 100-500 mg/day), vitamin D (50 ng/kg up to1 μg) and vitamin K (inconsistent) 1-10 mg/day) (Debray D., Deirdre K., Roderick H. et al., 2011).

Medical treatment

A major challenge for the clinical management of these patients is the early diagnosis of the liver disease. There is no clear correlation between specific mutations and the risk of liver complications, but among the recognized risk factors, there is the presence of more severe mutations (ie, classes I–III) and the association with pancreatic insufficiency, followed by male sex and meconium ileum at birth, even though the latter is controversial (Lamireau T., Monnereau S., Martin S. et al., 2004). Patients with CFLD are routinely treated with Ursodeoxycholic acid (UDCA) (Cheng K., Ashby D., Smyth R.L., 2017).

- ***Chronic Infections with Hepatitis B and C Viruses***

Liver disease is an early complication in children with CF. Steatosis is a common liver lesion associated with CF that does not seem causally related to CF's defective secretion. CF steatosis has been considered a benign condition without demonstrating any relation to the subsequent development of cirrhosis. Despite this, the available data regarding the role of non-alcoholic steatohepatitis as a cause for cirrhosis in adults may lead to reconsidering this issue in patients with CF.

Changes in intrahepatic bile ducts like sclerosing cholangitis have been described both in children and adults with CFLD. These lesions are the consequence of the inflammatory process in the bile ducts, of the accumulation of proteins and mucus, and the narrowing of the intrahepatic bile ducts due to fibrosis. Current data show that CFLD is the consequence of bile duct obstruction due to the CFTR cholangiocyte defect, the retention of toxic substances leading to peribiliary fibrosis, and the increase in the "sludge" amount in the bile duct, which generates microlithiasis.

Changes in the tests evaluating the liver function are inconsistent and are not correlated with the liver lesions' severity. 20-30% of the children with CF have increased liver enzymes during the disease's evolution. Transitory increase in the liver enzymes is correlated to hypoxemia, infection, and antibiotic treatment during pulmonary exacerbations.

Other causes for hepatic cytolysis must be excluded: viral infections (A, B, C, D hepatitis virus, cytomegalovirus, Epstein Barr virus), alpha1 anti-tripsine deficit, the celiac disease, autoimmune hepatitis, Wilson disease, nutritional causes (malnutrition, obesity, diabetes mellitus). It is mandatory to do periodical evaluations of the liver enzymes that originated in the biliary epithelium (gamma-glutamyl transferase, 5-nucleotidase alkaline phosphatase), whose change is much more specific to CFLD in comparison to ALT and AST. Other important investigations for hepatic steatosis include liver elastography (FibroScan), Hepatobiliary scintigraphy, CT, MRI, liver biopsy (Debray D., Deirdre K., Roderick H. et al., 2011).

As mentioned before, liver steatosis can be caused by chronic infections with HBV and HCV. The relationship between liver steatosis and chronic hepatitis C – CHC is intensely studied, steatosis being a common finding in 50% of adults with CHC. There have been identified 6 HCV genotypes. Genotype 3 directly affects the infected hepatocytes. This cytopathic effect leads to viral-induced steatosis, being identified in 74% of patients infected

with this type of HCV. In this case, the first step in the treatment plan of steatosis is antiviral therapy for achieving a sustained viral response. Unfortunately, due to its prediction of progression to fibrosis, steatosis is associated with a lower response rate to antiviral therapy. Regarding chronic hepatitis B – CHB, there are fewer studies about its correlation with steatosis, the frequency of hepatic steatosis in adults with CHB being estimated to vary in a large interval: 22% to 76%. The existing data indicate that steatosis in HBV infection is highly correlated with metabolic factors than viral determinants.

Regarding pediatric patients, they are ideal models to study hepatic steatosis as they are not often affected by other potential confounding risk factors responsible for its development, such as alcohol intake, metabolic syndrome, dyslipidemia, insulin resistance, or diabetes (Peng D., Han Y., Ding H. et al., 2008).

Social Impact and Economic Burden of Viral Infectious Diseases

The current configuration and variety of infectious diseases closely followed the combined evolutions in demography, environment, technology, social change and behaviours. Medicine itself has created new opportunities for bacteria and viruses through blood transfusions, organ transplants, or the use of hypodermic syringes, thus contributing to the induction of iatrogenic effects in some treatments for infections such as hepatitis B, C, HIV and others.

Also, the excessive use of antibiotics helped the expansion of the bacterial spectrum. In recent decades, old concerns have been reactivated at both the official and the general public levels regarding infectious diseases as a threat to public health. The factors that influence the emergence and resurgence of viral infections are numerous and complex and include environmental, sociological and economic changes.

Viruses are sub-microscopic intracellular parasitic particles of genetic material in a protein coat, totally dependent by host for cell replication, showing both living and non-living characteristics. The living characteristics of the viruses are represented by the high rate of multiplication (only in living host cells) and the ability to mutate. The non-living characteristics for viruses consist of acellularity (lack of cytoplasm and organelles), the replication only uses the host cell's metabolic machinery, and the composition with DNA or RNA (Van Regenmortel M.H.V., 2011).

According to Global Hepatitis database (WHO, 2019) viral hepatitis caused 1.34 million deaths in 2015, comparable to deaths caused by tuberculosis and higher than those caused by HIV. The difficulty in accurately quantifying and explaining the morbidity and mortality related to viral hepatitis stems from the fact that hepatitis deaths are caused by five distinct viruses (hepatitis A–E) with different routes of transmission, or from the fact that death occurs decades after infection, and that when people die with hepatitis-related liver cancer and cirrhosis, these deaths are not always linked to the underlying infection.

Every year, about 2 million new HBV infections in children younger than 5 years are reported, mainly through mother-to-child transmission, and horizontal transmission in early life. A major progress has been made concerning the elimination of HBV through universal infant hepatitis B immunization, that being very effective in reducing new infections in pediatric patients.

In comparison with adult patients, an insufficient consideration has been given to experiment treatment plans for chronic HBV infection among children and adolescents. This

lack of attention can be explained by the fact that most pediatric patients are in the immunotolerant phase of infection, in which a treatment is not necessary and due to the lack of evidence from low-income settings with high HBV prevalence to inform child-specific management practices. Most of them are in the high-replication, low-inflammation phase of infection, with normal or only slightly raised aminotransferases (Indolfi G., Easterbrook P., Dusheiko G. et al., 2019). Cirrhosis and hepatocellular carcinoma are rare, but unfourtunately children with a chronic hepatic infection can be affected by other more or less serious disease such as NAFLD.

The prevalence of HCV infection in children and adolescents is different worldwide from one region or continent to another; in certain developing countries from Europe has been reported to vary from 1.8% to 5.8%, while in United States is around 0.05%-0.36%. Despite, these reports do not provide a true estimate of this prevalence, as current finding practices consider only a small fraction of children who are expected to be infected with the virus. Moreover, this prevalence is difficult to be estimated accurately, because in time have been identified six distinct HCV genotypes. In the last decades, in United States, HCV infection in children has been mainly caused by transfusions procedures, but this fact has been stopped from some years (CDC, 2009). Nowadays, representing about 60% of cases, the most common way of catching HCV infection is mother to infant, the transmission mechanism being not clear (Dal Molin G., D'Agaro P., Ansaldi F., et al., 2002). Considering other viral diseases, it is very important to mention that a considerable determinant of the risk of perinatal transmission is the maternal HIV co-infection (Benova L., Mohamoud Y.A., Calvert C. et al., 2014).

Anti-Hepatitis Virus Agents

Interferons – IFNs are potent cytokines that possess antiviral, immunomodulatory and antiproliferative activities. These proteins are synthesized by host cells in response to various inducers and, in turn, cause biochemical changes leading to an antiviral state in cells. Currently, interferons α, β, and γ have antiviral activity, the first two being produced by nearly all cells as response to viral infections, while the third is restricted to T-lymphocytes and NK cells. IFN-induced proteins include 2′-5′-oligoadenylate [2-5(A)] synthetase and a protein kinase, either of which can inhibit protein synthesis in the presence of double-stranded RNA. The 2-5(A) synthetase produces adenylate oligomers that activate a latent cellular endoribonuclease (RNase L) to cleave both cellular and viral single-stranded RNAs. The protein kinase selectively phosphorylates and inactivates a protein involved in protein synthesis, eukaryotic initiation factor 2 (eIF-2). IFN-induced protein kinase also may be an important effector of apoptosis. In addition, IFN induces a phosphodiesterase that cleaves a portion of transfer RNA and thus prevents peptide elongation. A given virus may be inhibited at several steps, and the principal inhibitory effect differs among virus families. Certain viruses are able to counter IFN effects by blocking production or activity of selected IFN-inducible proteins (Biron C.A, 2001, Dalod M., Salazar-Mather T.P., Malmgaard L. et al., 2002).

Ribavirin is a purine nucleoside analogue with a modified base and D-ribose sugar. Ribavirin inhibits the replication of a wide range of RNA and DNA viruses, including orthomyxo-, paramyxo-, arena-, bunya-, and flaviviruses in vitro. Ribavirin is reported to have several mechanism of actions that lead to inhibition of viral RNA and protein synthesis as: inhibition of viral mRNA polymerase by ribavirin triphosphate (predominant metabolite resulting from activity of adenosine kinase) by binding to the nucleotide binding site of the

enzyme, preventing attachment of the correct nucleotides; inhibitory action on viral mRNA guanylyltransferase and mRNA 2′-O-methyltransferase of dengue virus; inhibition of host inosine monophosphate dehydrogenase and subsequent depletion of intracellular guanosine triphosphate – GTP pool, proposed to be another mechanism of action of Ribavirin (Te H.S., Randall G., Jensen D.M., 2007).

Adefovir dipivoxil (9- [2 [bis[(pivaloyloxy)methoxy]phosphinyl]methoxyl]ethyl] adenine, bis-POM PMEA) is a diester prodrug of adefovir, an acyclic phosphonate nucleotide analogue of adenosine monophosphate. is inhibitory in vitro against a range of DNA and RNA viruses, but its clinical use is limited to HBV infections. Adefovir dipivoxil is intracellulary deesterified to adefovir, which is converted by cellular enzymes to diphosphate, and acts as a competitive inhibitor of viral DNA polymerases and reverse transcriptases with respect to deoxyadenosine triphosphate and also serves as a chain terminator of viral DNA synthesis (Krečmerová M., 2017, De Clercq E., 2003).

Entecavir is a guanosine nucleoside analogue, with selective activity against HBV polymerase, requiring intracellular phosphorylation. Entecavir triphosphate competes with endogenous deoxyguanosine triphosphate and inhibits all three activities of the HBV polymerase (reverse transcriptase): base priming, reverse transcription of the negative strand from the pregenomic messenger RNA, synthesis of the positive strand of HBV DNA (Lee H.W., Park J.Y., Ahn S.H., 2016).

Telbivudine is a synthetic thymidine nucleoside analogue with activity against HBV DNA polymerase. Telbivudine is phosphorylated by cellular kinases to the active triphosphate form, telbivudine 5′- triphosphate that inhibits HBV DNA polymerase (reverse transcriptase) by competing with the natural substrate, thymidine 5′-triphosphate. Incorporation of telbivudine 5′-triphosphate into viral DNA causes chain termination (Matthews S., 2007, Amarapurkar D.N., 2007).

Nanotechnology: how does it face the antiviral therapy?

Up to fare we have concluded that current antiviral therapy did not reach yet the ideal shape and efficiency and also that the complex biological barriers are major obstacles, but can we critically say that nanotechnology could be the identified solution?

Nanotechnology search engine generates more than 114,000 items on specialized platforms (Science direct for example) that represents potential and challenges in different fields from biosensors and industry related applications up to nanomedicine and biomaterials. When the search keywords are “nanotechnology as antiviral therapy” the same engine turns out only 1,404 results starting from 1997. It is a given fact that nanotechnology is defined as the application of materials in the nanometer scale.

According to the literature data results, nanomaterials designed with different shapes and morphologies display numerous advantages for use in antiviral therapy, namely: nanometric size that permits drug delivery through impermeable barriers (Mahajan S., Law W., Aalinkeel R. et al., 2012), large surface area to volume ratios for large drug payloads incorporation (McNeil S.E., 2011) and improved efficacy, surface modification and/or backbone functionalization versatility that facilitates cellular membranes passage (Goldberg M., Langer R., Jia X., 2007), virucidal activity against a series of viruses (HIV, HSV, HBV, etc.) due to biomimetic properties (Gagliardi M., 2017), increased specificity, improved antiviral delivery and controlled drug release to the target (Muthu M.S. and Singh S., 2009)

through engineered moieties, decrease the emergence of drug resistance, personalized therapy possibility, protection of the drugs and low adverse drug side effects mainly due to the composition. The mechanism of nanomaterials mediated drug delivery is determined by the chemistry, the architecture and the specific properties of each nano-system (as presented in the schematic representation from Fig. 11).

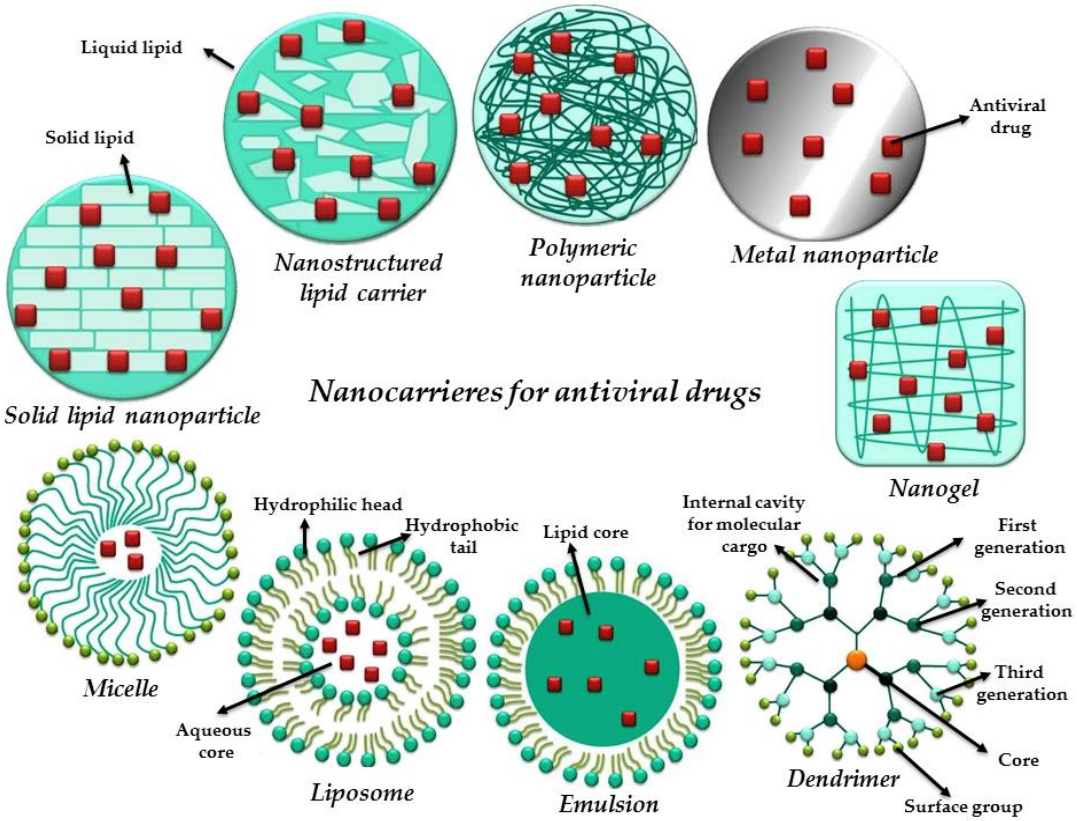

Figure 11. *Nanocarriers developed for antiviral therapy*

The design of new drug delivery systems for the antiviral therapy is focused on manipulating these features that are relevant in viral diseases where high drug doses are compulsory, implies high costs and the patient is depended on the administration protocol.
Lembo and Cavalli (Lembo D. and Cavalli R., 2010) present the status up to 2010 in the nanoparticulate delivery systems in antiviral therapy area, highlighting their perspective on the challenges that must be tackled before the nanotechnology can be translated into clinical use as safe and effective antiviral formulations. Therefore, the synthesised nanoparticulate antiviral systems up to 2010 consisted mainly of micelles, polymeric NPs, solid lipid NPs (SLNs), nanostructured lipid carriers (NLCs), liposomes, nanocapsules, vesicles, dendrimers, nanogels, cyclodextrines based systems and emulsions (Fig. 11).

Recently, Arca-Lafuente et al. (Arca-Lafuente S., Martínez-Román P., Mate-Cano I. et al., 2019) overview nanotechnology-based systems as reliable alternative diagnostic tools for HCV infectious disease. Even if our review does not cover screening, it is important to mention that new diagnostic methods are required to overcome current drawbacks of HCV under-diagnosed infection as highlighted in the above-mentioned review.

The nanotechnology-based tools described in the review seem to fulfil the necessary features for HCV elimination. Other examples of anti-hepatitis virus drugs incorporated in nanoplatforms are presented in Table VIII.

Table VIII. Nano-delivery platforms developed for antiviral drugs

Nanoplatform Type	**Nanoplatform Characteristics**	**Drug**	**Virus type**
- rHDL (recombinant high-density lipoproteins;)	rHDL-nosiheptide complex with a diameter < 30 nm (Feng M., Cai Q., Shi X. et al., 2008)	Nosiheptide	HBV
	rDHL-ACV palmitate complex size of 33.5 nm, around 10 times smaller than ACV-lipososmes (Feng, M., 2008)	Acyclovir (ACV)	
- cationic	viral gene expression reduces by 65-75% in liver after 2 dayts of administration at mice (Kim S.I., Shin D., Lee H. et al., 2009)	siRNA	HCV
- Human serum albumin + copolymers of maleic anhydride/alkyl vinyl ethers of oligo (ethylene glycol)	mean size of NPs in the range of 100 – 300 nm. NPs surface with targeting moieties able to interact with liver cells receptors (Chiellini E.E., Chiellini F., Solaro R., 2006)	INFs-α	HBV, HCV

Progress in nanomedicine: antiviral nanotherapeutics approved or under evaluation

Nanomedicine represents a fast-revolutionizing field that faces rapidly and constantly progress assessed by the numerous nanodrugs that have entered clinical practice and also by even more being investigated in clinical trials. Table IX presents the approved antiviral nanomedicines, from which half are vaccines.

Table IX. Examples of approved nanoplatforms for anti-hepatitis drug delivery by the FDA, EMA and other organizations
(updated table, after Singh L., Kruger H.G., Maguire G.E.M. et al., 2017)

Name	**Company/ Approval year Country/ Organization**	**Nanoplatform. Benefits**	**Virus**	**Route of administration**
Epaxal®	Crucell (former Berna Biotech Ltd.); 1994 Switzerland	Virosomes (around 150 nm spherical liposomal vesicles) – intrinsic adjuvant properties; reduced toxicity and superior tolerability (Boevir P.A., 2008, He H., Yuan D., Wu, Y., et al., Cao, Y. 2019)	HAV	Intramuscular vaccine
PegIntron®	Schering Corporation, 2001, U.S., FDA	PEG-interferon alfa-2b (polymeric NPs)–31.000 Daltons molecules; superior protein stability (Peginterferon alfa-2b, 2001)	HCV	Subcutaneous
Pegasys®	Genentech, 2002, U.S. FDA	PEG - interferon alfa-2a (polymeric NPs)–31.000 Daltons molecules; superior protein stability (Ventola C.L., 2017, Peginterferon alfa-2b, 2002)	HBV, HCV	Subcutaneous

According to Singh et al. review from 2017 and also to available information on the respective websites there are still several nanomedicines under evaluation, like the one from Arbutus Biopharma, lipid nanoparticles of ARB-001467 TKM-HBV containing three RNAi therapeutics for HBV genome targeting; in 2018 the company completed the phase 2a, single blind, randomized, placebo controlled, study evaluating the safety, anti-viral activity, and pharmacokinetics following multiple doses of intravenous ARB-001467 (number NCT02631096)(https://clinicaltrials.gov/ct2/show/NCT02631096?term=NCT02631096&draw=2&rank=1).

Perspectives to Design Next Generation of Nano-Based Antivirals for Clinical Translation

Since the development of "best" viral carriers involves a multidisciplinary team, virologists should be directly implicated in the development, offering specialized support on the following matters: identification of differentially expressed moieties virus cells for targeted delivery, elucidation of the type of desired targeted and the response from the host cells to nanodelivery platforms.

When thinking about the translation into the clinical practice, the nano-based future antiviral therapy must follow a specific flow-chart, starting with the optimization and scale-up practices according to the good manufacturing practice, the elaboration of suitable regulatory guidelines and finishing with the development of cost-effective and high-quality formulations available worldwide. Taken into consideration all these enhanced features, the road to clinical practice still has many addressed issues in order to provide effective and safe antiviral nano-formulations to patients. (Cojocaru F.D., Botezat D., Gardikiotis I. et al., 2020).

I.1.4.5. Conclusions

- ***Cystic fibrosis-Related Liver Disease***

All patients with liver dysfunction must performed sweet test. A major challenge for the clinical management of these patients is the early diagnosis of the liver disease. The liver function improvement influencing life quality and increasing the survival rate in CF patients. New therapeutic strategies are necessary to prevent CF liver disease and secondary malnutrition and its progression before the development of its many features and complications.

- ***Chronic Infections with Hepatitis B and C Viruses***

Treating or improving treatment success rate for viral diseases are fundamental responsibilities. The established potential and boosted progress of nanotechnology in antiviral therapy development generates great expectations for new therapeutic innovative strategies for attacking or eradicating viral disorders.

At present, studies explored numerous and diverse nano-platforms including nanoparticles, liposomes, micelles, with different compositions, size, with single or combined entrapped drugs that may serve as potential antiviral drug delivery transporters. The clinical use of a nano-based antiviral formulation to date based on our knowledge has turned out just a few approved or under clinical trials nano formulations, mainly vaccines, despite more than 22 years of constant efforts. It is expected in the upcoming years that part of this "success preclinical story" to be scaled-up, translated and applied for better outcome, convenience, and access for patients.

I.1.5. Risk cancer and Cystic Fibrosis

I.1.5.1. Introduction

Currently, many children with CF now reach adulthood, at which time the risk of cancer is increased. CF patients have an increased risk of gastrointestinal cancer, lymphoid leukemia and testicular carcinomas. (Vekens K., Vincken S., Hanon S. et al., 2020). Even though lung cancer (LC) is up to now not frequently observed in CF patients, the management and life expectancy of patients with CF have improved substantially in recent years, which will lead to increasing the number of these patients diagnosed with LC.

Mutations in the CFTR gene determined the dysfunction or absent CFTR protein, which impairs mucosal clearance mechanisms causing recurrent lung infections, inflammation, and airflow obstruction. Chronic inflammation, mutations and epigenetic alterations in the CFTR gene may contribute to develop lung cancer. But the association with CFTR-alteration will conduct probably to more aggressive lung tumors. Treating and monitoring lung cancer in CF patients will be difficult due to chronic colonization of airways with pathogens like *Pseudomonas aeruginosa* and multi-organ involvement. (Vekens K, Vincken S, Hanon S et al., 2020).

It is now known that patients with CF have an increased risk of developing hematological malignancies (Vijenthira A. and Trinkaus M., 2016.). Acute lymphoblastic leukemia (ALL) is the most common cancer in childhood.

ALL is a malignant disease of the bone marrow and effective treatments are now available. (Cooper S.L. and Brown P.A., 2015, Huang F.L., Liao E.C., Li C.L. et al., 2020, Pui C.H., Mullighan C.G., Evans W.E., et al., 2012, Pui C.H., Cheng C., Leung W. et al., 2003). There are concerns about the possible risk of radiation-induced cancer in CF patients because, from childhood, they undergo multiple radiographic and computed tomography examinations. Exposure to postnatal diagnostic X-rays has been associated with an increased risk of childhood acute lymphoblastic leukemia (Bartley K., Metayer C., Selvin S. et al., 2010).

We anticipated and confirmed an increase of ALL in CF patients, but the absolute increase remained exceptionally low (approximately 2 per 100,000 CF patients), particularly because CF is itself a life-threatening disease. 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 C.H., Mahmoud H.H., Rivera G.K. et al., 1998, Biondi A. and Rambaldi A., 1996, Borkhardt A., Repp R., Haas O.A., et al., 1997).

LC and chronic lung diseases are currently two of the main causes of death in the world. The relationship between LC and chronic lung diseases has generated a great degree of scientific interest over the past decade. Consequently, relevant knowledge has increased exponentially. Both conditions have common etiological factors and multiple research directions in the last decades demonstrated the presence of some common relevant biological mechanisms which can explain why some patients with chronic respiratory diseases are at higher risk of developing lung cancer. Recent evidence suggests that inflammatory processes play a central role in the carcinogenesis of LC. Chronic obstructive pulmonary disease (COPD),

pneumonia or tuberculosis are major sources of inflammation in pulmonary tissues. Such pathologies may trigger lung cancer and be correlated with its development. They share the same aetiology (Brenner D.R., McLaughlin J.R., Hung R.J., 2011).

Although the association between chronic pulmonary diseases and LC has been researched for several decades, the evidence remains uncompelling due to inconclusive results and insufficiently large cohorts (fewer than 500 cases in 65% of studies) (Durham A.L. and Adcock I.M., 2015). So, we would expect that chronic lung disease due to CF to be accompanied by an increased risk of long-term cancer.

Several factors such as chronic inflammation in association with an avalanche of inflammatory cytokines, recurrent infections with extremely aggressive germs, respiratory failure due to fibrosis lesions and bronchiectasis in the lung, repeated lung x-rays can increase the risk of LC. However, data from the literature show that LC incidence in patients with CF remains at a low level (Maisonneuve P., Marshall B.C., Knapp E. A. et al., 2013). In this sense, together with colleagues from the Medical Oncology discipline, we reviewed the relationship between chronic lung disease and LC. However, future studies on potential mechanisms that may increase or decrease cancer risk in this population are needed.

Part of the preoccupations related to relationship between cystic fibrosis and risk cancer were synthesized in the following articles:

1. Alecsa MS, Moscalu M, **Trandafir LM**, Ivanov AV, Rusu C, Miron IC. Outcomes in Pediatric Acute Lymphoblastic Leukemia - A Single-Center Romanian Experience. *Journal of Clinical Medicine / J Clin Med*, 2020; 9(12):4052 (IF = 3.303)

2. Miron O, Afrasanie VA, Paduraru MI, **Trandafir LM**, Miron L. The relationship between chronic lung diseases and lung cancer - a narrative review. *J BUON*, 2020; 25(4):1687-1692. (IF = 1.695)

Using the theoretical data mentioned above, the two articles aimed to:

- ***Pediatric Acute Lymphoblastic Leukemia in Children***

The research we have been publishing aims to appraise the clinical, hematological, and the molecular modifications associated with ALL in our Romanian patients.

- ***Chronic Lung Diseases and Lung Cancer***

The aim of this review was to discuss the role of chronic pulmonary diseases on LC development. Also, we wanted to inform the clinicians in this respect for a careful follow-up of this category of patients and for the application of a personalized treatment approach.

I.1.5.2. Material and methods

- ***Pediatric Acute Lymphoblastic Leukemia in Children***

Patients and Clinical Data Our research included patient data collected over a period of 6 years and with an average follow up of 60 months. Our retrospective observational study 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 January 2010 and 31 December 2016. Fourteen patients were excluded after quitting treatment, leaving a total of 125 cases eligible for analysis.

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

Cerebrospinal fluid 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 (BD Biosciences, San Jose, CA, USA) and the classification criteria issued by the European Group for the Immunological Characterization of Leukemia (Bene M.C., Castoldi G., Knapp W. 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.

Statistical Analyses 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 and overall survival, 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 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.

- ***Chronic Lung Diseases and Lung Cancer***

We revised the current literature concerning chronic pulmonary obstructive diseases as a potential risk factor of LC development. We revised the current literature concerning pain health challenge in CF patients. We analysed the literature data using “lung, cancer, chronic,

tuberculosis, asthma, fibrosis" keywords. We researched the multiple causes of LC, the possible mechanisms involved in this relationship and how these chronic diseases influence the prognosis of patients with LC.

I.1.5.3. Results

- ***Pediatric Acute Lymphoblastic Leukemia in Children***

Clinical, paraclinical and molecular characteristics of acute leukemia patients For thE 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 X.

Table X. 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.

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*103/µL). 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. 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.

Treatment outcomes In this study we analysed 125 cases 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.

The overall relapse rate was 11.2%. Most relapsed patients experienced an early relapse (11/14, 78%). 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. Death occurred 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. It is also worth mentioning that, whenever it occurred, death seemed to follow shortly after relapse, resistance to chemotherapy, or recurrence.

Based on the Kaplan–Meier analysis, we were able to compare the 1-year event-free survival rates between BCP-ALL cases (86.3%) and T-ALL cases (71.4%), as well as the 3-year event-free survival rates between the two groups (78.9% and 64.9%, respectively) and 5-year event-free survival rates: 76.1% in BCP-ALL vs. 64.9% in T-ALL. Similarly, we analyzed the 1-year overall survival rates for BCP-ALL cases (87.2%) vs. T-ALL cases (71.4%), the 3-year overall survival rates (82.9% vs. 71.4%, respectively), and the 5-year overall survival rates (81.6% vs. 71.4% respectively) (Fig.12).

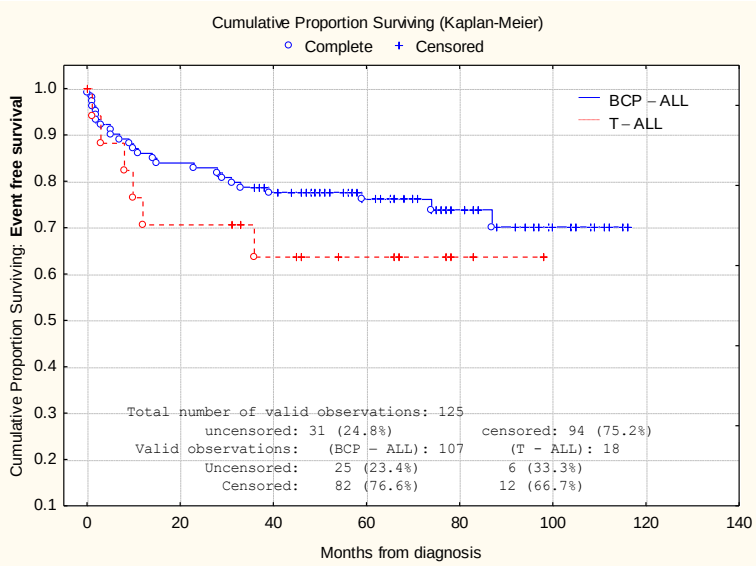

Figure 12. *Comparative analysis of Kaplan–Meier curves between precursor B-cell acute lymphoblastic leukemia (BCP-ALL) cases and T-cell acute lymphoblastic leukemia (T-ALL) cases. Comparison of event-free survival (EFS) rates.*

- ***Chronic lung diseases and lung cancer***

With a global incidence of 1.8 million cases in 2012, LC is among the leading causes of death worldwide (Ferlay J., Soerjomataram I., Dikshit R. et al., 2012). Recent evidence suggests that inflammatory processes play a central role in the carcinogenesis of LC. Chronic obstructive pulmonary disease (COPD), pneumonia or tuberculosis are major sources of inflammation in pulmonary tissues. Such pathologies may trigger LC and be correlated with its development. They share the same etiology (Brenner D.R., McLaughlin J.R., Hung R.J., 2011). Although the association between chronic pulmonary diseases and LC has been researched for several decades, the evidence remains uncompelling due to inconclusive results and insufficiently large cohorts (fewer than 500 cases in 65% of studies) (Durham A.L. and Adcock I.M., 2015).

Chronic obstructive pulmonary disease

COPD is a progressive deterioration of pulmonary function which can lead to death. In 2018 COPD was the third most common cause of death with the number of new cases growing. Pulmonary damage in COPD is generated by oxidative stress (both exogenous due to smoking, and endogenous), the release of inflammatory cytokines, the activation of proteases (protease-antiprotease imbalance) and the expression of antibodies. This lung disease may lead to the destruction and obstruction of airways, plus hyperinflation (Koshiol J., Rotunno M., Consonni D. et al., 2009).

COPD is an independent risk factor for LC, especially for squamous cell carcinoma (Young R.P. and Hopkins R.J., 2010). LC and COPD may share the same physiopathological mechanisms: a genetic predisposition, telomere shortening, mitochondrial dysfunction or early aging. In most smokers, the carcinogenic effect of smoking may be counterbalanced by the body's antioxidant defense mechanisms: superoxide dismutase, antiproteases and DNA repair mechanisms. However, these may fail, leading to cancer if the mutations are not repaired, or to COPD if cellular or protein destruction is too great. Alternatively, COPD can drive LC by increased oxidative stress, DNA mutations, chronic exposure to proinflammatory cytokines, suppression of DNA repair mechanisms and increased cellular proliferation (Barnes P.J. and Adcock I.M., 2011).

The epidemiology and risk of lung cancer in chronic obstructive pulmonary disease patients

Several reports have shown that the prevalence of LC in COPD patients ranges from 8% to 50% (De Torres J.P., Marin J.M., Casanova C. et al., 2011). Skillrud et al. conducted a case-control study evaluating the risk of LC in COPD patients and estimated an 8.8% cumulative probability for COPD patients to develop LC in the first 10 years, compared to 2% in patients with normal pulmonary function (Skillrud D.M., Offord K.P., Miller R.D., 1986). This indicates that approximately 1% of COPD patients will develop LC (the risk being five times higher). In another study, De Torres et al found that 215 of 2,507 COPD patients developed LC (incidence of 16.7 cases per 1,000 person-years with a median follow-up of 60 months).

Their result suggests that, in a population of smokers diagnosed with COPD and with a history of admissions to a pneumology clinic, the incidence of LC was higher than previously reported (De Torres J.P., Marin J.M., Casanova C. et al., 2011). In yet another study of 2,100 LC patients, the risk of LC was superior in patients with chronic bronchitis (OR 2.0, 95% CI, 1.5-2.5), emphysema (OR 1.9, 95% CI, 1.4-2.8), and COPD (OR 2.0, 95% CI, 1.06-2.59). After a 20-year follow-up of 448,600 smokers, Turner et al. reported that mortality due to LC

correlated significantly with emphysema (HR 1.66, 95% CI, 1.06-2.59) as well as with emphysema associated with chronic bronchitis (HR 2.44, 95% CI, 1.22-4.9), but not with chronic bronchitis alone (HR 0.96, 95% CI, 0.72-1.28) (Skillrud D.M., Offord K.P., Miller R.D., 1986). In both studies, COPD had been diagnosed based on the patients' clinical symptoms of emphysema or chronic bronchitis and their answers to questionnaires, not by means of spirometry or CT scans.

Last but not least, LC incidence is also influenced by the severity of COPD. Data collected from 5,402 study participants over a period of 22 years revealed a significant correlation between the degree of airway obstruction and LC incidence (Tockman M.S., Anthonisen N.R., Wright E.C., et al. 1987). Mild COPD was demonstrated to generate a relatively higher level of risk compared to the absence of comorbidities (HR 1.4, 95% CI, 0.8-2.6), while moderate and severe COPD was linked to higher rates of LC compared to normal pulmonary function (HR 2.8, 95% CI, 1.8-4.4) (Skillrud D.M., Offord K.P., Miller R.D., 1986). De Torres et al found that LC incidence in advanced stages of COPD (9.2 cases per 1,000 person-years) was less than half of that in stage I of COPD (19.9 cases in 1,000 person-years). The authors presumed that an active, intolerant immune system would act as a barrier against cancer development and progression (De Torres J.P., Marin J.M., Casanova C. et al., 2011).

Clinical and molecular features of lung cancer associated with COPD

Squamous cell carcinoma is most frequently associated with COPD or with emphysema and LC and its location is typically central in these patients. However, lower emphysema grades seem to correlate with central lung tumors, while more severe grades tend to predict peripheral location (Lim J., Shin K.M., Lee K. et al., 2015).

There appears to be a strong relationship between the tumor location and the area with the higher grade of emphysema (Hohberger L.A., Schroeder D.R., Bartholmani B.J. et al. 2014). Murakami et al have asserted that neoplasms which develop in the vicinity of the emphysema exhibit more aggressive tumor biology (Murakami J., Ueda K., Sano F. et al., 2016).

Another research demonstrated that the doubling time of pulmonary nodules was shorter in smokers with reduced pulmonary function, thus suggesting that COPD is a useful clinical marker for appraising LC aggressiveness. Several studies have revealed that both EGFR mutations and ALK rearrangements were less frequent in COPD-associated LC, and that the presence of EGFR mutations correlated negatively with the severity of airway obstruction. KRAS mutations occurred regardless of COPD status (Dai J., Yang P., Cox A. et al., 2017; Sekine Y., Yamada Y., Chiyo M. et al., 2007).

The prognosis for patients with LC and COPD

The prognosis for COPD patients is poorer compared with patients without COPD as a result of added treatments, altered pulmonary function and reduced quality of life [19]. The long-term postsurgical survival of patients with COPD and stage IA LC has been subject to research, and the 5-year survival of COPD patients proved to be significantly lower than that of patients with normal pulmonary function due to a higher rate of recurrence (77% vs. 91.6%, $p<0.0001$). Such results suggest that LC in patients with COPD tends to be more aggressive (Sekine Y., Yamada Y., Chiyo M. et al., 2007).

Other studies have found that COPD is a significant risk factor for respiratory complications and poorer long-term survival on account of the respiratory insufficiency following the resection of the cancerous tumors (Cardarella S., Ogino A., Nishino M. et al.,

2013). Lopez-Encuentra et al reported that despite the similar survival rates of patients with and without COPD soon after LC surgery after 2 and then 3 years, the survival rate of stage I LC patients with underlying COPD was significantly lower compared to that of patients without COPD (Paik P.K., Arcila M.E., Fara M. et al., 2011).

Although COPD patients suffer from substantially more cardiopulmonary comorbidities, postsurgical physical and mental evaluations did not seem to differ between patients with and without COPD (Tse H. and Tseng C. 2014, Namajika T., Sekine Y., Yamada Y. et al., 2009). Because long-term results are yet to be reported, the effects of comorbidities upon survival remain controversial (Lopez-Encuentra A., Astudillo J., Cerezal J. et al., 2005).

Lung cancer and tuberculosis

According to the World Health Organisation, tuberculosis (TB) occurs with an incidence of 9.4 cases yearly and causes approximately 1.7 million deaths. TB is the result of the reactivation of a latent infection with Mycobacterium tuberculosis, which currently colonizes a third of the global population. Pulmonary infections may contribute to cancer etiology. TB increases the risk of LC by prolonged and substantial pulmonary inflammation which damages the host tissue, causes fibrosis, scarring and genetic alterations (Engels E.A, Shen M., Chapman R.S. et al., 2009).

Two cohort studies and other case-control studies investigated the relationship between TB and LC. Although one study found the risk of LC to be six times higher in patients with TB than in those without it, another cohort study did not establish any such correlation. Some case-control studies reported notable associations between TB and LC, rating the level of risk between 1.6 and 4.2, while others did not. When patients with LC were stratified based on histology, TB related significantly to squamous cell carcinoma (Nalbandian A., Yan B.S., Pichugin A. et al., 2009).

A review of 41 studies aiming to establish if preexisting TB increases the risk of LC found TB to be associated with the adenocarcinoma group mostly in non-western countries. Inconclusive results justify the need for more research regarding the relationship between TB and LC (Keikha M. and Esfahani B.N.,2018).

Lung cancer and sarcoidosis

Sarcoidosis is a granulomatous inflammatory disease whose cause is yet unknown. Some characteristics point to it having an infectious origin, others suggest genetic susceptibility.

In Scandinavian and Caucasian patients, there is a strong correlation between genetic rearrangement for the specific T cell receptor, HLA-DR17, and favorable prognosis, thus indicating the presence of a single antigen and underlining the importance of host predisposition. Chronic inflammation is associated with a high risk of malignant lymphoma or cancer in the affected tissue. Theoretically, this could apply to sarcoidosis as well, which most often involves intrathoracic organs, the liver and the skin (Askling J., Grunewald J., Eklund A. et al. 1999).

Lung cancer and asthma

Asthma is one of the most common diseases of childhood, with a global prevalence of 10% among children aged 6-7. It is characterized by chronic pulmonary inflammation, bronchial hyperreactivity, excessive mucus production, and airway obstruction. Some studies found a significant association between asthma and LC, but these results are inconclusive.

A meta-analysis of 22 studies conducted in order to establish if LC is indeed associated with asthma, indicated a high level of risk (OR=1.44).

In addition, non-smoking asthma patients were also found to be at high risk of LC (OR=1.28). The analysis of racial subgroups revealed elevated risks in both Caucasians and Asians (OR=1.53, 95% CI, 1.37-1.72, p<0.001, I2=56%, and OR=1.52, 95% CI, 1.15-2.01, p<0.001, I2=93%). However, asthma was not found to increase the risk of lung adenocarcinoma (OR=1.01, 95% CI, 0.69-1.50, p=0.95, I2=45%).

These results suggest that asthma may be an independent risk factor for LC. Further studies are needed to assess the relationship between asthma and other histopathological types of LC (Scagliotti G.V., Longo M., Novello S., 2009, Qu Y-L., Liu J., Zhang L.X., et al. 2017). Meta-analyses concluded that asthma may be associated with LC (Hung Y.P., Teng C.J., Liu C.J. et al. 2014).

Lung cancer and pneumoconioses

Pneumoconioses are a group of lung diseases caused by the inhalation of mineral dusts. There are three types of inorganic mineral dusts responsible for the typical form of pneumoconiosis: coal, asbestos and silica powders. Coal dusts may trigger pneumoconiosis and chronic inflammatory disease such as massive progressive pulmonary fibrosis, COPD, or emphysema. Inhaled silica crystals have been associated with silicosis, tuberculosis, fungal infections, COPD, malignant illnesses, autoimmune diseases, and kidney diseases. Exposure to asbestos dusts may cause pulmonary fibrosis. The International Agency for Cancer Research has classified them as part of the group I carcinogens (Qu Y-L., Liu J., Zhang L.X. et al. 2017).

Lung cancer and idiopathic pulmonary fibrosis

Idiopathic pulmonary fibrosis (IPF) is an interstitial lung disease with poor prognosis and undefined etiopathogenesis. Several genetic and environmental risk factors have been identified to be involved in its pathogenesis: smoking, inorganic dusts, genetic mutations and polymorphisms (Bargagli E., Bont V., Ferrari K. et al. 2017). Survival in IPF is less than 5 years (King T.E., Pardo A., Selman M., 2011). LC incidence is much higher among patients with IPF than in the general population. For IPF patients, Ozawa et al reported rates of LC incidence of 3.3% at the one-year mark, 15.4% at 5 years, and 54.7% after 10 years. Mechanisms shared by both diseases are yet to be discovered (Ozawa Y., Suda T., Naito T. et al. 2009).

I.1.5.4. Discussion

- ***Pediatric Acute Lymphoblastic Leukemia in Children***

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 H., Abuirmeileh N., Rihani R. 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 D.A., Henley S.J., Li J. et al., 2017), Nordic countries (Hjalgrim L.L., Rostgaard K., Schmiegelow K. et al., 2003), or Surveillance, Epidemiology, and End Results database (Barrington-Trimis J.L., Cockburn M., Metayer C. et al., 2017). Similar male preponderance was reported in Pakistan (66.1%) and in some areas of India (Fadoo Z., Nisar I., Yousuf F. et al., 2015).

We believe studies such as ours provide important information regarding 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.

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 G., Botta L., Rossi S. 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 J.E., Lensing S., Zhou Y. 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 H., Sun H., Sun X., 2015).

- ***Chronic Lung Diseases and Lung Cancer***

LC represents approximately 13% of all newly diagnosed cases and 26% of all cancer-related fatalities (Ferlay J., Soerjomataram I., Dikshit R. et al. 2015). The high mortality is caused by the absence of symptoms, substantially delaying diagnosis.

COPD has been reported as a risk factor for LC. Despite substantial scientific interest in the association of these major lung diseases, the molecular details and their clinical implications have only begun to be understood during the last decade (Raviv S., Hawkins K.A., DeCamp M.M. Jr. et al., 2011, Mapel D.W., Hurley J.S., Frost F.J. et al. 2000). COPD and LC share several pathways. All neoplastic tissues exhibit inflammation, which is why many inflammatory diseases also predispose to cancer. Chronic inflammation in COPD may act as a potent driver for LC, as suggested by the efficacy of non-steroidal anti-inflammatory drugs. Inflammation is the main source of reactive oxygen species which are persistent in COPD (Lim J., Shin K.M., Lee K. et al., 2015).

Most of the previous research consisted of case-control studies and there is little prospective data on TB and the risk of LC. TB infection triggers a widespread immune response in the host, and inflammatory cells generate cytokine signalling cascades, reactive nitrogen and oxygen species, prostaglandins and proteases which destroy the tissues (Nalbandian A., Yan B.S., Pichugin A. et al., 2009). Even TB patients undergoing treatment may suffer from prolonged pulmonary inflammation because the symptoms have already occurred months before diagnosis, and treating tuberculosis requires lengthy periods of medication. Such prolonged pulmonary inflammation may cause tissue damage and genomic alteration. The repair of the TB-damaged tissue may lead to pulmonary fibrosis and scaring, which also

increase the risk of LC. It is worth mentioning that men diagnosed with TB sequelae are exposed to such elevated risk. This diagnosis is highly likely in men suffering from severe lung damage due to TB, and this observation is meant to underscore the role of fibrosis in facilitating carcinogenesis (Keikha M. and Esfahani B.N., 2018).

The literature is scarce on the risk of cancer after sarcoidosis. One retrospective cohort study tested the hypothesis of elevated risk for LC and included 8541 patients from the Swedish Patient Registry (Berlin M., Fogdell-Hahn A., Olerup O. et al. 1997). For LC, the relative risk was double in the first decade of follow-up. Also, sarcoidosis was noticed to increase the risk of cancer in the affected organs, with chronic inflammation being an important mediator of this risk (Askling J., Grunewald J., Eklund A. et al., 1999, Berlin M., Fogdell-Hahn A., Olerup O. et al., 1997). LC significantly reduces the survival of IPF patients. Generally, it occurs in the peripheral areas of the inferior lobes, where fibrotic modifications are common. Specifically, LC develops in honeycombs or at the border between the honeycombs and the non-fibrotic areas. Squamous cell carcinoma is the predominant histological type (Ozawa Y., Suda T., Naito T. et al., 2009).

Clinicians are faced with a dilemma: whether to treat LC in patients with IPF. Lee et al found that radiotherapy and surgical treatments for LC can further reduce the survival of IPF patients (Tomassetti S., Gurioli C., Ryu J.H. et al. 2015). Voltolini et al. showed that surgical treatments have a negative impact on these patients (Voltolini L., Bongiolatti S., Luzzi L. et al., 2013).

I.1.5.5. Conclusions

- ***Pediatric acute lymphoblastic leukemia in children***

Regardind the response to therapy and follow up of pediatric patients with ALL, we should consider both (para)clinical characteristics and genetic profiling. 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.

- ***Chronic lung diseases and lung cancer***

Deeper understanding is likely to be gained in the foreseeable future with regard to inflammation, oxidative stress, the genetic and molecular mechanisms which increase susceptibility to the disease, and new insights will lead to discoveries enabling early diagnosis and more effective treatments. Thus, there is hope that the quality of life and survival of these patients will improve.

I.2. RELATIONSHIP BETWEEN CELIAC DISEASE AND CHILD MALNUTRITION

I.2.1. Background

Celiac disease (CD) is an autoimmune multisystemic condition triggered by gluten and related prolamins intake (wheat, rye, barley) in genetically predisposed patients, which may impair any organ of system and which has a wide range of clinical signs of variable severity

(Fasano A., Araya M., Bhatnagar S. et al., 2008). CD used to be considered a pediatric condition with typical gastrointestinal signs and symptoms due to intestinal malabsorption (chronic diarrhea, abdominal distension, anorexia, failure to thrive) and non-gastrointestinal abnormalities (abnormal liver function tests, iron deficiency anemia, bone disease, skin disorders) (Rampertab S.D., Pooran N., Brar P. et al., 2006, Telega G., Bennet T.R., Werlin S., 2008).

Due to the wide range of symptomatology, an accurate diagnosis it is not always easily. Use of serologic screening tests (anti-gliadin antibodies - AGA, anti-endomisium antibodies - AEA, anti-tissue transglutaminase antibodies - ATTGA and anti-deamidated gliadin antibodies - ADGA) allowed identifying all forms of CD: atypical, silent or latent, as well as disease identification among associated pathological conditions. Since it often has atypical symptoms (recurrent abdominal pain, constipation, iron deficiency anemia, increased level of liver enzymes, arthritis, etc.) or is clinically asymptomatic, CD may go undiagnosed or even entail complications if exposure to gluten continues. Knowledge by pediatrician of all forms of manifestation of the CD is essential for proper diagnosis setting before the development of any complications that may affect patients' quality of life or even death (intestinal lymphoma).

Part of the preoccupations related to features and comorbidities of Celiac Disease were synthesized in the following papers:

Articles:

1. Trandafir LM, Cirdeiu E, Oltean C, Mihaila D, Anton-Paduraru DT. Polymorphism of the clinical signs of celiac disease in children. *Romanian Journal of Pediatrics.* 2015; 64(4): 413-417. (EBSCO)

2. **Trandafir LM**, Teslariu O, Diaconescu S, Iorga M, Miron I, Sarbu I, Ciongradi IC. Unusual presentation of celiac disease in a toddler from a pediatric surgery unit. *International Journal of Celiac Disease.* 2016; 4(2): 74-76. (Scopus)

3.Ozkan M, **Trandafir L**, Mindru E, Moraru E. Evolution of nutritional status and lipid metabolism parameters in gluten free diet. *The Medical-Surgical Journal*. 2012; 116(1): 103-107. (Pubmed)

4.Ozkan M, **Trandafir L**, Bozomitu L, Azocai A, Murgu A, Popovici P, Stana B, Tunza-Enea HA, Moraru E. Liver involvement in celiac disease in children. *The Medical-Surgical Journal*. 2011; 115(4): 1030-1034. (Pubmed)

5.Ghimpu S, Bozomitu L, Cardei E, Oltean C, Burlacu M, Anton D, **Trandafir L**, Mihaila D, Moraru D. Helicobacter pylori infection in children with celiac disease, *The Medical-Surgical Journal*. 2009; 113(4): 1093-1098. (Pubmed)

6. **Trandafir LM**, Anton-Paduraru DT, Rusu D, Burlea M. Oral manifestations in celiac disease children. *Romanian Journal of Oral Rehabilitation*. 2014; 6(1): 33-37. (DOAJ)

7. Diaconu G, Burlea M, Grigore I, Anton DT, **Trandafir LM**. Celiac disease with neurologic manifestations in children. *The Medical-Surgical Journal*. 2013; 117(1): 88-94. (Pubmed)

7.Ghimpu S, Bozomitu L, Cardei E, Oltean C, Burlacu M, Anton D, **Trandafir L**, Mihaila D, Moraru D. Helicobacter pylori infection in children with celiac disease. *The Medical-Surgical Journal*. 2009; 113(4): 1093-1098. (Pubmed)

Book chapter:

1.„Boala celiacă" - **Laura Mihaela Trandafir**, în Pediatrie, (editor Ingrid Miron). Ed. "Gr. T. Popa", UMF Iaşi, 2016, ISBN 978-606-544-429-4, pg. 308-313.
2. "Boala celiacă" - E. Cîrdei, Laura Trandafir – în Tratat de Pediatrie (editor Constantin N. Iordache), Ed. "Gr.T.Popa" UMF Iaşi, 2011, ISBN 978-606-544-046-3, pg. 622-629.

I.2.2. Celiac disease and nutritional pathology in children

I.2.2.1. Introduction

The CD diagnosis can be challenging because symptoms can vary significantly from patient to patient (Caio G., Volta U., Sapone A. et al., 2019). Also, CD is underdiagnosed due to the varied presentation of clinical signs and symptoms. Thus, nutrient deficiencies have been described in CD both before and after diagnosis, due to intestinal malabsorption and specific limitations of the gluten free diet, respectively.

CD diagnosis can be accurately and safely established with or without duodenal biopsies. According to the New Guidelines for the Diagnosis of Paediatric Coeliac Disease of ESPGHAN, for initial testing, the combination of total IgA and IgA class antibodies against transglutaminase 2 is recommended. The no-biopsy approach for CD diagnosis is confirmed to be safe in children with high IgA class antibodies against transglutaminase 2 $\geq$ 10 times the upper limit of normal. Children with positive ATTGA-IgA but lower titers (<10 times upper limit of normal) should undergo biopsies to decrease the risk of false positive diagnosis. ESPGHAN recommends that the decision whether to perform duodenal biopsies in patients with high ATTGA-IgA should be made during a shared decision-making process. This should be between the paediatric gastroenterologist specialist, the parent(s)/carer(s) and, if appropriate, the child (New Guidelines for the Diagnosis of Paediatric Coeliac Disease of ESPGHAN, 2020).

Part of the preoccupations related to celiac disease and nutritional pathology were synthesized in the following article:

1.Trandafir LM, Cirdeiu E, Oltean C, Mihaila D, Anton-Paduraru DT. Polymorphism of the clinical signs of celiac disease in children, *Romanian Journal of Pediatrics*. 2015; 64(4): 413-417 (EBSCO).

Objective: presentation of clinical polymorphism and nutritional deficiencies of CD in children.

I.2.2.2. Material and Methods

It is now known that CD onset may occur at any age, from infants to adult, and have a polymorphic clinical presentation due mainly to its autoimmune component. Thus, in this article, we reviewed the importance of early recognition of all forms of CD by exemplifying a series of four clinical cases in which CD presented with liver impairment, chronic constipation,

recurrent abdominal pain and one case in which CD onset was associated with type 1 diabetes mellitus (type 1 DM) onset.

I.2.2.3. Results

Clinical case I: 5 years and 2 months old male patient hospitalized in the 3rd Pediatric Department of the "Saint Mary" Children Emergency Hospital of Iasi due to recurrent abdominal pain, lack of appetite and weight gain decrement. The child had normal height and weight gain increase in the first two years of his life. During his hospitalization he had a relatively good general state, weight - 13kg, height - 119cm, BMI=9.2, lack of appetite, soft depressible abdomen, periumbilical spontaneous and palpation pain, and physiological intestinal transit. However, the thing which came to our attention was his behavioural disorders (short-tempered introverted child who did not fit in with the other children). The biological tests performed did not reveal any inflammatory syndrome, but they show hypochromic microcytic iron-deficiency anemia and the titers of ATTGA IgA and IgG were pathological, as they were 53.5UI/l and 13.4UI/l, respectively. The diagnosis of BC was supported by presence of HLA DQ2. After a gluten-free diet was introduced, the clinical-biological evolution was positive. The patient regained his appetite, the abdominal pain disappeared, the weight gaining curve was slightly ascending and his behavioural disorders vanished. After 6 months of gluten-free diet, the ATTGA were negative (1.5 UI/l) and after 8 months of gluten-free diet, the patient weighed 18 kg and BMI was 12.3.

Clinical case II: 1 year and 6 months old female patient living in rural areas admitted to the 3rd Pediatric Department of the "Saint Mary" Children Emergency Hospital of Iasi for failure to thrive. From her personal history we retain that she is the second child of the family, delivered at term naturally (weight on birth = 3100 grams), breastfed for 2months and then fed with age-adapted Morinaga powder milk formula. The additional feeding was initiated at the age of 4 months and food containing gluten was added at the age of 5 months. We find out from her personal pathological history that at the age of 9 months she started her failure to thrive. The patient's objective clinical examination revealed a relatively good general state, weight = 9kg, -2.15 SD and height =83cm, - 1.03 SD, which were lower than the normal percentages specific to this age category. The paraclinical examinations conducted revealed hypochromic microcytic iron-deficiency anemia, increased level of liver enzymes. AGA presented pathological values. The duodenal intestinal biopsy revealed total villous atrophy, inflammatory lymphoplasmacytic infiltrate in the chorion, numerous intra-epithelial lymphocytes in the lamina (MARSH IIIC).

The positive diagnosis was CD associated with reactive hepatopathy and iron deficiency anemia. As a result, a gluten-free diet was introduced, after which the weight curve began to go up, and the liver enzymes became normal after 6 months of gluten-free diet.

Clinical case III: 1 year and 2 months old male patient hospitalized in the 3rd Pediatric Department of the "Saint Mary" Children Emergency Hospital of Iasi on 31 August 2006 for failure to thrive and chronic constipation. His physical examination conducted on hospitalization revealed a relatively good general state, nutritional short stature (weight = 7.5kg, height = 76cm, Ponderal Index=0.68), pale teguments, reduced subcutaneous cell tissue on the abdomen, thorax and limbs, rickets sequelae (forehead humps, flared lower thorax, rachitic

rosary), muscular hypotonia, lack of appetite, swollen abdomen, slow intestinal transit (highly consistent stools every 4-5 days). Laboratory tests revealed IgA AGA values of 38 UI/l (NV below 6 UI/l). The X-ray revealed delayed bone age as compared to the chronological age of 8 months. Endocrine screening excluded congenital hypothyroidism. The pathology results from duodenal intestinal biopsy were subtotal villous atrophy, inflammatory lymphoplasmacytic infiltrate in the chorion, numerous intra-epithelial lymphocytes in the lamina - suggesting MARSH IIIB. In this case, the positive diagnosis was atypical CD associated with 2nd degree protein-caloric malnutrition and constipation. After a gluten-free diet had been initiated, the weight curve started to rise, the intestinal transit was resumed, and the IgA AGA values became normal after 3 months of gluten-free diet.

Clinical case IV: 9 years and 7 months male patient hospitalized in the 3rd Pediatric Department of the "Saint Mary" Children Emergency Hospital of Iasi for abdominal distension and altered general state. During his stay, the child had altered general state, weigh - 34 kg, heigt -141 cm, BMI - 17.17, swollen abdomen, hepatomegaly, postprandial abdominal distension and occasional diffuse abdominal pain, stools full of mucus and undigested food, depigmented patches of skin on the upper and lower limbs. Paraclinical investigations revealed: no inflammatory syndrome, hyperglycaemia, HbA1c = 13.53% (NV<6%), glycosuria = 150 mg/dl and ketone bodies +, cholesterol = 202 mg/dl, total proteins = 59.51 g/l, albumins = 29 g/l, ATTGA Ig A > 200 U/ml and Ig G = 35.6 U/ml antibodies (NV < 20 UI/ml). An upper digestive endoscopy and duodenal intestinal biopsy were performed, and it showed massive chronic duodenitis with partial villous atrophy, which were suggestive of MARSH III A.

I.2.2.4. Discussions

Following these results, the authors reported four clinical cases of CD that associated symptoms such as recurrent abdominal pain, impaired hepatic function, constipation and one case of type 1 DM associated with CD. These patients had common growth impairment, regardless of their clinical (constipation, recurrent abdominal pain), or biological symptoms (elevated liver enzymes, hyperglycaemia with glycosuria).

CD is diagnosed from infants to children and adults and its clinical signs vary depending on the patient's age on disease onset. Clinical signs are especially correlated with the patient's age on disease onset, as they are often not considered as suggestive of this affliction (Stănescu Popp A., 2006).

The onset of classic CD in children occurs around the age of 18 months, weeks and even months after cereals have been included in the children's diet. In babies, the onset of CD is insidious and consists of steatorrhea, faded, doughy, massive stools with bits of undigested food. An increasing amount of rarely we meet the classic triad formed by chronic diarrhea, swollen abdomen and malnutrition. Else ways, atypical forms have been an increasing amount of common (Walker-Smith J.A., 2000). Our patients associate several atypical manifestations, among which recurrent abdominal pain was frequently found that delayed diagnosis until the age of 5, while constipation and hepatic cytolysis syndrome associated with malnutrition was diagnosed in the second year of life.

Early onset, during infancy, is characterised by vomiting and watery long-recovery stools. During the illness, the patients may experience recurrent episodes of severe diarrhea, with watery stools and severe dehydration, or coeliac attacks, which have been increasingly

rarer. After patients reach the age of 3–5 years, digestive symptoms on disease onset are replaced by weight and later height growth stagnation. With age, CD is one of the major causes of weight and height hypotrophy due to anorexia, malabsorption and intestinal nutrient losses. Growth disorders are associated with anorexia, recurrent abdominal pain, constipation, marked physical asthenia and neuro-behavioural changes (children are lethargic, introvert or, in contrast, even violent; their focus and academic performance are also affected).

The consequences of intestinal malabsorption are fits of hypocalcaemia tetany, osteopenia or osteoporosis, spontaneous fractures of long bones (vitamin D and calcium malabsorption), Beri-Beri neuritis, glossitis, stomatitis, cheilitis, tegument pallor (B group vitamins malabsorption), bruising, nosebleeds, gum and intestinal bleeds (vitamin K malabsorption), as well as dermatosis and hair dystrophy (vitamin A malabsorption). Tooth enamel erosion, recurrent aphthous ulcer, delayed tooth eruption, cheilitis, are oral CD signs that, if timely recognised, allow early diagnosis setting (Greetje J.T., Wieke H.M.V, Marco W.J. et al., 2010). Even if the most common form at present is characterised by CD onset in children aged 5 to 7 years, in some patient's CD is diagnosed during adolescence or even adulthood (Fasano A., 2005). CD adolescents suffer from delayed sexual maturity, hypo- or amenorrhea. Data from the literature also show higher rates of malignancies, including intestinal lymphoma, squamous cell esophageal carcinoma, melanoma and adenocarcinoma, in patients with CD than in the general population (Green P.H.R., 2005, Dewar D.H. and Ciclitira P.J., 2005). The following clinical forms of CD have been approved (adapted according to the OSLO – 2013 classification):

- the classical form shown by signs and symptoms due to intestinal malabsorption: chronic steatorrheic diarrhea, failure to thrive, abdominal distension, anorexia, coeliac attacks.

- the atypical form includes both gastrointestinal symptoms, which are not due to intestinal malabsorption (recurrent abdominal pain, vomiting, constipation, irritable bowel syndrome) and extra-intestinal symptoms (increased level of liver enzymes, treatment-refractory anemia, recurrent aphthous stomatitis, height hypotrophy, tooth enamel erosion, hypogonadism and delayed puberty, arthritis, dermatitis herpetiformis, epilepsy with intracranial calcifications, ataxia, autism, depression).

- the subclinical form, formerly known as silent or asymptomatic CD, in which the patient is asymptomatic, but the intestinal mucosa of the patient has specific intestinal lesions. This category also includes the latent CD form defined by the existence of normal intestinal mucosa in children with gluten intake who will develop the disease subsequently manifested due to their genetic predisposition (the only intestinal mucosa change is intraepithelial lymphocytes increase) (Walker-Smith J. A., 2000). This form does not have enough clinical signs and symptoms that would justify routine serological CD testing.

- the potential form ("potential coeliac disease") in which the patients' intestinal biopsy is normal, but they have a high risk of CD, which is supported by the positive serology [15, 16].

- the refractory form characterized by the fact that patients experience persistent or recurrent signs and symptoms of intestinal malabsorption with intestinal villi atrophy, which are not improved by a strict gluten-free diet adopted for more than 12 months.

- non-coeliac gluten sensitivity is characterized by one or more clinical, immunological or morphological signs triggered by gluten intake in people in whom CD was ruled out; this

category may include non-IgE mediated food allergy (Ludvigsson J.F., Leffler D.A., Bai J. et al., 2013).

CD is associated with a series of autoimmune (type 1 DM, autoimmune thyroiditis, Sjorgen syndrome, psoriasis, alopecia areata) or non-autoimmune (selective IgA deficiency, Down syndrome, Williams syndrome, Turner syndrome, IgA nephropathy, neuro-psychiatric conditions like autism, depression, ataxia, epilepsy with intracranial calcifications) diseases, first degree relatives of CD patients (Fasano A., 2005).

Type 1 DM associated with CD is very strongly documented and supported by common genetic predisposition. CD-specific gastrointestinal signs (diarrhea, abdominal distension) are rare in diabetic patients, with predominant atypical, isolated signs and symptoms: iron deficiency anemia, height hypotrophy, late puberty onset, epilepsy, hypertransaminasemia, dyspeptic symptoms, dermatitis herpetiformis or recurrent aphthous stomatitis (Smyth D.J., Plagnol V., Walker N.M. et al., 2008).

Immunological screening tests are used for diagnosis purposes, to reduce the need for biopsy of the intestinal mucosa and to monitor the development of CD under treatment and to verify adherence to gluten-free diet. Serological screening tests have shown that CD is now much more frequent than it was initially believed, with its prevalence rate being 1% of the general population (McGough N., Cummings J.H., 2005, Roma E., Panayiotou J., Karantana H. et al., 2009). Patient diagnosis using screening tests showed a higher incidence of atypical or silent forms, while the classical diarrhea form was less frequent (Rampertab S.D., Pooran N., Brar P. et al., 2006, Lo W.,Sano K., Lebwohl B., 2003).

I.2.2.5. Conclusions

In all four cases, the diagnosis of CD was established lately due to atypical forms and association with type 1 DM. In children with atypical features or with associated diseases, use of immunological tests has been increased the incidence of atypical or silent forms of CD in children and adolescents. Recognition of all digestive and extra digestive manifestations by the general practitioner and pediatrician is extremely important for early diagnosis of the CD and prevention of its long-term complications: osteoporosis, infertility, intestinal lymphoma.

CHAPTER II. CHILDHOOD OBESITY AND HEALTH CONSEQUENCES

Introduction

We are currently witnessing a new nutritional reality, a real paradox that refers to the dual problems of malnutrition and obesity, known as the double burden of malnutrition (Hawkes C., Ruel M.T., Salm L. et al., 2020). Malnutrition refers to deficiencies, excesses or imbalances in a person's intake of energy and/or nutrients. The term malnutrition covers two broad groups of conditions. One is 'undernutrition'—which includes stunting (low height for age), wasting (low weight for height), underweight (low weight for age) and micronutrient

deficiencies or insufficiencies (a lack of important vitamins and minerals). The other is overweight, obesity and diet-related noncommunicable diseases (such as heart disease, stroke, diabetes, and cancer). Many children do not eat enough nutritious foods like fresh fruit and vegetables, legumes, meat, and milk, while foods and drinks high in fat, sugar, and salt are cheaper and more readily available, leading to a rapid rise in the number of children and adults who are overweight and obese. Globally, people are consuming foods and drinks that are more energy-dense (high in sugars and fats), and engaging in less physical activity (WHO, 2014).

The prevalence of childhood overweight is increasing in all regions of the world. If these increasing trends continue, it is estimated that the prevalence of overweight in children under 5 years of age will rise to 11% worldwide by 2025. Overweight and obesity are complex and multifaceted problems. Childhood overweight and obesity also increase the risk of obesity, noncommunicable diseases (NCDs), premature death and disability in adulthood. Finally, the economic costs of the escalating problem of childhood overweight and obesity are considerable, both in terms of the enormous financial strains it places on health-care systems and in terms of lost economic productivity (WHO, 2014).

Undernutrition, obesity, and diet-related NCDs are intrinsically linked through early-life nutrition, diet diversity, food environments, and socioeconomic factors (Hawkes C., Ruel M.T., Salm L. et al., 2020). Action to prevent and control childhood overweight and obesity, therefore, needs to go hand in hand with the actions to achieve other global nutrition targets of increasing the rate of exclusive breastfeeding and reducing stunting, anaemia in women of reproductive age, wasting and low birth weight. Optimizing nutrition early in life—including the 1000 days from conception to a child's second birthday—ensures the best possible start in life, to ensure good physical and mental development and long-term health (WHO, 2014).

II.1. CHILDHOOD OBESITY: THE NEW EPIDEMY

II.1.1. Background

Obesity represents a chronic disorder characterized by an excessive adipose tissue, due to the calorie intake, which exceeds the caloric needs of a body. Childhood obesity represents one of the most critical global public health challenges of the 21st century for two reasons: firstly, the rapid increase in the prevalence of obesity worldwide, and secondly, the severe consequences it generates for public health. It is widely known that obesity represents a risk factor for multiple chronic diseases: cardiovascular and lung pathologies, type 2 DM, liver disease or cancer. The number of obese children has increased remarkably since the 1970's until now, and probably the number will continue to grow in the future. If new global strategies for treating obesity will not be developed and implemented, the number of overweight children is expected to reach. It is currently estimated that 381 million children worldwide are overweight or obese (McPhee P.G., Singh S, Morrison K.M., 2020). It seems that obesity also affects children under the age of 5, showing that in 2017, over 38 million children worldwide suffer from this disease. Recently, the number of obesity cases has escalated in underdeveloped countries, although previously, obesity was present mainly in countries with a higher standard of living. That is why governments have tried to develop management strategies with the same common goal: to stop the increase in the number of overweight and obese children by 2025 in all pediatric age groups (WHO, 2018).

Personal contribution related to study of childhood obesity was synthesized in the following papers:

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: 735. (IF=3,11)

2.Cojocaru E, Magdalena Leon-Constantin M, Ungureanu C, Trandafirescu MF, Maștaleru A, **Trandafir LM,** Dumitru Petrariu F, Viola Bădulescu O, Filip N. Hypolipemiant Actions and Possible Cardioprotective Effects of Valine and Leucine: An Experimental Study. *Medicina* (Kaunas). 2021; Mar 5;57(3):239. doi: 10.3390/medicina57030239. PMID: 33807510. (IF= 1.205)

3.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.* 2020; 99:30. (IF=1.552)

4.Trandafir LM, Baciu G, Grigore M, Gafitanu D, Scripcariu IS, Moscalu M, Ivan A, Temneanu O. Early Nutrition for a Healthy Future Generation. *Review of Research and Social Intervention*. 2018; 63: 389-402. (IF= 1.076)

5.**Trandafir LM,** Baciu G, Frasinariu OE, Mihalache L, Bogdan-Goroftei R, Moscalu M. The Impact of TV Exposure and Computer Use on Obese Adolescents. *Review of Research and Social Intervention*. 2018; 62: 173-184. (IF= 1.076)

6.**Trandafir LM,** Grigore M, Preda C, Gafitanu D, Temneanu OR, Petroaie AD, Scripcariu SI, Ungureanu MC. Hormonal Contraception in Teenager Girls. The Role of Counseling to Ensure Effective Contraceptive Use. *Review of Research and Social Intervention.* 2019; 67: 223-233. (IF= 0.736)

7.**Trandafir LM**, Baciu G, Constantin MML, Mastaleru A, Temneanu OR, Mihai B, Novac O, Frasinariu OE, Ivan A, Tudorachi NB, Al Hiary R, Moscalu M. Predictive Biological Markers in Post-therapeutic Evolution in Obese Patients. *Rev. Chim. (Bucharest).* 2018; 69(11): 3048-3051. (IF= 1.22)

8.Trandafir LM, Anton-Paduraru DT, Miron I, Indrei LL Psychosocial Implications of Childhood Obesity. *Review of Research and Social Intervention.* 2015; 49: 205-215. (IF=0.424)

9.**Trandafir LM**, Baciu G, Leon-Constantin MM, Novac O, Barbacariu LC, Slanina AM, Petroaie AD, Pohaci-Antonesei LS, Mastaleru A, Temneanu OR. Implementing a healthy lifestyle at paediatric age - a preventive condition for obesity. *International Journal of Medical Dentistry*. 2019; 23(1): 7-11. (Web of Science Core Collection)

10.**Trandafir LM**, Frasinariu OE, Chiriac MI, Miron I. Management of dyslipidemia in childhood may prevent cardiovascular events in adults. Medical-Surgical Journal. 2017; 121(2): 313-320. (Web of Science Core Collection)

11.Russu G, Frasinariu OE, **Trandafir L** Cardiovascular suffering in childhood obesity. *Romanian Journal of Pediatrics*. 2017; 66(1): 18-23. (EBSCO)

12.**Trandafir LM**, Temneanu OR. Pre and post-natal risk and determination of factors for child obesity. Journal of Medicine and Life. 2016; 9(4): 386-391. (Pubmed)
13.Temneanu OR, **Trandafir LM**, Purcarea MR. Type 2 diabetes mellitus in children and adolescents: a relatively new clinical problem within pediatric practice. *Journal of Medicine and Life*. 2016; 9(3): 235-239. (Pubmed)

ISI Proceedings
1.Trandafir LM, Frasinariu OE. Nutrition Recommendations in Hepatic Steatosis in Children, Proceedings of the 4th Congress of the Romanian Society for Minimal Invasive Surgery in Ginecology / Annual Days of the National Institute for Mother and Child Health Alessandrescu-Rusescu, 2019, 648-653. (Web of Science Core Collection)
2.Trandafir LM, Frasinariu OE, Corciova C, Boiculese LV, Moscalu M. Prevention of Cardiovascular Risk Factors in Childhood Obesity, 2017 IEEE International Conference on E-Health and Bioengineering Conference (EHB), 2017, 717-720. (Web of Science Core Collection)
3.**Trandafir LM**, Frasinariu OE, Subotnicu M, Miron IC. Gastrointestinal Disorders in Childhood Obesity, NEUROGASTRO 2017 - Meeting of the Romanian Society of Neuro-gastroenterology with Rome IV Regional Central East European Meeting, 2017, 142-146. (Web of Science Core Collection)

Book chapter
1. Childhood Obesity - **Laura Mihaela Trandafir**, Ileana Ioniuc, Ingrith Miron in "Adiposity - Epidemiology and Treatment Modalities", Publisher: InTech book edited by Jan Oxholm Gordeladze, ISBN 978-953-51-2996-7, Print ISBN 978-953-51-2995-0, Published: March 15, 2017 under CC BY 3.0 license
2. Obezitate, Dana Teodora Anton Păduraru, **Laura Mihaela Trandafir**, în Pediatrie (editor Ingrid Miron). Ed. "Gr. T. Popa", UMF Iasi, 2016, ISBN 978-606-544-429-4, pg. 317-320.
3. Cerealele integrale – un aliat în lupta împotriva obezității copilului - **Laura Mihaela Trandafir,** Maria Magdalena Leon, Doina Azoicăi, "Prevenția obezității la vârsta copilăriei", Ed."Gr.T.Popa", Iași, 2018, Veronica Mocanu (coordonator), ISBN 978-606-544-521-5, pg. 39-47.
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II.1.2. CHILDHOOD OBESITY: UPDATE ON ETIOLOGY AND PREDISPOSING FACTORS

II.1.2.1. Introduction

Obesity is considered one of the most common causes, which affect children's health and may be responsible for premature death. Serious health consequences were reported in connection with this disease. (Swinburn B.A., Kraak V.I., Allender S. et al., 2019) and many studies show that childhood obesity will affect a future adult's health (Trandafir L.M., Cojocaru E., Moscalu M. et al., 2021).

In children and adolescents between 2–18 years old obesity and overweight are defined as a body mass index (BMI) greater than or equal to the 95th percentile and the 85th to 94th percentile for age and sex. (de Ferranti S.D., Steinberger J., Ameduri R., Baker A. et al., 2019). It is very important to follow the pattern of growth over time, using these cut-points to identify children who require more frequent follow-up and further assessment rather than to assign a diagnosis. Annual assessment for obesity is recommended by the American Academy of Pediatrics and others via measurement of height and weight to calculate BMI and plotting the results on growth charts from the Centers for Disease Control and Prevention (de Ferranti S.D., Steinberger J., Ameduri R. et al., 2019, Barlow S.E., 2007.). Once the diagnosis of obesity in children and adolescents has been established, screening for associated cardiovascular risk factors is mandatory (Brickman W.J., Huang J., Silverman B.L. et al., 2010). BMI must be considered and monitored in every child because it is a major risk factor for many diseases often referred to as NCDs such as type 2 DM, cardiovascular disease, liver disease and different types of cancers (colorectal cancer, oesophageal cancer or kidney cancer). The monitoring of WC waist circumference can improve sensitivity for the detection of adiposity-related CVD risk, even before BMI criteria are met (Mokha J.S., Srinivasan S.R., Dasmahapatra P. et al., 2010). The risk factors causing NCDs affect all age groups. Insulin resistance (IR) and chronic inflammation have an essential role in the pathogenesis of obesity-associated comorbidities (Lopez-Sandoval J., Sanchez-Enriquez S., Rivera-Leon E.A. et al., 2018, Sinaiko A.R., Steinberger J., Moran A. et al., 2005).

Fortunately, there are two modifiable risk factors for NCDs and obesity: unhealthy diets and physical inactivity. Thus, behavioural changes in dietary and physical activity patterns may be an adequate first step in lowering the prevalence of obesity. On the other hand, teasing, bullying, and social isolation have been observed in overweight and obese children, which most certainly affect their quality of life.

EPIDEMIOLOGICAL DATA

Lately, epidemiological studies have reported an increased prevalence of childhood obesity worldwide. Although obesity is more common in specific sex, age, ethnic, and socioeconomic status groups, the result is similar: an obese adult. It has been reported that a high prevalence of obesity in the age group 0–6 years increases the chances of obesity in adolescence and adulthood.

In 2016, about 124 million children and adolescents, between 5–19 years, were suffering from obesity, and 213 million were overweight (NCD-Risc, 2017, Lobstein T., Jackson-Leach R., Moodie M.L. et al., 2015).

Due to current hygiene diets, the prevalence of obesity has risen in all countries, regardless of their degree of development. The first countries that reported the growing number of obesity cases were the United States of America. In 2010, the National Health and Nutrition Examination Surveys indicated that childhood obesity in the United States has approximately doubled during the past three decades, and adolescent obesity has more than tripled during the same time frame. (Ogden C.L., Carroll M.D., Curtin L.R. et al., 2010). Despite public health strategies and policies, we are currently only witnessing a slowdown in the cases of obesity, and the United States is far from stopping this phenomenon. Prevalence of overweight increased from 20% to 30% and the prevalence of obesity from 5% to 15% in North America and some countries in Europe. However, recent surveys indicate that starting from 2005, prevalence levels have been stationary. (Trandafir L.M, Ioniuc I., Miron I., 2017)

One of the biggest epidemiological studies was published in The Lancet in 2017, which evaluated BMI variation during 1975-2016 of children with overweight and obesity worldwide. Two thousand four hundred sixteen studies were analysed, and anthropometric assessments representative of weight and height were checked in a total of 128.9 million participants over the age of five. The study concluded that in the last forty years, the BMI of children and adolescents had risen globally. The prevalence of obesity increased from 0.7% in 1975 to 5.6% in 2016 in females and males from 0.9% to 7.8%. In Eastern European countries, it has been observed that the growth of BMI has not changed significantly in the last forty years, and in the industrialized countries of North-West Europe, the growth curve of BMI has flattened in both females and males. In contrast, BMI had an upward slope in the pediatric population over five years of age in East and South Asia for both sexes.

In Romania, there are relatively few statistical and epidemiological data on childhood obesity. The first Health Behaviour in School-aged Children research in Romania took place in 2005-2006 (study published in a report by IASO, London, 2009) for children between 11 and 15. This study showed that the prevalence of overweight was 14.7% in girls and 8.7% in boys. According to data provided by the National Center for Assessment and Promotion of Health in Romania, the prevalence of obesity in children between 3-16 years increased from 2004 to 2010 from 0.7% in rural areas to 1.6% and in urban areas from 1.5% to 3.1%, respectively. According to the COSI - WHO study report from 2017, in 7-year-old children, the prevalence of overweight was increased in girls (18.10%) compared to boys (13,00%). In the same age group, the prevalence of obesity was lower in the girls (10.70%) than in boys (14.90%).

Unfortunately, there are no newer studies on the prevalence and incidence of obesity and overweight children in Romania. Still, if data from around the world is analysed, the numbers are continually growing. This reality is highly worrying because the vast majority of overweight children will become obese adults.

Determinants of childhood obesity

Childhood obesity involves cardiovascular, gastrointestinal, endocrine, musculoskeletal, neurological, pulmonary, and renal complications, and psychosocial problems. All these factors together will affect the children's progress throughout adulthood, possibly with severe consequences (Yach D., Stuckler D., Brownell K.D., 2006). Obesity is

considered a significant health problem, which is why the authors of many studies try to debate and establish the aetiology of childhood obesity (Olshansky S.J., Passaro D.J., Hershow R.C. et al., 2005).

Despite extensive research, many aspects of obesity remain unknown because this condition is much more complex, and our understanding of it is still somewhat limited. Lately, several fascinating discoveries were made, and the most important ones attract attention about adipose tissue and the gut flora, which are considered organs and interact continuously with the brain. A multitude of factors contributes to obesity, but genetically heritable traits must be carefully evaluated. Genetics, epigenetics, metagenomics and the environment influence these traits. The "obesogenic" environment is translated by a sedentary lifestyle and unhealthy dietary behaviours. (Smith J.D., Fu E., Kobayashi M.A., 2020)

a) Genetics and epigenetics in obesity

Analysis of the genetic causes underlying obesity will help develop new prevention strategies and improve pediatric obese patients' treatment and reduce the risk of occurrence of associated comorbidities.(Chesi A. and Grant S.F.A., 2015) Genetic causes of obesity are classified into: monogenic with a single gene mutation, syndromic obesity characterized by severe obesity associated with other phenotypes such as neurodevelopmental abnormalities and other organs malformations, and polygenic obesity: many genes whose effect is amplified by the "weight gain promoting" environment (Thaker V.V., 2017). "Obesity genes" contribute to the phenotype, but the environmental factors play a significant role in developing body mass, adjusting the balance between caloric intake and physical activity (Rao K.R., Lal N., Giridharan N.V., 2014, Deram S. and Villares S.M.F., 2009).

Over the past 40 years, researchers have developed a real "science of obesity". Gene expression is continuously modified under the action of many environmental factors, starting with nutrition, psychosocial stress, or toxins, tobacco, and alcohol. Simultaneously, endocrine disruptors and microbiota or other geographic, political, or socioeconomic influences were incriminated. It is well known that environmental factors influence the epigenetic programming of parental gametes, foetus, and early postnatal development, or through the various periods of life.

This process starts in utero, which explains why the mother's lifestyle before birth is so important. Environmental factors can affect at least three generations: the mother and father, their children, and grandchildren. The health of the conception product depends on the mother's diet, hormonal changes, metabolism, or exposure to stress, and all this can affect the descendant's tissue development (Deram S. and Villares S. M. F., 2009).

The periods of fetal development and infancy are considered the most important and can influence future adults' health. Hence, new strategies are needed to prevent obesity and specific public health measures focused on this period of life because they have a long-term impact. Studies suggest that maternal nutritional status is the most effective and sustainable means of improving health across the next three generations (WHO, 2016).

b) Prenatal determinants of childhood obesity

Maternal nutritional status

Many studies have reported that maternal nutritional status during fetal development and infancy can influence growth and development. This aspect is due to changes in fetal epigenetic programming for the responsiveness to environmental factors.

So, maternal health and nutritional status can affect intergenerational chronic disease susceptibility. Maternal malnutrition in the first trimester of pregnancy promotes obesity, glucose intolerance, dyslipidemia, cardiovascular disease, hypertension, and affective disorders. Also, maternal undernutrition in the second trimester of pregnancy is associated with glucose intolerance, lung, kidney disease, and in the third trimester with glucose intolerance and cardiovascular disease in children (Boekelheide K., Blumberg B., Chapin R.E. et al., 2012).

On the other hand, maternal obesity and gestational diabetes are strong predictors of overweight and obesity in children. They are associated with newborn macrosomia and a high risk of obesity and metabolic syndrome in adulthood (Kim S.Y., Sharma A.J., Sappenfield W., et al., 2016, Rayanagoudar G., Hashi A.A., Zamora J. et al., 2016, Bellamy L., Casas J.P., Hingorani A.D. et al., 2009, Norman K., Stobäus N., Gonzalez M.C. et al., 2011, Godfrey K.M., Reynolds R.M., Prescott S.L. et al., 2017, Oken E., Ning Y., Rifas-Shiman S.L. et al., 2007, Villamor E., Saathoff E., Mugusi F. et al., 2006).

Infant birth weight

Recent studies suggest that birth weight (low birth weight or high birth weight) is associated with childhood obesity. Newborns "deprived of nutrition" in utero present an accelerated growth rate, especially if they received infant formula after birth. This rapid postnatal growth in the first few months of life is associated with an increased risk of obesity. High birth weight, primarily related to maternal gestational diabetes, correlates with elevated plasma insulin levels, childhood obesity, and metabolic syndrome development (Haire-Joshu D. and Tabak R., 2016).

Maternal pregnancy smoking status

Data from the literature suggests that passive smoking of the infant also increases the risk of developing obesity or other pathologies related to it (Nadhiroh S.R., Djokosujono K., Utari D.M., 2020).

Family history

A positive family history of obesity increases the risk of children who developed obesity in the first years of life to become obese adults by 80% (for those with both parents obese) and 40% for children with one obese parent. Disturbed intrauterine environment and fetal malnutrition and other epigenetic risk factors disrupt the cardiovascular and metabolic gene controls. So, it increases the risk of developing metabolic syndrome, hypertension, hyperinsulinemia, and dyslipidemia, known as programmed fetal diseases (Smith C.J. and Ryckman K.K., 2015).

c) The postnatal determinants of childhood obesity

Nutrition in the first years

Inadequate milk formula with excess protein in the first year of life is considered a risk factor for further developing obesity and insulin resistance by IGF1. Breastfeeding is a key factor in the prophylaxis of cardiovascular pathology, obesity, and other numerous metabolic disorders. (Skilton M.R., Siitonen N., Würtz P., et al., 2014) The WHO meta-analysis (2013) of 75 observational studies demonstrated the positive effect of breastfeeding on preventing obesity and hypertension in adulthood.(Bernardo L. H. and Cesar G. V., 2013) The survey of 822 young Dutch people (18-28 years old) shows that breast milk's exclusive diet had a significant protective effect on the body and visceral fat mass (BMI, abdominal circumference, and abdominal circumference ratio hip circumference). Moreover, the literature data

demonstrated a significant correlation between preterm infants' breastfeeding and the subsequent standard lipid profile. Early introduction of complementary food (before six months) increased the risk for childhood obesity. (Taveras E.M., Gillman M.W., Kleinman K. et al., 2010) Also, hypercaloric, hyperglycemic, hyperprotein diet, low in dietary fiber, in the first year of life are involved in increasing the risk of obesity.

Improper nutrition in childhood

It is well known that the combination of consuming too many calories and lack of physical activity leads to weight gain. In the last years, the most spectacular changes in children's diets are not only qualitative but also quantitative: high-calorie foods, high consumption of sour juices, concentrated sweets, fast food, some family eating habits. In older children and adolescents, consuming fast food is frequently found to play a significant role in the variation of children's body weight (Vonzie N., 2009).

A fast-food menu has an average energy density of 263 kcal/100g, two times more than the energy intake recommendations for a healthy diet (125 kcal/100g). People have a low ability to recognize foods with a high energy density and to adjust the amount ingested to adequately meet the body's energy requirements (Prentice A.M. and Jebb S.A., 2003). Also, fast food is unhealthy from other points of view: it is high in fat, high in salt, high in cholesterol, high in red meat, and processed meat. Children who eat this type of food have a higher daily caloric intake and a lower intake of fiber, fruits, and vegetables (Anderson J.W., Baird P., Davis R.H. Jr. et al., 2009).

In addition to the high caloric content, the portion size in fast-food restaurants also plays an important role. It is known that offering larger portions leads to a larger consuming pattern, and in recent decades portions of french fries, pizza, burgers have increased 2 to 5 times (Young L.R. and Nestle M., 2002). A critical factor in preventing obesity may be reducing sugar-sweetened beverages, particularly carbonated soft drinks because it has high-added sugar content and low satiety. Each additional serving of carbonated juice can lead to an increase in BMI, given that an amount of 500 mL of juice contains the equivalent of 15-18 teaspoons of sugar (Goryakin Y., Monsivais P., Suhrcke M., 2017).

Children who were offered such sweet drinks more than three times a week in the age range 10 - 12 months displayed a double risk of obesity than those who did not consume juices during this period (Pan S.Y., Litscher G., Gao S.H. et al., 2014). High salt intake is incriminated in the appearance of overweight. Many studies report a positive association between salt intake and variation in obesity assessment parameters, such as BMI and WC. Data from the literature shows that salt intake correlates with the consumption of sweets and high-calorie drinks. This link is thought to be due to the effect of salt intake on thirst, given that experimental studies in both animals and humans show an increase in fluid intake in the context of a high-sodium diet (Grimes C.A., Bolhuis D.P., He F.J. et al., 2016).

Sedentary lifestyle

Reducing energy expenditure by decreasing physical activity, inactivity, lack of exercise, and sport are factors that predispose to obesity. Often, the inactivity of obese children is also linked to the parent's lifestyle. A sedentary lifestyle is a significant risk factor for obesity, dyslipidemia, or cardiovascular disease (Lakka T.A. and Bouchard C., 2005).

Electronic entertainment devices and obesity

Numerous studies show that the increased period of watching TV or using any other electronic device (laptop, tablet, smartphone, video games) has been associated with overweight, obesity, or adiposity, primarily until preschool. Also, watching TV during meals leads to ingesting a more considerable amount of food, especially junk food, to the detriment of consuming fruits, vegetables, and whole grains (Harris J.L. and Bargh J.A., 2009). The American Academy of Pediatrics (AAP) does not recommend children under two to watch TV.

Sleep deprivation and obesity

There is an opposite relationship between sleep duration and adiposity markers, overweight and obesity recorded until preschool age (Morrissey B., Taveras E., Allender S., et al., 2020). Sleep is an essential modulator of neuroendocrine function and glucose metabolism. Sleep loss has been shown to result in metabolic and endocrine alterations, including decreased glucose tolerance, decreased insulin sensitivity, increased evening concentrations of cortisol, decreased levels of leptin, resulting in excessive hunger with increased appetite, increased amount of ingested foods and implicitly the increase of the BMI (Guglielmo C.G., McGuire L.P., Gerson A.R. et al., 2011).

Microbiota

Current research has shown that the intestinal microbiota is a determining factor in the occurrence of obesity. Knowing the relationship between obesity and the intestinal microbiota has allowed the identification of biomarkers that predispose the child to weight gain and has also contributed to early therapeutic prevention strategies. The intestinal microbiota of infants is quite different from that of adults. After one year of age, the infant has an adult-like microbiota (Palmer C., Bik E.M., DiGiulio D.B. et al., 2007). The microbiota participates in developing and maintaining obesity by changing the host's eating behaviour, storing energy and fat. Other additional mechanisms are the supply of extra energy by converting dietary fiber into short-chain fatty acids and increasing intestinal permeability. All these mechanisms cause metabolic endotoxemia and low-grade chronic inflammation, and, finally, metabolic syndrome onset (Bäckhed F., Ding H., Wang T. et al., 2004).

Medications

It is well known the association between the chronic administration of glucocorticoids and the weight gain. The causes of weight gain are multiple: increased appetite by activating the endocannabinoid system, decreases costs of energy and increases the accumulation of fat in the liver. In the hepatic tissue, glucocorticoids stimulate the cannabinoid receptor 1 causing lipogenesis, hepatic steatosis dislipidemia. Also, in the liver, the stimulation of the activity by 11-beta-hydroxysteroid-dehydrogenase-1, increases the production of endogenous glucose and thus insulin resistance and promotes obesity, especially the visceral obesity. (Verhaegen M.E., Mangelberger D., Harms P.W. et al., 2017) Another class of medications, such as *antidiabetic agents (*insulin, sulfonylurea, and thiazolidinediones) caused substantial weight gain. (McFarlane S.I., 2009). Literature data show that obesity is two to three times more frequent in psychiatric patients due to multiple causes: unhealthy diet, sedentary lifestyle, high prevalence of smoking, and added the effects of psychiatric drugs. Obesity in psychiatric patients is most often due to increased appetite and high food intake, especially with high-calories, sweet and fat components ("food craving") (Meule, A., 2020). Also, psychiatric drugs affect energy metabolism and satiety by interfering with central appetite neurotransmitters (dopaminergic,

serotonergic, and histaminergic neurotransmissions). (Schmidt F.M., Weschenfelder J., Sander C. et al., 2015)

Hormonal contraceptives are currently the most common form of combating unwanted pregnancies and sexually transmitted diseases. Although they contribute to teenagers' and women's health, contraceptives also have some side effects, such as intermenstrual spotting, nausea, breast tenderness, headaches, migraine, and weight gain. Currently, the effects on weight represent o problem of debate. Clinical studies in this area show that hormonal contraceptives could contribute to weight gain by increased appetite, fluid retention, increased body fat.

Among the contraceptive methods, it has been observed that depot-medroxyprogesterone acetate (DMPA) causes significant weight gain in both obese and non-obese adolescents. Still, the risk of severe obesity is higher in already obese adolescents. Regardless of the nutritional status, prolonged administration of DMPA causes significant weight gain and body structure changes. It has been observed that DMPA-associated increased weight is due to increased fat mass and not lean mass (Berenson A.B. and Rahman M., 2012) Several studies (Berenson A.B.and Rahman M., 2009, Clark M.K., Dillon J.S., Sowers M. et al., 2005) showed an increase in visceral fat on normal-weight women who used DMPA as a contraception method. Because the visceral fat is a critical element of metabolic syndrome, the risk for women that use DMPA to develop cardiovascular complications of type 2 diabetes is increased. Also, evaluating other factors such as total caloric intake, amount of protein, fat, and carbohydrate consumed per day showed that an increase in protein intake was protective against gains in weight and body fat among DMPA and oral contraceptive (OC) users. OC administration is not associated with significant weight gain but has caused an increase in body fat and a decrease in lean body mass (Berenson K.R., Gyurak A., Ayduk O. et al., 2009).

Regarding the administration of progestin-only contraceptives (POCs), weight gain is essential. (Lopez L.M., Edelman A., Chen M. et al., 2013) Therefore, the choice of contraceptive method in obese adolescents must consider several individual risk factors for the occurrence of cardiometabolic complications. The health of adolescents is significant both for their health and for the health of future descendants. When we talk about adolescent health, an essential practical aspect is represented by reproductive health. WHO recommends improving access to receive information about contraception of teenage girls and encourages the use of different measures to prevent pregnancies.

Part of the preoccupations related to childhood obesity determinants were synthesized in the following articles:

Articles:

1.**Trandafir LM**, Baciu G, Grigore M, Gafitanu D, Scripcariu IS, Moscalu M, Ivan A, Temneanu O. Early Nutrition for a Healthy Future Generation, Review of Research and Social Intervention, 2018, 63: 389-402. (IF= 1.076)

2.**Trandafir LM**, Baciu G, Frasinariu OE, Mihalache L, Bogdan-Goroftei R, Moscalu M. The Impact of TV Exposure and Computer Use on Obese Adolescents, Review of Research and Social Intervention, 2018, 62: 173-184. (IF= 1.076)

3.**Trandafir LM,** Grigore M, Preda C, Gafitanu D, Temneanu OR, Petroaie AD, Scripcariu SI, Ungureanu MC. Hormonal Contraception in Teenager Girls. The Role of Counseling to Ensure Effective Contraceptive Use, Review of Research and Social Intervention, 2019, 67: 223-233. (IF= 0.736)

Objectives

Using the theoretical data mentioned above, the three articles aimed to:

- ***Early Nutrition During the First 1000 Critical Days***

To highlight some data regarding the relationship between mothers and young children's optimal nutrition during the first 1,000 critical days and the child's and future generations' health.

- ***Electronic Entertainment Devices***

Due to an increased period of watching TV or using any other electronic entertainment device, a sedentary lifestyle represents risk factors for overweight obesity.

- ***Hormonal Contraception in Teenager Girls***

Hormonal contraception is essential to teenagers' health, but one of the contraceptives' side effects is weight gain and obesity.

II.1.2.2. Materials and methods

- ***Early Nutrition During the First 1000 Critical Days***

The current literature concerning the importance of the first 1000 days of life revealed that this is the "window of opportunity" for long-term health in the first to third generation. A mother's lifestyle before birth is the opportunity to influence the child's optimal health. To conceptualize the importance of the effects and nutritional benefits of interventions in this period of life, we researched the articles published in various databases.

The keywords used in our research were: early nutrition, the first 1,000 days, healthy development, malnutrition. We have shown that women's nutritional status at the time of conception and during pregnancy is essential for the growth and development of the product of conception. Moreover, the child's nutritional status in the first 24 months of life, the peculiarities of the diet during this period contribute to the development of obesity and other chronic diseases (obesity, cardiovascular diseases, diabetes mellitus, dyslipidemia, etc.). Thus, in this article, we reviewed the importance of nutrition throughout life, from adolescence to pregnancy and later to childhood, highlighting the implications on adult health.

- ***Electronic Entertainment Devices***

The prospective study covered the period 1st of April and the 30th of June 2017 and included 38 obese adolescents, aged 12 to 18 years (mean age 13.7 ± 2.6 years) with the sex ratio (M/F) 1.1, and most of them from urban areas. The evaluation was made using a questionnaire with 15 items referring to the exposure of the electronic device, and the adolescents were grouped into three categories based on the final score as it follows:

- under 34 points – the minimal impact of computer and TV use on adolescents' behaviour.

- between 35-38 points – the moderate impact of computer and TV use on adolescents' behaviour.
- above 39 points – major impact of computer and TV use on adolescents' behaviour.

- ***Hormonal Contraception in Teenager Girls***

Reproductive health care plays a crucial role in adolescent life. Therefore, contraceptive counselling is of utmost importance, offering the opportunity to choose from various methods. In such circumstances, confidentiality should be taken very seriously and respected. Given that most adolescents trust health providers with such sensitive information, it is recommended to discuss sexual history with the adolescent alone. There are "5 P's" which must be the subject of such a conversation: partners, prevention of pregnancy, protection from sexually transmitted infections (STIs), sexual practices, and history of STIs and pregnancy. (Centers for Disease Control and Prevention, 2005). Ideally, adolescents should be educated on all these topics by a team composed of a general practitioner, a pediatrician, and a gynecologist. All of them play a significant role in explaining and encouraging young people to actively use contraception and protect themselves from unwanted sexually transmitted diseases.

II.1.2.3. Results and Discussions

- ***Early Nutrition During the First 1000 Critical Days***

The research has identified the vulnerable periods that can affect the health of the conception product both in the short and long term. Significant are both the preconception period and the pregnancy period, when exposure to environmental factors, including malnutrition or over nutrition, can trigger adaptations to the growing foetus. While these effects may be adaptive in the short term, they may also be associated with adverse outcomes in childhood and later life, including an increased risk of developed obesity, cardiovascular disease, and type 2 diabetes mellitus.

Early life nutrition refers to nutritional exposures before conception and during pregnancy, infancy, and early childhood. It is now well known that the "fetal origin of the disease hypothesis" has demonstrated the link between fetal undernutrition and the increased risk of development of many chronic diseases: obesity, cardiovascular disease, DM, dyslipidemia, etc. (Deepak S., Sweta S., Pradeep S., 2016).

So, the consequences of intrauterine growth restrictions will influence individual well-being and the social and economic development of nations (Black R.E, Victoria C.G., Walker S., 2013). Moreover, Prentice et al., consider that adolescence is an "additional window of opportunity" because the nutritional deficiencies of the future mother will affect the future child and two more generations of offspring, a phenomenon known as the intergenerational cycle (Victora C.G., de Onis M., Hallal P.C. et al., 2010).

Intergenerational cycle of growth failure

If initially the term malnutrition was used only for underweight, it includes both malnutrition and obesity, both of which have multiple nutritional and immunological consequences for the body. Improper dietary intake is "a scourge in our world" (Fanzo J., 2015).

and affects health from conception and later in certain parts of the life cycle: infants and young children, adolescent girls, and pregnant and lactating women (Fig. 13) (UNSCN, 2000).

The vicious circle of malnutrition can be interrupted during adolescence if adolescents receive a balanced diet in micro and macronutrients. Teenage mothers have an increased risk of birth to stillbirths, premature, or low birth weight due to gestational age. Proper nutrition in adolescence will improve the health of women and future generations of children and ensure optimal growth and the physical and mental development of offspring.

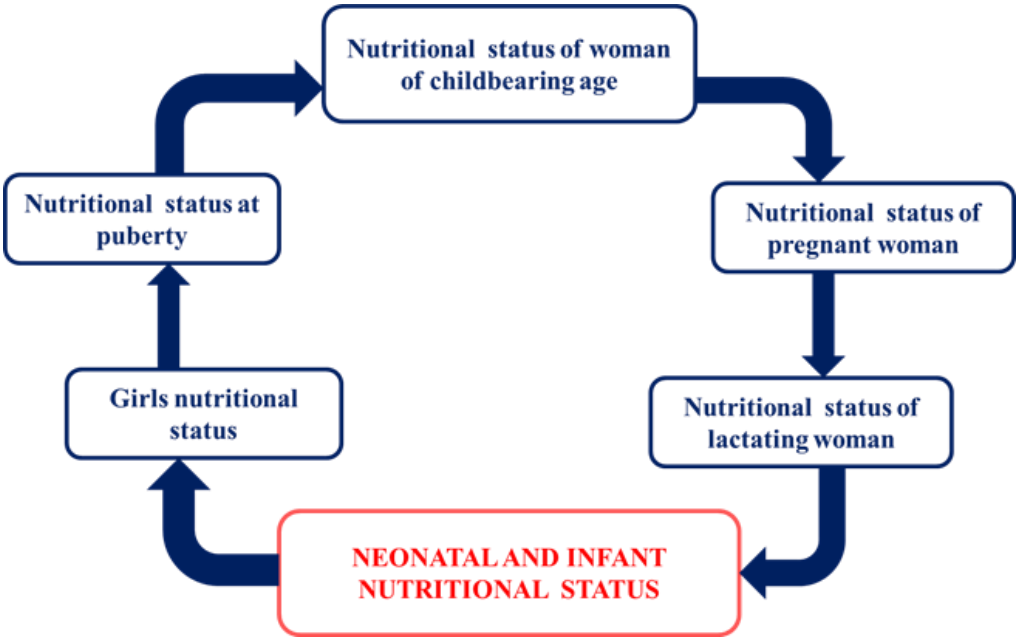


Fig. 13. Poor nutrition throughout the lifecycle (adaptation)

The Baby-friendly Hospital Initiative

The importance of breastfeeding for both the baby and the mother are well known. Thus, in 1991 WHO and UNICEF launched the "Baby-friendly Hospital Initiative" (BFHI), based on "Ten Steps for Successful Breastfeeding (The Ten Steps)". This concept aims to ensure the protection, promotion, and support for breastfeeding in maternity facilities. (WHO, 1999). The first few hours and days of a newborn's life are a critical window for initiating breastfeeding. Thus, maternity hospitals that have opted for the implementation of this concept support mothers to breastfeed and provide information about short and long-term benefits, both for the child, the mother, and the whole family. The Ten Steps summarizes a package of policies and procedures, which should be implemented to support breastfeeding. A systematic review about new-born care in maternity, which adopting the BFH concept, demonstrated that adherence to the Ten Steps impacts early initiation of breastfeeding and improves the rate and compliance to breastfeeding (WHO, 2018b).

Breastfeeding: the gold standard for infant nutrition

Breastfeeding represents "the gold standard" and "the ideal food" for infant nutrition, with many short- and long-term benefits. The mother needs to know that she can give the child all the micro-and macronutrients necessary for healthy infant's growth. The WHO currently recommends to infant's nutrition exclusive breastfeeding up to 6 months of age, followed by

complementary feeding in parallel to breastfeeding, up to 2 years. Breastfeeding is an essential component of all programs aimed at ensuring the child's rights. To date, the current literature shows that breast milk is the best food, both for babies born in term but also for premature or low birth weight babies. Human milk contains antibodies that reduce morbidity, mortality, and hospital admissions. Breastfeeding reduces the risk of obesity in childhood and adolescence, decreases the risk of developing diabetes and cancers (WHO, 2013).

If breastfeeding is not possible, human milk banks play an essential role. Worldwide, there is a growing interest in the importance of human milk banks for proper infant nutrition (Haiden N. and Ziegler E.E., 2016). If access to a human milk bank is not possible, infant formula will be used, always adapted individually, according to the baby's particularities. Milk formulas provide the qualitative and quantitative needs appropriate to each infant's development and growth stage while respecting European standards (EFSA, 2014).

After 6 months, infants present a period of rapid growth and are susceptible to nutrient deficiencies or over nutrition. That is why it is recommended to introduce complementary feeding. The European Society for Paediatric Gastroenterology, Hepatology, and Nutrition published in 2017 a position paper regarding complementary feeding. Thus, there are well-defined recommendations regarding the starting of complementary feeding, the use of cow's milk, the introduction of potentially allergenic foods, and gluten (Fewtrell M., Bronsky J., Campoy C., 2017).

In Romania, the prophylactic administration of vitamin D and iron in the first 18 months of life is recommended, according to the Romanian Ministry of Health (Protocols for the prophylaxis of anemia and rickets in children, 2010).

The proper nutrition in teenagers and pregnancy period represents "the future key" to break the vicious cycle of intergenerational malnutrition and chronic diseases (Das J.K., Salam R.A., Thornburg K.L. et al., 2017). Thus, the development of National Health Programs for teenagers' health care can significantly contribute to improving the population's health. In 2016, WHO recommended pregnant women healthy eating patterns and physical activity to prevent obesity during pregnancy (WHO, 2016). These recommendations include fresh fruits and vegetables, regular meals, reducing intake of products containing caffeine, candy, snacks, and sweet beverages.

- ***Electronic Entertainment Devices***

The results obtained after processing the data from the questionnaires are synthesized in Table XI.

Most obese adolescents, 94,7% are spending their free time in front of the TV, and 68.4% are using the computer. The daily activity of adolescents is affected by the TV/ computer (I3) but was only recognized by 7.9%.

Analysing the type of TV shows (I4), many adolescents (78,9%) preferred films and cartoons. A large proportion (78.94%) of adolescents spent 1-3 hours in front of the TV (I5) and 21,05% between 3-5 hours. Also, 42.1% of the adolescents spent more than 1 hour in front of the computer (I6), and only four (10.5%) spent more than 5 hours. The reasons adolescents spent many hours in front of electronic devices (I7) are relaxation (21.1%), and only 10.55% of them are using the computer to obtain information quicker.

Regarding preventing excessive use of the TV or computer (I8), school activities are noted (31.6 %) while spending time with the family and socializing with friends was only found

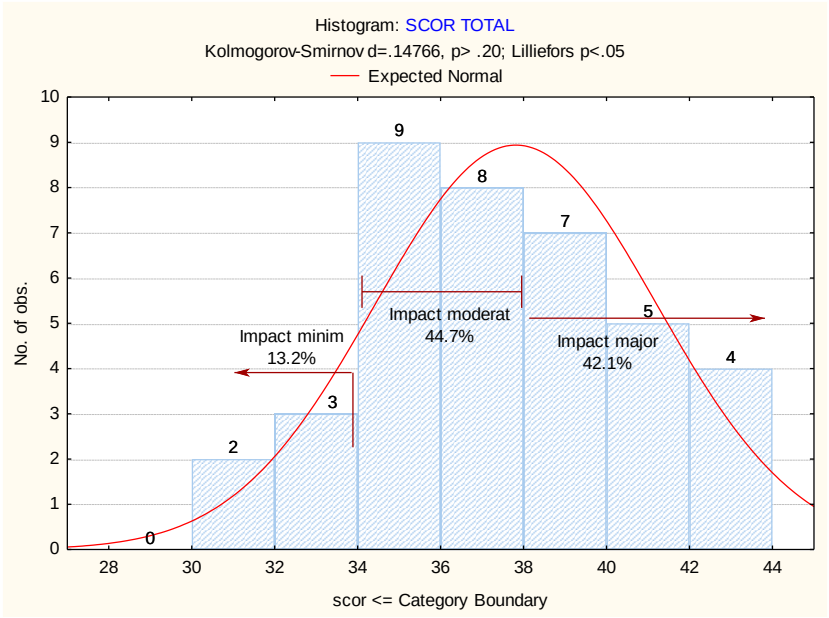
in 20.1% of cases. The consequences of a long time spent in front of the electronic devices in the study group (I9) were fatigue (57.9%), addiction (21.05%), isolation, and boredom (10.5% for each). On the other hand, the positive effects (I10) included: relaxation (47.36%), information (31.57%), and communication (10.52%). By negative effects of TV/computer use (I11), were mentioned violence/aggression (28.9%), learning problems (21.05%) and attention difficulties (18.42%).

Other activities that replace spending time on TV/computer (I12) are meeting with friends (21.05%), listening to music (10.5%), and practicing a sport (10.5%). Regarding activities performed in front of the computer (I13) are mentioned social networks (31.6%), searching for information (10.5%), and watching movies/music videos (26.3%). Almost half of the adolescents (42,1%) consider that the impact of TV/computer on school activity (I14) has significant importance. In comparison, parents try to limit the exposure to TV/computer (I15) in only 47.36% of cases.

Table XI. Results of the questionnaire on the impact of computer and television use on adolescent behaviour

Questionnaire item	**N (%)**
I1 YES/NO	36/2 (94.7%/5.3%)
I2 YES/NO	26/12 (68.4%/34.6%)
I3 To a great extent/Largely/Moderately/To a small extent	12/16/7/3 (31.6%/42.1%/18.4%/7.9%)
I4 Documentation / Cartoons / Advertising / Advertisements / Sports	14/16/4/4 (36.8%/42.1%/10.5%/10.5%)
I5 Less than one hour/ Within 1-3 hours	30/8 (78.9%/21.1%)
I6 None / Less than one hour/ Between 1-3 hours / More than 5 hours	14/8/12/4 (36.8%/21.1%/31.6%/10.5%)
I7 Defeat boredom / I'm interested in some shows /Provide faster information / I'm having fun / Other	8/8/4/4/14 (21.1%/21.1%/10.5%/10.5%/36.8%)
I8 I have many homework to do / My parents forbid me / I meet with friends / I spend time with my family / Do other things	12/4/4/4/14 (31.6%/10.5%/10.5%/10.5%/36.8%)
I9 Fatigue / Isolation / Boredom / Addiction	22/4/4/8 (57.9%/10.5%/10.5%/21.1%)
I10 Communication / Information / Relaxation / Education-Learning	4/8/12/4(10.5%/31.6%/47.4%/10.5%)
I11 Difficulties in Communication / Attention Difficulties / Learning Issues / Violence / Aggression	12/7/8/11 31.6%/18.4% /21.1%/28.9%
I12 Read / I'm walking through the park / Drawing / Painting / Listening to music / Meeting with friends / Preparing for school / Others	6/4/8/4/8/4/4 (15.8%/10.5%/21.1%/10.5%/21.1%/10.5%/10.5%)
I13 Staying on social networks / Looking for information / downloading movies, music / exploring various WEB pages / solving tasks for school	12/4/8/4/10 (31.6%/10.5%/21.1%/10.5%/26.3%)
I14 To a great extent/Largely/Moderately/To a small extent	10/8/16/4 (26.3%/21.1%/42.1%/10.5%)
I15 Are against exaggerated use / They impose program compliance / They use as much as I do	18/12/8 (47.4%/31.6%/21.1%)

A score over 40 points (25% -Q75 of cases), is indicating that the use of electronic devices has a significant impact on adolescent behaviour and demonstrates that 75% of the adolescents have a score higher than 35 points, which corresponds to a moderate and major impact (Fig. 14).



Mean Scor	Mean -95%	Mean +95%	Dev. std.	Er. std.	Min	Max	Q10	Q25	Me	Q75	Q90
37.8	36.7	38.9	3.4	0.55	31.0	43.0	33	35	37	40	43

Figure 14. *Histogram of total score values of the impact of computer and TV use on adolescent*

A direct correlation was found between the adolescents' age and the total score (r=0.41, p=0.0195, 95%CI) (Fig.15).

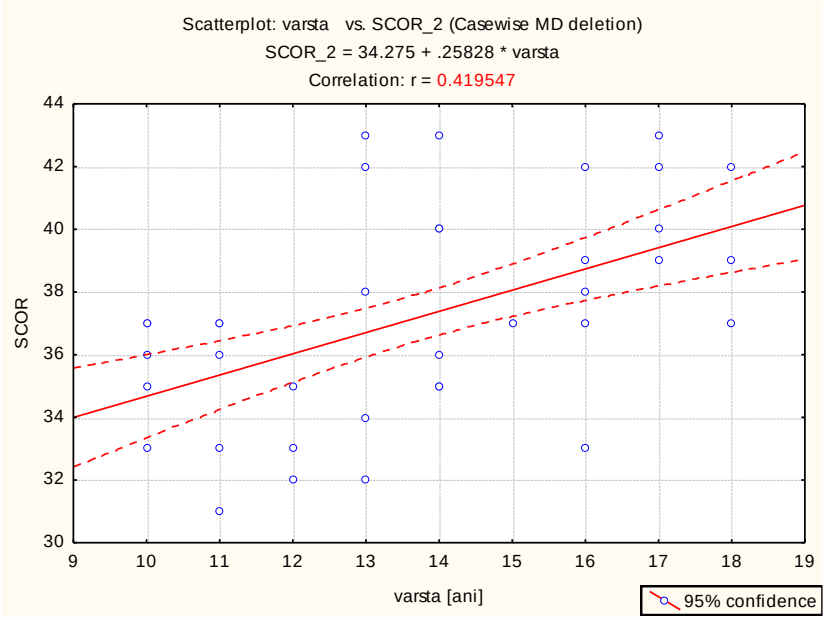


Figure 15. *Regression line in age correlation vs. total score*

The prevalence of infant obesity is three times higher in the urban area (76.4%) when compared to the rural area (23.6%), more in the male sex (51.3%) (Table XII).

Regarding family history, 5% of adolescents do not have a family history of obesity, while 70% of them have single obese parents, and 25% have both obese parents.

Table XII. Anamnestic characteristics and other relevant aspects of studied patient group

Characteristics	N (%)
The area of origin (Urban/ Rural)	29/9 (76.4%/23.6%)
Male / female	20/18(52.6%/47.6%)
Family history (negative / positive: a parent/obese parents)	2/36 (5%/95%: 70%/25%)
Psychological investigation	
Low self-esteem	18(47.4%)
Hyper-protective mother	8(21.1%)
Tendency to antisocial behavior	2(5.3%)
Feelings of inferiority	8(21.1%)
Depression	2(5.3%)

† Values were expressed as number (%) or percent at %

The results of multivariate analysis (multiple linear regression) show that the urban background was an important risk factor for adolescent's lifestyle (β = 11.058, p = 0.001) next to age (ß = 6.54, p = 0.004), male gender (ß = 3.86, p = 0.029) and low self-esteem (β2.098, p = 0.015) (Table XIII).

Table XIII. Multiple linear regressions. The evaluation of factors de risk

Dependent variable:	Predictors:	Coeff. Beta	Std. Error	t	p-value
Score † (value increase)	Age	6.541	0.104	9.847	0.004*
	The area of origin (Urban / Rural)	11.581	0.278	21.641	0.001*
	Masculine	3.864	0.386	5.274	0.029*
	Family history (a parent /obese parents)	0.654	0.018	0.643	0.083
	Psychological Survey (Low Self-Esteem)	2.098	0.095	2.008	0.015*

(*) Marked effects are significant at p <0.05

The development of technology involves the widespread use of electronic devices (televisions, computers, mobile phones) by children, adolescents, and adults. This access to technology led to the development of sedentary behaviour and unhealthy, fast-food food habits (Verloigne M., Van Lippevelde W., Maes L. et al., 2012). Data from the American paediatric population showed that most adolescents (98.5%) aged 12 to 15 are watching TV daily, and 91.1% use the computer. Nowadays, many studies show that children spend at least 4 hours daily in front of the TV (AAP - American Academy of Pediatrics, 2001) and this was showed in our study because 21.05% of children spent over 3 hours in front of the TV.

During the week, students are watching 2 hours or more on TV while the percentage is higher over the weekend, according to Health Behaviour in School-aged Children by 2014. Romanian students are spending 12-14 hours/day for sedentary activities (at least 2 hours/day

on TV, 2 hours game/ computer, 2 hours Internet socialization, with additional 6 hours spent in the classroom and at least 2 hours for lessons) (Baban A., Balaszi R., Taut D., 2014).

Prolonged exposure to TV or computer is contributing to the increase in weight in the child and adolescent. Other health problems like insomnia, anxiety, confusion, depression, phobias, psychotic behaviour, type 2 diabetes, cardiovascular and bone diseases may appear secondary to low levels of physical activity (Tremblay M.S., LeBlanc A.G., Kho Saunders T. J., et al., 2011). When exposure to TV/computer exceeds two h/day, the risk of depression enhances (Liu M., Wu L., Yao S., 2016).

Another study showed that sedentary children could have the first signs of heart disease and hypertension since six ages. The risk of hypertension increases by 10% for every extra hour of inactivity a day. In terms of nutritional status, a recent study has shown that time spent on TV can be correlated with the intake of dietary elements like protein, minerals, vitamins, and dietary fiber (Tsujiguchi H., Hori D., Kambayashi Y. et al., 2018).

A large-scale study has shown that caffeine intake is significantly higher in children who spent over 2 hours/day in front of the TV (Ahluwalla N., Frenk S.M., Quan S.F., 2018).

Other studies demonstrate a direct relationship between the presence of televisions in adolescents' bedroom and the time spent in front of the TV, contributing to mobile phones or personal tablets (Ye S., Chen L., Wang Q. et al., 2018).

A Canadian study demonstrated that the quality of sleep is lower in children using electronic devices before bedtime. The risk of obesity was double in those who used the TV or computer in their bedroom (Dube N., Khan K., Loehr S., et al., 2017).

Parents, teachers, and physicians' role in educating children about how to prevent a sedentary lifestyle is essential. An active lifestyle must be implemented by the family from the age of childhood to avoid obesity and avoid its long-term comorbidities (de Jong E., Visscher T.L.S., HiraSing R.A. et al., 2013). WHO recommends at least 60 minutes of moderate physical activity a day to limit sedentary of the small aged children (WHO, 2010) because early intervention will improve outcomes in the medium/long term, especially in preventing obesity complications or comorbidities.

- ***Hormonal Contraception in Teenager Girls***

Choosing the right contraceptive method is an important step that must take several critical aspects into account, such as safety, effectiveness, availability, and acceptability. Another part worth mentioning is communication, which should always take place openly and freely between adolescents and their partners and their parents. This matter was further demonstrated by Amialchuk and Gerhardinger in 2015 proving that adolescents who had the opportunity to engage in a dialogue with their parents regarding contraception were less likely to deal with unwanted pregnancies (Amialchuk A. and Gerhardinger L., 2015).

One of the most common and safe contraception methods in pregnancy prevention is represented by hormonal contraceptives. Knowledge and awareness must be paid attention to because risks and benefits should be fully understood. Frequent issues are related to hormonal side effects or confusion about how the pills should be administrated (Apter D., 2018). The best contraceptive should have a few main characteristics, such as being cheap, fully reliable, and convenient to use, resulting in as few side effects as possible. The choice of contraceptive method should not be influenced by the age of the adolescent, who has all the rights to make this decision for herself.

Combined hormonal contraceptive methods are usual among women and adolescents and are based on a combination between estrogens and progesterone. They can take different forms, such as pills, patches, and vaginal rings. A preliminary discussion about side effects and possible disadvantages should occur, given that the adolescent must identify them correctly. A few relevant examples in this context include nausea, breast tenderness, headaches, and breakthrough bleeding. It is necessary to inform adolescents about a crucial aspect regarding oral combined hormonal contraceptives: they do not protect against sexually transmitted diseases.

A newly extended-cycle oral contraceptive was presented as a potential alternative, consisting of 84 tablets containing 0.15 mg of levonorgestrel and 0.03 mg of Ethinylestradiol. Seven tablets containing 0.01 mg of Ethinyl estradiol each. This is recommended for adolescents with conditions (for example, anemia or endometriosis). Adolescents are more likely to observe a failure of such oral contraceptives due to a lack of attention for continuous administration or a lower rate of adherence (White K.O, 2011). Thus, there are other options for those who find it challenging to stick to a tablet-based contraceptive plan: the vaginal ring, the dermal patch, or the transdermal delivery (Raine T.R., Foster-Rosales A., Upadhyay U.D. et al.,1998).

Progestin-Only Contraception. It must be administrated every day at the same hour and considered when other health issues prevent the usage of more common methods, namely obesity or arterial hypertension. DMPA is a highly effective injectable contraceptive appealing to adolescents because of its dosing scheduled four times per year. This method's effectiveness is superior to that of estro-progestatives contraceptives, possibly due to administration (Winner B, Peipert J.F., Zhao Q. et al., 2012). Two issues should be discussed with adolescents regarding the use of DMPA. The first one is represented by the common side effect described by amenorrhea. They should be explained about this side effect. The second issue is that DMPA can decrease bone mineral density. These methods are the first choices for adolescents who have difficulty taking their pills regularly or for teenagers with developmental disabilities.

Depot medroxyprogesterone acetate stands for a perfect and efficient contraceptive practice based on injections, making it more tempting for adolescents who do not have to worry about daily administration. Side effects such as amenorrhea and a decrease in bone mineral density must be addressed and explained thoroughly before reaching a decision. DMPA is preferred in cases of teenagers displaying developmental disabilities or an evident struggle with taking pills regularly.

Hormonal intrauterine devices *(IUD*s): when discussing the IUD method, the chances of contracting a sexually transmitted disease are much higher, which further requires dual protection. The Contraceptive CHOICE Project, which involved patients with ages ranging from 14 to 19 years old, proved that more than 80% of reversible contraceptive methods continued with this method as opposed to only one-half focusing on the short-active ones (Klein D.A., Arnold J.J., Reese, E.S., 2015). Once a contraceptive method was preferred and agreed upon, it is necessary to decide on a date for the follow-up appointment, which usually takes place after 3 to 6 weeks. It represents the best opportunity to discuss all possible side effects, potential changes if the adolescent is not content with the choice made and, more broadly speaking, solve all problems that might have occurred in that period.

II.1.2.4. Conclusions

- ***Early Nutrition During the First 1000 Critical Days***

There is growing evidence that nutrition is one of the environmental factors influencing chronic diseases from childhood to adulthood. The effect of nutritional deficiencies begins in the prenatal stage up to the epigenetic level. It continues during pregnancy and in the first months of life when breastfeeding is the crucial element. Measures to prevent childhood and adult obesity must begin in the early 1000 days of life through national health strategies and programs to ensure future generations' health.

- ***Electronic Entertainment Devices***

TV and computer exposure had an essential negative impact on 86.85% of adolescents' health in our study. Therefore, prevention programs for obesity need to become a priority in the public health area. These programs can include promoting physical exercise in schools or in free time for reducing the number of hours spent in front of the TV/computer. The effects of these lifestyle changes are beneficial for the medium and long term to prevent childhood obesity.

- ***Hormonal Contraception in Teenager Girls***

Contraceptive counselling is a pivotal element that sustains proper health and nutritional status for adolescents. Unfortunately, teenage pregnancy is common worldwide, and the short- and long-term consequences for adolescent health are disastrous. Hormonal contraception is widely used, and great attention must be paid when explaining the correct manner of regular administration and possible side effects, like weight gain and obesity. Teenagers' contraceptive needs should be taken very seriously and addressed in programs implemented for their safety and protection. Excess weight is often a reason for low compliance with oral contraception use among adolescent girls.

II.1.3. THE PRACTICAL APPROACH OF CHILDHOOD OBESITY DIAGNOSIS

II.1.3.1. Introduction

The diagnostic criteria for obesity are different for children than adults. High BMI is not enough to diagnose obesity at pediatric age. The adipose tissue and its distribution should be evaluated and compared to percentiles for age and sex. To assess complications and the best course of treatment, a specialist must consider the psychological, physical, and biological particularities of each obese child (Sahoo K., Sahoo B., Choudhury A.K. et al., 2015).

The initial child evaluation is critical to establish the correct diagnosis. The complete anamnesis should evaluate the personal and family history of obesity: the perinatal data, the personal antecedents, the family history, and behavioural particularities. The perinatal anamnesis includes information related to the mother's health (like obesity, GDM, treatments during pregnancy) and, respectively, about the child (birth weight, feeding in the first year of life). The family history should include data about obesity, dyslipidemia, cardiovascular diseases, type 2 DM, obstructive sleep apnea, polycystic ovary syndrome, bariatric surgery. The behaviour particularities important for obese children evaluation are represented by family

eating habits, activity patterns, and sleep programs (Sahoo K., Sahoo B., Choudhury A.K. et al., 2015).

The anamnesis should be followed by a complete physical exam, including anthropometric indices (weight and height, BMI, WC, and skin-fold thickness), body fat distribution, measurement of blood pressure, identification of clinical signs of potential comorbidities, endocrine disturbances, etc (Pereira P.F., Serrano H.M., Carvalho G.Q. et al, 2015).

In children, the nutritional status is evaluated by plotting the BMI on a chart for age and sex. In Europe, researchers classified overweight as at or above 85th percentile and obesity as at or above 95^{th} percentile of BMI (Ghosh A., 2014). WC represents a clinical parameter used to evaluate the risk of cardio metabolic comorbidities associated with obesity. WC must be performed in all overweight and obese patients older than five years.

Accurate measurement of the blood pressure in obese children is essential. For that, the practitioner should use a suitable cuff, and the values of systolic or diastolic blood pressure must be higher than 95^{th} percentile for age, gender, or height for a minimum of 3 measurements for the diagnosis of hypertension (von Ruesten A., Steffen A., Floegel A., et al., 2011).

Any clinical signs of endocrine complication should be early diagnosed and treated. Thus, the acanthosis nigricans is associated with IR, hirsutism and excessive acne with polycystic ovary syndrome, violaceous striae and moon facies with Cushing's syndrome. Other clinical manifestations such as polydipsia, polyuria, and nocturia draw attention to T2DM.

Long-term obesity evolution predisposes to respiratory diseases (sleep apnea or asthma), hepatomegaly or abdominal pain (NAFLD or gastroesophageal reflux), musculoskeletal impairment (pain in the hip or the knee, genu valgum, Blount disease, slipped capital femoral epiphysis), and psychological diseases (the sign of depression, distorted body image, eating disorders like bulimia nervosa, distorted social relationships) (Flynn M.A., McNeil D.A., Maloff B., et al., 2006).

The obese child's first assessment should include a standard set of investigations, which could be combined with other examinations and analyses depending on the anamnesis and physical examination results. The standard serum biochemical parameters include total and all fractions of cholesterol (LDLc, HDLc, VLDLc), triglycerides, liver function tests, fasting plasma glucose, insulinemia, urea (Freedman D.S., Kahn H.S., Mei Z., et al., 2007).

The second-line tests contain the abdominal ultrasound, the oral glucose tolerance test (OGTT), HbA1c, hormonal dosages, polysomnography (Umer A., Kelley G.A., Cottrell L.E. et al., 2017). The OGTT is necessary for every overweight pediatric patient who has a value of the fasting blood glucose higher than 100 mg/dL, or has family members with DM, or has any indicators of IR, metabolic syndrome or polycystic ovary syndrome (NCD-RisC, 2017). If the value obtained in this test is higher than 126 mg/dL, a second test is required. As these patients are at risk of developing diabetes, the value of HbA1c should be investigated further.

Metabolic syndrome should be suspected and further investigated when a minimum 3 of the following criteria are met: BMI suggestive for obesity, WC > 90th percentile, hypertension, high values of serum triglycerides (>95th percentile), decreased values of serum HDL_c (Tabel XIV).

Table XIV. The International Diabetes Federation (IDF) criteria for defining the MS in pediatric patients starting with age six

Age	Recommendations
6 to 10 years	-WC > 90th percentile -even if the diagnosis of MS is not clear, if there are family members with MS, hypertension, obesity, dyslipidemia or T2DM, the evaluation should be like in a MS -IDF recommends losing weight in the patients with abdominal obesity.
10 to 16 years	-WC > 90th percentile (or adult cut-off if lower) -serum values of triglycerides >1.7 mmol/L -serum values HDL_c <1.03 mmol/L -high values of blood pressure (systolic blood pressure >130 mmHg, diastolic blood pressure >85 mmHg) -serum glucose >5.6 mmol/L (further investigations are required - OGTT)
>16 years	IDF uses criteria from adults. Defining MS requires central obesity (measured through MS) and any 2 of the following: -blood levels of triglycerides >150 mg/dL (1.7 mmol/L) or previous treatment for this disturbance. -decrease blood levels of HDL_c <40 mg/dL (1.03 mmoL/L) in males and < 50 mg/dL (1.29 mmoL/L) in women or previous treatment for this disturbance. -high values of blood pressure (systolic blood pressure >130 mmHg, diastolic blood pressure >85 mmHg) or previous treatment or diagnosis of hypertension -increased fasting plasma glucose >100 mg/dL (5.6 mmol/L) or anterior diagnosis of T2DM.

In obese patients with high blood pressure (BP) values, complete cardiac examination (ECG, echocardiography), urine exam, and assess creatinine and potassium serum levels are compulsory (Rolland-Cachera M.F., 2011).

Even though vitamin D deficiency is frequently correlated with obesity and the risk of developing T2DM is higher in these patients, the guidelines still do not include routine vitamin D dosage among their recommendations (de Onis M. and Lobstein T., 2010).

The abdominal ultrasound exam to evaluate the liver's structure and size is necessary for every child and adolescent with obesity. If the value of ALT is higher than 40 IU/L or the physician feels hepatomegaly at the abdomen examination, other investigations for diagnosing liver injury are required (Alpert M.A., Omran J., Bostick B.P., 2016). Also, there are many psychological disorders associated with weight change: depression, uncontrolled eating, distorted body image, unhealthy methods of losing weight, bulimia nervosa, binge-eating disorder. A carefully psychological and psychiatric examination is necessary in these cases.

Increased BMI is not enough to diagnose obesity at paediatric age. To assess complications and the best course of treatment, a specialist must consider the psychological, physical, and biological particularities. We know that insulin resistance (IR) and chronic inflammation are involved in the pathogenesis of obesity-associated comorbidities. Early CVD predictors include BMI, hypertension, dyslipidemia, IR and elevated circulating inflammatory molecules (Raj M., 2012). Inflammatory changes during the early stages of childhood obesity impair metabolic and cardiovascular health (Kim J., Bhattacharjee R., Kheirandish-Gozal L. et al., 2010). The adipose tissues release many inflammatory mediators which predispose people to a proinflammatory 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. Studies have shown that high levels of IL-6 are associated with metabolic or cardiac comorbidities in adulthood. Additionally, systemic, low-level elevations of gut-derived endotoxin has a role in metabolic changes in obesity (Boutagy N.E., McMillan R.P., Frisard M.I. et al., 2016). 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 K.A., Hätönen K.A., Lehto M. et al., 2015). Early identification of signs of cardiovascular impairment and IR in obese children and adolescents is essential for the precocious establishment of lifestyle correction measures and ensuring long-term health.

The scientific interest related to clinical and biological evaluation of obese children were synthesized in the following articles:

1.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, 2020, 99:30. (**IF=1.552)**

2. 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, 735. **(IF=3,11)**

Objective

Using the theoretical data mentioned above, the two articles aimed:

- ***Waist Circumference a Clinical Criterion for Prediction of Cardiovascular Complications***

To establish if WC represents a clinical criterion for predicting vascular and cardiac impairment in obese children.

- ***Predictive Markers of Early Cardiovascular Impairment and Insulin Resistance***

One of the crucial aims of preventing the comorbid conditions associated with obesity is to detect children with early higher cardiovascular risk. In this study, we evaluated the presence of early markers of cardiovascular risk represented by IL-6, Intercellular Adhesion Molecules (ICAM) and endotoxemia and their correlation with metabolic markers of IR represented by insulinemia, HOMA index and plasma cortisol.

II.1.3.2. Materials and methods

- ***Waist Circumference a Clinical Criterion for Prediction of Cardiovascular Complications***

One hundred sixty pediatric patients hospitalized in the Saint Mary Emergency Children Hospital Iași, Romania, were included in this retrospective study. The inclusion criteria

employed consists of overweight and obesity diagnosis without any associated pathologies. All participants have signed an informed consent form before taking part in the research. The patients were divided into two age groups: Group A: children between 6–11 years old; Group B: adolescents between 12–18 years old.

The authors evaluated anthropometric data that have included height, weight, WC, and the body mass index (BMI) in these patients. According to the BMI Z score, the patients were diagnosed with overweight (BMI Z score >+1SD or BMI percentiles between 85-97th), obesity (BMI Z score >+2SD or BMI percentiles between 97-99,9th) or severe obesity (BMI Z score > +3 SD or BMI percentiles> 99.9th). Values over the 90th percentile of WC defined visceral obesity (Bassali R., Waller J.L., Gower B., Allison J. et al., 2010).

The biochemical evaluation included: total cholesterol (TC), low-density lipoprotein cholesterol (LDLc) and high-density lipoprotein cholesterol (HDLc), triglycerides (TG), glucose levels, alanine aminotransferase values, ureea and creatinine values. The children's BP value was measured, and systolic BP and diastolic BP over the 95th percentile defined hypertension. Usually, high blood pressure was considered the values between the 90-95 percentile values. According to the "National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents, 2004", BP values ≥ 90th percentile were defined as "elevated BP" or vascular impairment (The Fourth Report on the Diagnosis, Evaluation, and Treatment of High Blood Pressure in Children and Adolescents, 2004).

Imaging evaluation included echocardiography for every patient. Patients who were diagnosed with concentric (LVM index more significant than the 95th percentile and elevated RWT >0.41) or eccentric (increased LVM index and normal RWT <0.41) LV hypertrophy, concentric remodelling (standard LVM index and normal RWT <0.41), and epicardial fat (over 4.1 mm) were considered to have a cardiac impairment.

Statistical Analysis

Data analysis was performed using SPSS 20.0 (Statistical Package for Social Sciences, Chicago, Illinois). For the continuous variables, data were presented as mean ± standard deviation (SD). Comparisons were performed using Student's t-test or Mann-Whitney U Test for continuous variables, and the comparisons between groups were made with McNemar, Yates Chi-square, or Fisher's exact test. Analysis of prognostic factors was performed using the Logistic regression model. The significance level calculated (P-value) was considered significant for P values <0.05.

- ***Predictive Markers of Early Cardiovascular Impairment and Insulin Resistance***

We conducted a prospective study on two groups of pediatric patients: the study group and the control group. The study group included 85 obese pediatric patients between 6 and 18 years old with obesity without associated pathologies followed in Children′s Hospital "Sfânta Maria" Iaşi between January 1st and December 31st, 2019. The control group included 30 pediatric patients with normal BMI. The inclusion criteria were patients with newly diagnosed obesity who did not benefit from dietary and/or pharmacological treatment. 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), and/or administration of

any chronic therapy in the previous three months. Only children and adolescents who provided informed consent (including parental consent) were included in the study.

Anthropometric and Biochemical Measurements included body weight, body height, WC and BMI. The interpretation of BMI values was made according to the BMI percentile, applicable for age and sex, according to the CDC standards. We defined visceral obesity by WC values above the 90th percentile (Bassali R., Waller J.L., Gower B., Allison J., et al., 2010). In all patients, the BP value was determined, and the obtained values were evaluated according to the percentiles for age and sex. In our study, BP values ≥90th percentile were defined as "elevated BP" or vascular impairment (The Fourth Report on the Diagnosis, Evaluation, and Treatment of High Blood Pressure in Children and Adolescents, 2004).

Total lipid profiles, liver function tests, total protein, blood glucose and creatinine were measured. The cardiovascular risk parameters were defined by age and sex. Borderline cardiovascular risk was considered when the TC was between 180–199 mg/dL. Medium cardiovascular risk was defined as a TC value between 200–249 mg/dl or LDLc value between 130–159 mg/dl according to Italian Society for Paediatric Nutrition, 2000 (Guidelines for the prevention of atherosclerosis in pediatric age, 20008). High cardiovascular risk was defined by TC value over 250 mg/dL or LDLc value over 160 mg/dL.

IL 6, ICAM, endotoxemia, insulinemia, plasma cortisol and HOMA-IR (Homeo-stasis model assessment) evaluated the inflammatory and metabolic status. We used enzyme-linked immunosorbent assay (ELISA) kits for the quantitative detection of human IL-6, sICAM-1 and cortisol. The ELISA kits are for research use only, not for diagnostic or therapeutic procedures. Cortisol was determined using an ELISA kit in human serum. The reference values for cortisol in serum were 60–230 ng/mL. For determining IlL-6 and ICAM-1, we used BioVendor kits. The amount of IlL-6 or ICAM-1 was determined extrapolating OD values against IlL-6/ICAM-1 standard concentrations using a standard curve. The minimum detectable concentrations were 0.10 pg/mL for IL-6, 2.2 ng/mL for ICAM-1 and the interassay coefficient of variation was 7.0% for all kits. Endotoxin (ET) was determined using the Abbexa ELISA kit. The sensitivity is <0.005 EU/mL and the concentrations range of ET in serum 0.015 EU/mL–1.0 EU/mL using the Abbexa ET kit.

Informed consent was obtained from all participants and their parents in accord-ance with the Helsinki Declaration revised in 2013. The study was approved by the Ethical Committee of the Saint Mary Children Hospital (14055 / 19.06.2018).

Statistical Analysis

Statistical analysis of the data was performed using the STATA 16 software (StataCorp LLC, College Station, TX 77845-4512, USA). Continuous variables were pre-sented as mean values and standard deviation or as median with lower and upper quartile (Q1; Q3). To compare the values of the continuous type parameters corre-sponding to the two groups of patients we applied the ANOVA test or Mann–Whitney U Test depending on the homogeneity of the data series (Levene's test). Qualitative variables were compared based on Pearson Chi-square test results. To verify the corre-lations between the continuous type variables, we applied the Pearson univariate cor-relation 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 char-acteristic (ROC) curve and the AUC value (area under the ROC curve). In the statistical analysis, the reference threshold for the level of significance p was 0.05. A p value <0.05 indicated with 95% confidence that there was statistical significance.

II.1.3.3. Results

- ***Waist Circumference a Clinical Criterion for Prediction of Cardiovascular Complications***

In this article we analysed the clinical, biological, and imagistic characteristics of all 160 pediatric patients included in this study.

Group A included 97 patients aged 6 to 11 years (mean age 9.82 ± 2.2 years), and group B included 63 patients aged 12 to 18 years (mean age 14.7 ± 1.6). We observed the predominance of the male sex in both groups (59.8% in group A compared with 60.3% in group B) (Table XIV)..

Table XIV. Baseline characteristics

	Study lot (n = 160)			
Baseline characteristics†	**Group A: 6-11 years (n = 97)**	**Group B: 12-18 years (n = 63)**	**Statistical test**	**P-value**
Age: years	9.82±2.2	14.7±1.6		
Gender, (men/women)	58/39 (59.8%/40.2%)	38/25 (60.3%/39.7%)	0.0041ǂ	.9473
Environment (urban/rural)	47/48 (49.5/50.5%)	36/26 (58.1%/41.9%)	1.1142ǂ	.2911
BMI (kg/m2)	24.4±3.5	27.5±3.8	27.3245†	<.001*
Percentiles BMI	98.67±2.16	96.80±3.14	-5.1565†	<.0001*
WC (cm)	80.7±11.6	93.6±9.8	34.2075†	<.001*
Percentiles WC (median)	99.6	97.7		
Visceral obesity (No/Yes)	39/58 (40.21%/59.79%)	23/40 (36.51%/63.49%)	0.2207ǂ	.6385
Nutritional status				
Overweight	13 (13.44%)	28 (44.44%)	28.4990ǂ	<.001*
Obesity	52 (53.61%	31 (49.21%)		
Severe obesity	32 (32.99%)	4 (6.35%)		
Cholesterol (mg/dl)	166±29.4	177.7±43.5	3.0882†	.0814
Triglyceride (mg/dl)	96.05±63.03	125.87±73.69	-2.4507†	.0142*
Vascular impairment (No/Yes)	**76/21 (78.35% /21.65%)**	**32/31 (50.79%/49.21%)**	**13.1127ǂ**	**.0002***
Systolic BP	126.94±24.32	133.89±26.74	0.8198†	.3700
Diastolic BP	80.31±15.39	85.25±12.33	1.4810†	.2299
Normal value BP	75 (77.3%)	31 (49.2%)	16.1997ǂ	.0003*
Pre-hypertension	8 (8.3%)	19 (30.2%)		
Hypertension	14 (14.4%)	13 (20.6%)		
Cardiac impairment (No/Yes)	**37/60 (38.14%/61.86%)**	**22/41 (34.92%/65.08%)**	**0.1710ǂ**	**.6792**
IVS thickened (normal/>9/>1.2)	90/7/0 (92.8%/7.2%/0%)	56/6/1 (88.9%/9.5%/1.6%)	2.1738ǂ	.3372
IVS (cm)	0.76±0.16	0.85±0.17	12.4642†	.0005*
Pw (cm)	0.80±0.17	0.92±0.23	14.8411†	.0001*
DdLV (cm)	3.98±0.461	4.62±0.573	59.1216†	<.001*
RWT	0.39±0.08	0.38±0.09	0.3550†	.5521

RWT>0.42	31 (31.96%)	17 (26.98%)	0.4535‡	.5006
Epicardial fat (yes)	54 (55.67%)	35 (55.56%	0.0002‡	.9886
Epicardial fat (cm)	3.06±1.53	3.24±1.5	0.2885†	.5925
LVM (g)	118.4±37.12	180.9±52.95	76.5654†	<.001*
LVM index (g/m$^{2.7}$)	1.30±1.04	1.24±1.04	0.1002†	.7522
Concentric LVH	87 (89.69%) 10 (10.31%)	53 (84.13%) 10 (15.87%)	1.0599	.3032
Concentric remodeling LV	75 (77.32%) 22 (22.68%)	55 (87.30%) 8 (12.70%)	2.6027	.1066
Eccentric LVH	79 (81.44%) 18 (18.56%)	47 (74.60%) 16 (25.40%)	1.0540	.3045

Continuous variables were expressed as: mean ± standard deviation; categorical variables: number (%)
† Student's t-test or Mann-Whitney U Test for continuous variables
‡ Chi-square test (McNemar Chi-square/Yates) or Fisher's exact test;
(*) Marked effects are significant at P <.05

Obesity was prevalent in both groups (53.61% in children and 49.21% in adolescents). Severe obesity was commonplace in study group A, 32.99%, while in group B, 44.44% of adolescents had overweight (Table XIV). Obesity and severe obesity were associated (P <.001) with the age of fewer than 12 years, and at this age category, BMI had significantly higher mean values (Table XIV). BP values shows that at obese adolescents (8.3% vs. 30.2%; P = .0003) pre-hypertension and hypertension are more frequent (20.6% vs. 14.4%; P = .0003). Statistically significant differences between children and adolescents were observed only regarding triglycerides values (p = .0142) (Table XIV). Table XV evaluated the correlation between epicardial fat and visceral obesity in children and adolescents.

Table XV. Evaluation of the association of epicardial fat *vs.* visceral obesity

	Visceral obesity			
	Absent	**Present**	**Statistical test‡**	**P-value**
Pathological epicardial fat				
No (n/%)	45/97.83%	72/78.26%	11.7281	0.0006*
Yes (n%)	1/2.17%	20/21.74%		
Visceral obesity vs. pathological epicardial fat		**Asymptotic 95% Confidence Interval:Lower Bound-Upper Bound**		
Area Under the Curve	0.668	0.562-0.775		0.014*

‡ Chi-square test (McNemar Chi-square/Yates)

Visceral obesity was associated (χ^2 = 11.72, P=0.0006) with the presence of pathological epicardial fat in 21.74% of 92 cases.

Pathological epicardial fat has an increased predictive power (AUC = 0.668, 95% CI: 0.562-0.775, P = 0.014) in the presence of visceral obesity (Figure 16).

The evaluation of the AUC revealed that BMI is not a significant predictor for vascular impairment for either children or adolescents (AUC = 0.57, P = 0.327 vs. AUC = 0.54, P =

0.53) but is a predictive factor for the occurrence of cardiac impairment in children (AUC= 0.62, P =0 .041) and adolescent (AUC = 0.66, P = 0.036) (Table XVI).

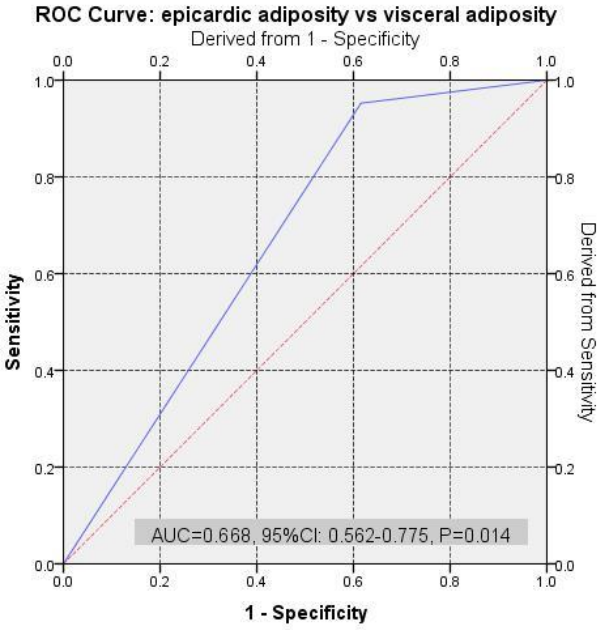


Figure 16*. AUC: Epicardial fat vs visceral obesity*

Table XVI. The estimated parameters in the evaluation of the predictability of the clinical and biological parameters on the vascular and cardiac impairments

	Study cohort (n = 160)			
	Group A: 6-11 years (n = 97)		**Group B: (12-18 years) (n = 63)**	
	AUC (95%CI)	**P-value**	**AUC (95%CI)**	**P-value**
	Vascular impairment			
BMI (kg/m^2)	0.570 (0.426-0.714)	.327	0.547(0.389-0.695)	.530
Visceral obesity	**0.635(0.508-0.762)**	**.059**	**0.669(0.534-0.804)**	**.021***
Cholesterol (mg/dL)	0.518 (0.352-0.683)	.837	0.485(0.316-0.655)	.864
Triglyceride (mg/dL)	0.558 (0.386-0.731)	.488	0.637 (0.477-0.797)	.107
LDL_c (mg/dL)	0.533 (0.291-0.776)	.799	0.650 (0.350-0.950)	.329
HDL_c (mg/dL)	0.489 (0.242-0.735)	.932	0.636 (0.323-0.950)	.366
	Cardiac impairment			
BMI (kg/m^2)	0.620 (0.505-0.735)	.041*	0.666 (0.529-0.802)	.036*
Visceral obesity	**0.559 (0.419-0.700)**	**.432**	**0.697(0.533-.861)**	**.037***
Cholesterol (mg/dl)	0.427 (0.290-0.563)	.296	0.572 (0.400-0.743)	.419
Triglyceride (mg/dl)	0.447 (0.313-0.581)	.453	0.677 (0.513-0.842)	.044*
LDL_c (mg/dl)	0.364 (0.158-0.569)	.198	0.667 (0.565-0.851)	.038*
HDLc (mg/dl)	0.556 (0.335-0.778)	.595	0.308 (0.006-0.609)	.258

Area Under the Curve – AUC; 95%CI - Confidence Interval);
(*)Marked effects are significant at P < 0.05

The evaluation of the AUC revealed that BMI is not a significant predictor for vascular impairment for either children or adolescents (AUC = 0.57, P = 0.327 vs. AUC = 0.54, P = 0.53) but is an predictive factor for the occurrence of cardiac impairment in children (AUC= 0.62, P = 0.041) and adolescent (AUC = 0.66, P = .036) (Table XVII).

For adolescents, visceral obesity is an important predictive factor for the occurrence of both vascular (AUC = 0.669, P = 0.021) and cardiac (AUC= 0.697, P = 0.037) impairment. Also, for adolescents, increased levels of TG and LDLc are predictable for the occurrence of cardiac impairment (AUC = 0.67, P = .044; AUC = 0.66, P = 0.038) (Table XVI).

Table XVII. Estimated parameters in evaluating the predictability of visceral obesity on cardiac impairment

	Group A: 6-11 years (n = 97)			
	LVM index ($g/m^{2.7}$)	**Concentric hypertrophy of LV**	**Concentric remodeling of LV**	**Eccentric hypertrophy of LV**
Visceral obesity				
AUC (95%CI)	0.594 (0.521-0.767)	0.664 (0.621-0.806)	0.408 (0.232-0.585)	0.576 (0.434-0.719)
P-value	.024*	.013*	.327	.314
	Group B: (12-18 years) (n = 63)			
	LVM index ($g/m^{2.7}$)	**Concentric hypertrophy of LV**	**Concentric remodeling of LV**	**Eccentric hypertrophy of LV**
Visceral obesity				
AUC (95%CI)	0.53 (0.509-0.721)	0.716 (0.695-0.836)	0.630 (0.349-0.911)	0.451 (0.285-0.617)
P-value	.035*	.026*	.286	.564

Area Under the Curve – AUC; 95%CI - Confidence Interval); (*)Marked effects are significant at P <0 .05

The results show that visceral obesity can be predictive for incresed the LMV index values in both children (AUC = 0.594, P = .024) and adolescents (AUC copil = 0.53, P = .035) and concentric LV hypertrophy is influenced by the presence of visceral obesity (AUC= 0.664, P = .013 children: AUC= 0.716, P = .026 adolescents) (Table XVII).

- ***Predictive markers of early cardiovascular impairment and insulin resistance***

We included 115 pediatric patients aged between 6 and 18 years old. There were no significant differences in the two study groups related to age and gender of the children. 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.

In Table XVIII, we present the main characteristics of the included patients. Regarding the lipid profile, we observed significant higher values of the triglycerides and lower levels of HDL cholesterol in the obese patients.

We observed significant higher levels of HbA1c, insulinemia and HOMA IR index in obese patients. Moreover, inflammatory markers, IL-6, ICAM 1 and endotoxemia were significantly higher in obese patients versus the control group.

Table XVIII. Comparison of clinical and biochemical parameters between the group of obese pediatric patients and the control group.

Baseline Characteristics	Study Group (*n* = 115)				Statistical Test	*p*-Value
	Control Group (*n* = 30)		Obese Pediatric Patients (*n* = 85)			
	Mean ± SD	Std.Err.	Mean ± SD	Std.Err.		
Age: Years †	**13.4 ± 2.47**	0.34	12.1 ± 3.4	0.29	2.40	0.142
Gender, (boys/girls) ‡	12/18 (40%/60%)		44/41 (51.8%/48.2%)		1.24	0.267
BMI (kg/m^2) †	19.2 ± 1.8	0.28	29.2 ± 5.2	0.47	27.64	<0.001*
Percentiles BMI †						
mean ± SD	40.6 ± 2.3	0.75	97.52 ± 2.4	0.21	65.16	<0.001*
median (Q1; Q3)	40.5 (17; 58)		98 (97; 99)			
Percentiles W C †						
mean±SD	42.8 ± 4.6	0.50	93.9 ± 4.6	0.41	35.78	<0.001*
median (Q1; Q3)	44.5 (34; 50)		95 (92; 97)			
Lipid profile and liver function tests †						
Total serum cholesterol (mg/dl)	152.3 ± 14.1	2.57	165.7 ± 30.7	2.63	5.29	0.023*
Triglycerides (mg/dl)	44.1 ± 5.6	1.02	104.1 ± 51.96	4.33	39.67	<0.001*
LDL cholesterol	64. 3± 5.5	0.98	79.9 ± 24.4	2.03	12.16	0.0006*
HDL cholesterol	79.2 ± 11.7	1.65	64.9 ± 15.2	1.34	22.03	<0.001*
ALT	13.6 ± 8.7	1.58	24.1 ± 15.9	1.39	11.58	<0.001*
AST	18.7 ± 7.2	1.31	22.4 ± 8.9	0.78	4.34	0.039*
TP	70.8 ± 4.1	0.62	73.3 ± 3.4	0.30	10.27	0.001*
Glucidic profile and insulin resistance †						
Plasma glucose level	85.3 ± 9.5	1.03	89.3 ± 11.2	1.82	0.65	0.438
Hb A1c	4.1 ± 0.4	0.07	4.8 ± 0.5	0.04	14.06	0.0002*
Insulin, μU/mL	13.8 ± 9.2	1.57	23.3 ± 14.8	1.36	10.95	0.001
HOMA index	3.17 ± 2.3	0.32	4.94 ± 2.9	0.21	8.85	0.003*
Inflammatory markers and the hormones profile †						
IL6	1.68 ± 1.3	0.22	9.08 ± 15.1	1.25	7.14	0.008*
ICAM 1	385.6 ± 71.7	8.09	481.6 ± 79.7	6.94	33.80	<0.001*
Endotoxemia	3.98 ± 0.1	0.01	3.83 ± 0.2	0.01	24.08	<0.001*
Plasma cortisol	184.7 ± 108.4	9.78	176.1 ± 105.9	9.63	0.145	0.704
Blood pressure †						
SBP, mm Hg	102.3 ± 5.2	0.94	117.7 ± 14.1	1.22	34.13	<0.001*
DBP, mm Hg	60.7 ± 1.6	0.30	73.7 ± 12.5	1.07	32.3	<0.001*
Blood pressure value ‡						
Normal	30 (100%)		46 (54.1%)		20.83	0.0003*
Borderline hypertension	0 (0%)		16 (18.8%)			
Hypertension	0 (0%)		23 (27.1%)			

- Continuous variables were expressed as: mean ± standard deviation; Std.Err.—standard error; categorical variables: number (%). Q1; Q3—lower quartile; upper quartile. † Student's *t*-test or Mann-Whitney U Test for continuous variables. ‡ Pearson Chi-square test. * Marked effects are significant at $p < 0.05$.

Correlations between the BMI Percentile and Biochemical Markers with Inflammatory Markers of Early Cardiovascular Risk (IL-6, Endotoxemia and ICAM 1).

Regarding the changes of the inflammatory markers of the obese patients included in the study, we identified significant correlations between them and BMI. Significant correlations were also identified between certain biochemical parameters and the level of the analyzed

inflammatory markers. Thus, IL-6 correlates significantly with blood glucose (r = -0.334, p = 0.001) and BMI percentile (r = 0.252, p = 0.031) (Table XIX).

We also found a significant correlation between ICAM and serum TG values (r = 0.253, p = 0.001), plasma glucose level (r = -0.145, p = 0.044) and with BMI (r = 0.302, p = 0.037). Additionally, 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 XIX).

Table XIX. Univariate analysis showing correlations between inflammatory markers and biochemical parameters and BMI percentiles.

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	0.253	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 $p < 0.05$.

Thus, we observed that IL-6 was significantly correlated with blood glucose and BMI percentile, these being significant predictive factors for cardiometabolic diseases.

The link between low-grade inflammation, IR and endothelial dysfunction and obesity is currently being studied extensively. Low-grade inflammation was assessed in our study by measuring both IL-6 and ICAM. We did not find a positive correlation (r = 0.09, p = 0.297) between the values of IL-6 and ICAM in the analyzed groups (Fig. 17).

Starting from the results of the univariate analysis, we made a multivariate analysis to evaluate the contribution of each biochemical parameter but also of BMI percentile in the variation of the inflammatory markers. Given the correlations between inflammatory markers and the BMI percentile, we used an ROC curve analysis to evaluate the predictive power of BMI percentile, plasma glucose level and serum triglycerides on IL-6, ICAM and endotoxemia. The results indicated a significant predictive power of BMI percentile on inflammatory markers: IL-6 (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) (Table XX, Fig. 18a,b).

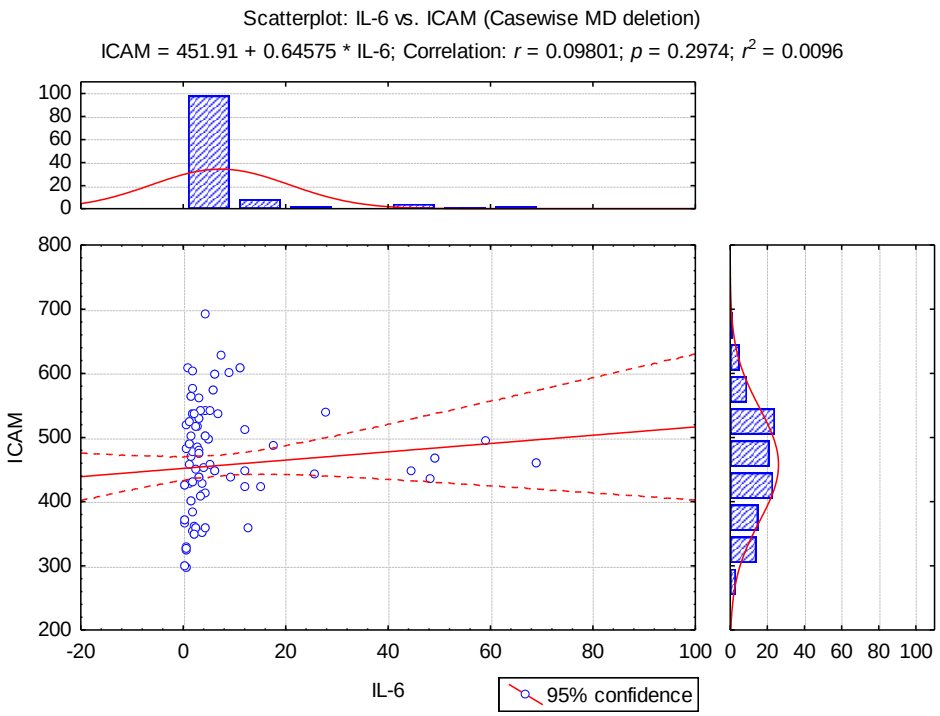
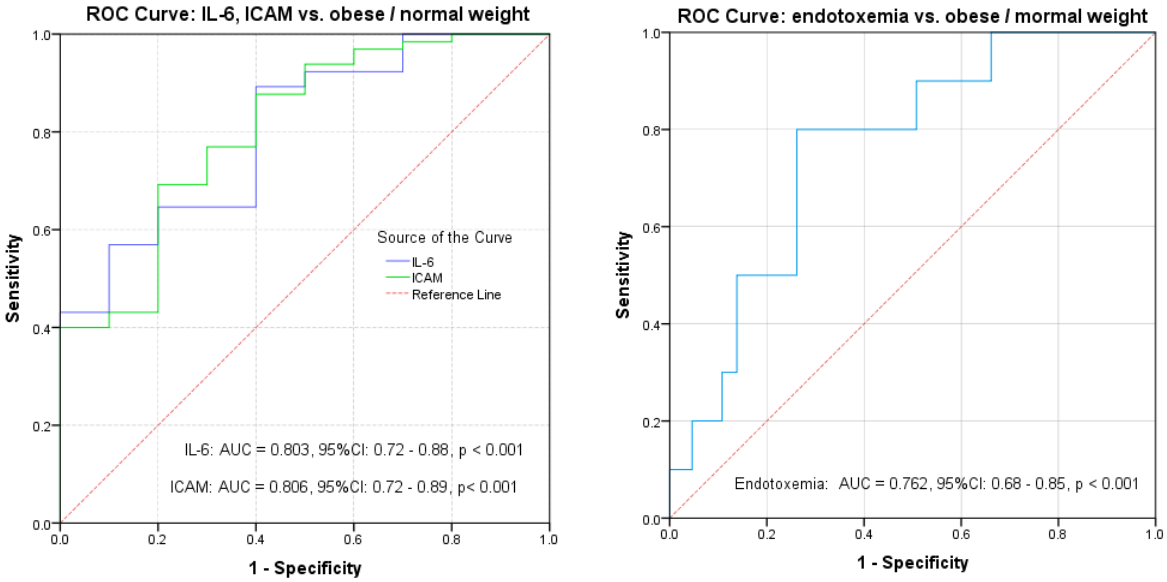


Figure 17. *The regression line in the correlation of IL-6 and ICAM values.*

Table XX. The coefficients of multiple linear regression regarding the correlations between early inflammatory markers of cardiovascular risk and BMI percentile and biochemical parameters

Multiple Linear Regression	Unstandardized Coefficients		Standardized Coefficients	t–Value	*p*–Value
	B	Std. Error	Beta		
Dependent Variable: IL-6					
Total serum cholesterol	0.026	0.042	0.054	0.607	0.545
LDL-cholesterol	−0.126	0.016	−0.207	−1.192	0.235
Triglycerides	−0.050	0.026	−0.184	−1.898	0.060
Plasma glucose level	−0.413	0.091	−0.254	−3.520	0.024*
BMI percentile	0.631	0.054	0.421	2.411	0.017*
Model verification: ANOVA, F = 4.815, p = 0.001*					
Dependent Variable: ICAM					
Total serum cholesterol	0.095	0.168	0.032	0.355	0.723
LDL-cholesterol	1.053	0.154	0.267	1.610	0.109
Triglycerides	0.429	0.064	0.250	2.621	0.010*
Plasma glucose level	−0.726	0.177	−0.100	−1.257	0.211
BMI percentile	0.787	0.142	0.244	2.883	0.005*
Model verification: ANOVA, F = 4.098, p = 0.003*					
Dependent Variable: Endotoxemia					
Total serum cholesterol	0.001	0.001	0.110	1.347	0.180
LDL-cholesterol	0.021	0.001	−0.012	−0.149	0.881
Triglycerides	−0.002	0.001	−0.014	−1.174	0.083
Plasma glucose level	−0.032	0.001	−0.013	−0.195	0.694
BMI percentile	0.452	0.001	0.276	3.185	0.038*
Model verification: ANOVA, F = 5.873, p = 0.028*					

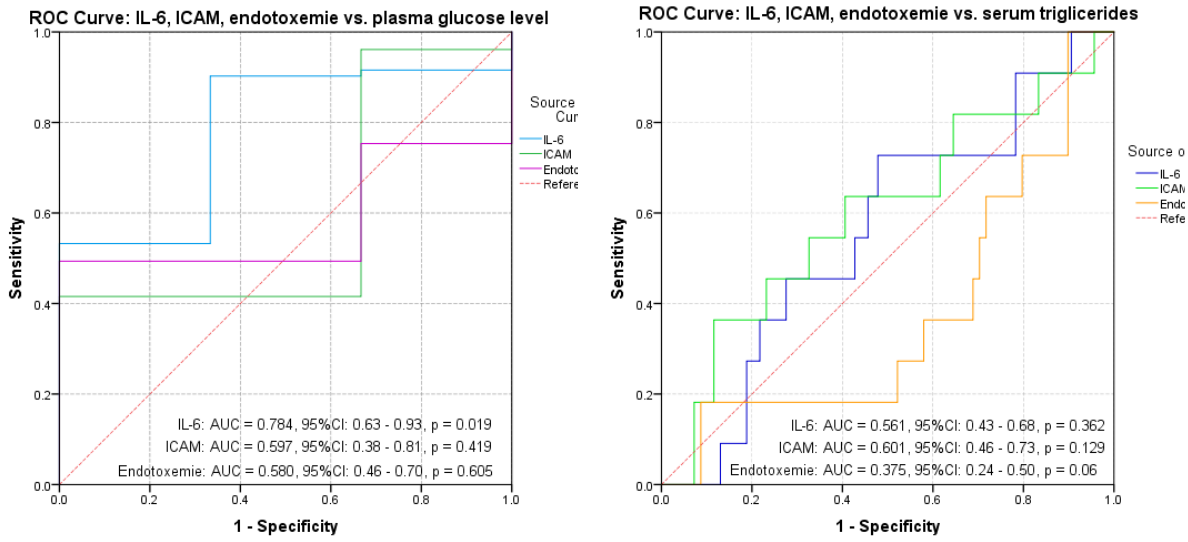
* Marked effects are significant at $p < 0.05$.



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

Figure 18. *ROC curves for BMI vs. (**a**) IL-6 and ICAM and (**b**) endotoxemia.*

Plasma glucose level shows a significant prediction for IL-6 (AUC = 0.784, 95% CI: 0.63–0.93, p = 0.019) (Table XX, Fig. 19a). Although a significant correlation with ICAM was observed in the case of serum TG (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) (Table XX, Fig. 19b).



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

Figure 19. *ROC curves for IL-6 and ICAM and endotoxemia vs. (**a**) plasma glucose level and (**b**) serum triglycerides.*

Identifying Cut-Off Values for Inflammatory Markers in Obese Children and Adolescents

Demonstrating the predictive power of BMI percentile and plasma glucose levels, we identified baseline cut-off values for IL-6, ICAM and endotoxemia for obese children and adolescents with early vascular damage (Fig. 20).

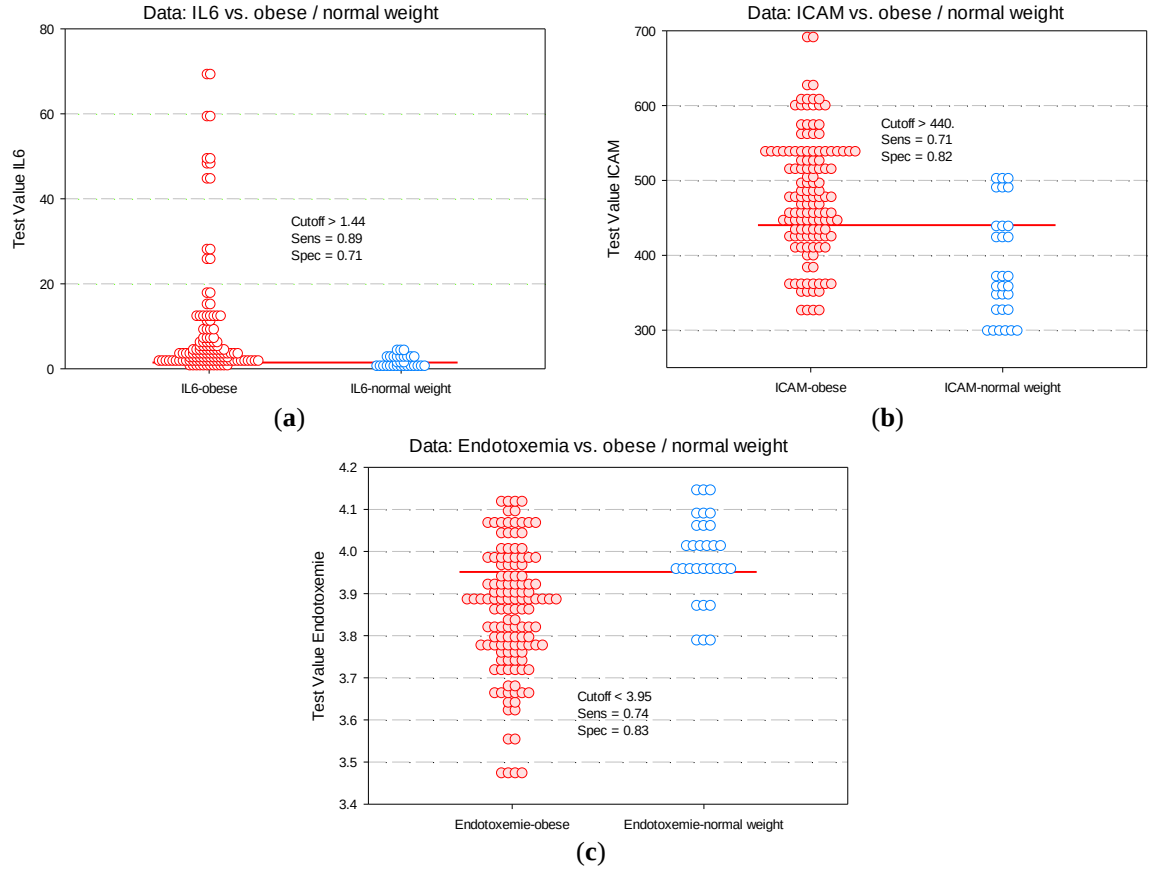


Figure 20. *Identifying cut-off values predictive for vascular impairment in obese children (dot histogram) for: (**a**) IL-6; (**b**) ICAM; (**c**) endotoxemia.*

Correlation of BMI Percentile and Biochemical Markers with Metabolic Markers for Insulin Resistance (Insulin, HOMA Index and Hormones Profile).

Evaluating the correlation between BMI percentile, metabolic markers and the analyzed biochemical parameters, we found that insulin value correlates significantly with BMI ($r = 0.52$, $p = 0.001$), TC ($r = 0.265$, $p = 0.022$) and TG level ($r = 0.228$, $p = 0.006$) (Table XX).

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. Thus, the significant correlation between the HOMA index and the BMI percentile confirms that obesity is a major risk factor for the development of IR. The HOMA index also shows a significant correlation with TC ($r = 0.273$, $p = 0.017$) and with serum TG ($r = 0.205$, $p = 0.009$) (Table XXI).

Table XXI. Univariate analysis showing correlations between metabolic markers of insulin resistance and biochemical parameters and 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 $p < 0.05$.

Considering the results of the multivariate analysis, we could see that between BMI and the analyzed biochemical parameters, only the BMI percentile has a significant predictive power for metabolic markers of insulin resistance (insulin value: AUC = 0.72, $p < 0.001$ and HOMA index: AUC = 0.68, $p = 0.003$) (Table XXII, Fig. 21). This aspect was noted although the univariate analysis revealed other correlations between biochemical parameters (total cholesterol and triglycerides level) and insulin value or HOMA index. We noted that at insulin cut-off values of 14.3 (Fig. 22a) and HOMA index cut-off of 3.32 (Fig. 22b), respectively, the risk of vascular damage in obese children increases.

Table XXII. The coefficients of multiple linear regression regarding the correlations between metabolic markers of insulin resistance and BMI percentile and biochemical parameters.

Multiple Linear Regression	Unstandardized Coefficients		Standardized Coefficients	t–Value	*p*–Value
	B	Std. Error	Beta		
Dependent Variable: Insulin Value					
Total serum cholesterol	0.061	0.049	0.115	1.231	0.220
Triglycerides	−0.018	0.030	−0.060	0.603	0.548
Plasma glucose level	−0.105	0.106	−0.081	−0.992	0.323
BMI percentile	1.217	0.063	0.512	6.820	<0.001*
Model verification: ANOVA, F = 14.712, $p < 0.001$*					
Dependent Variable: HOMA Index					
Total serum cholesterol	0.011	0.010	0.101	1.085	0.280
Triglycerides	−0.004	0.006	−0.072	−0.718	0.474
Plasma glucose level	0.031	0.021	0.119	1.475	0.142
BMI percentile	0.430	0.013	0.520	7.019	<0.001*
Model verification: ANOVA, F = 16.186, $p < 0.001$*					
Dependent Variable: Plasma Cortisol					
Total serum cholesterol	0.111	0.370	0.029	0.301	0.764
Triglycerides	−0.013	0.228	−0.006	−0.441	0.660
Plasma glucose level	0.339	0.796	0.036	0.425	0.571
BMI percentile	2.767	0.472	0.060	0.668	0.062
Model verification: ANOVA, F = 4.815, $p = 0.001$*					

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

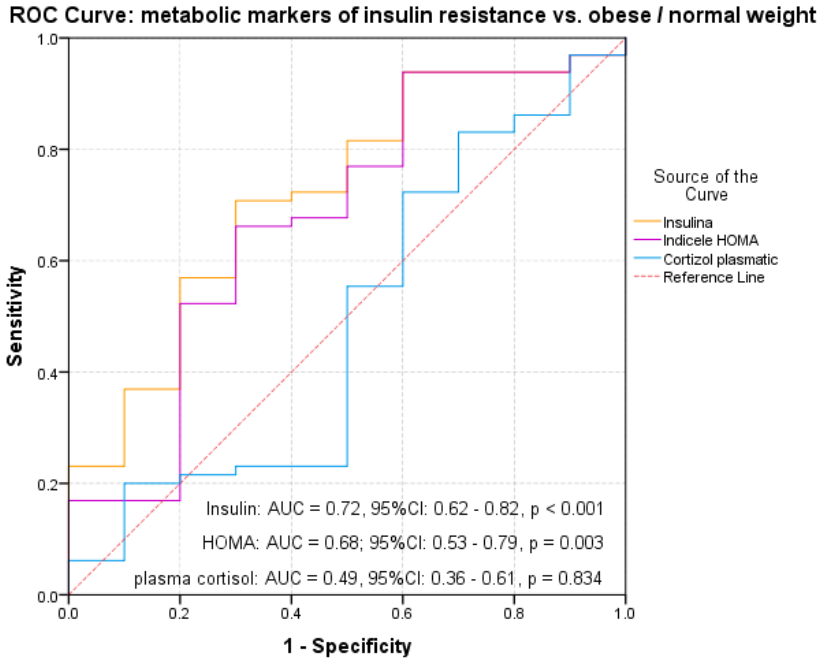
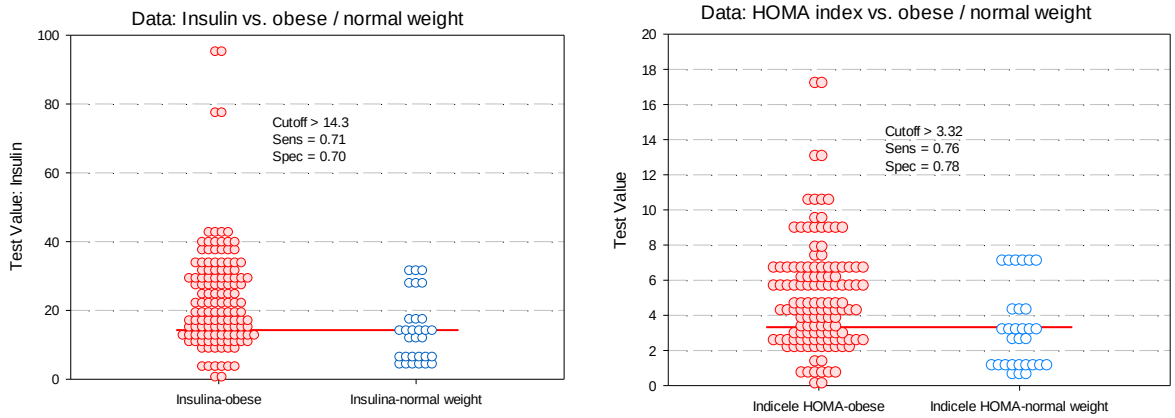


Figure 21. *ROC curves for metabolic markers of insulin resistance and obesity in children and adolescents.*



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

Figure 22. *Identifying cut-off values predictive for vascular impairment in obese children and adolescents (dot histogram) for metabolic markers of IR: (**a**) insulin; (**b**) HOMA index.*

Because the evolution of obesity in our pediatric patients' group is relatively short, we noticed no correlation between all these markers (HOMA index and IL-6, ICAM and endotoxemia) (Fig. 23a–c).

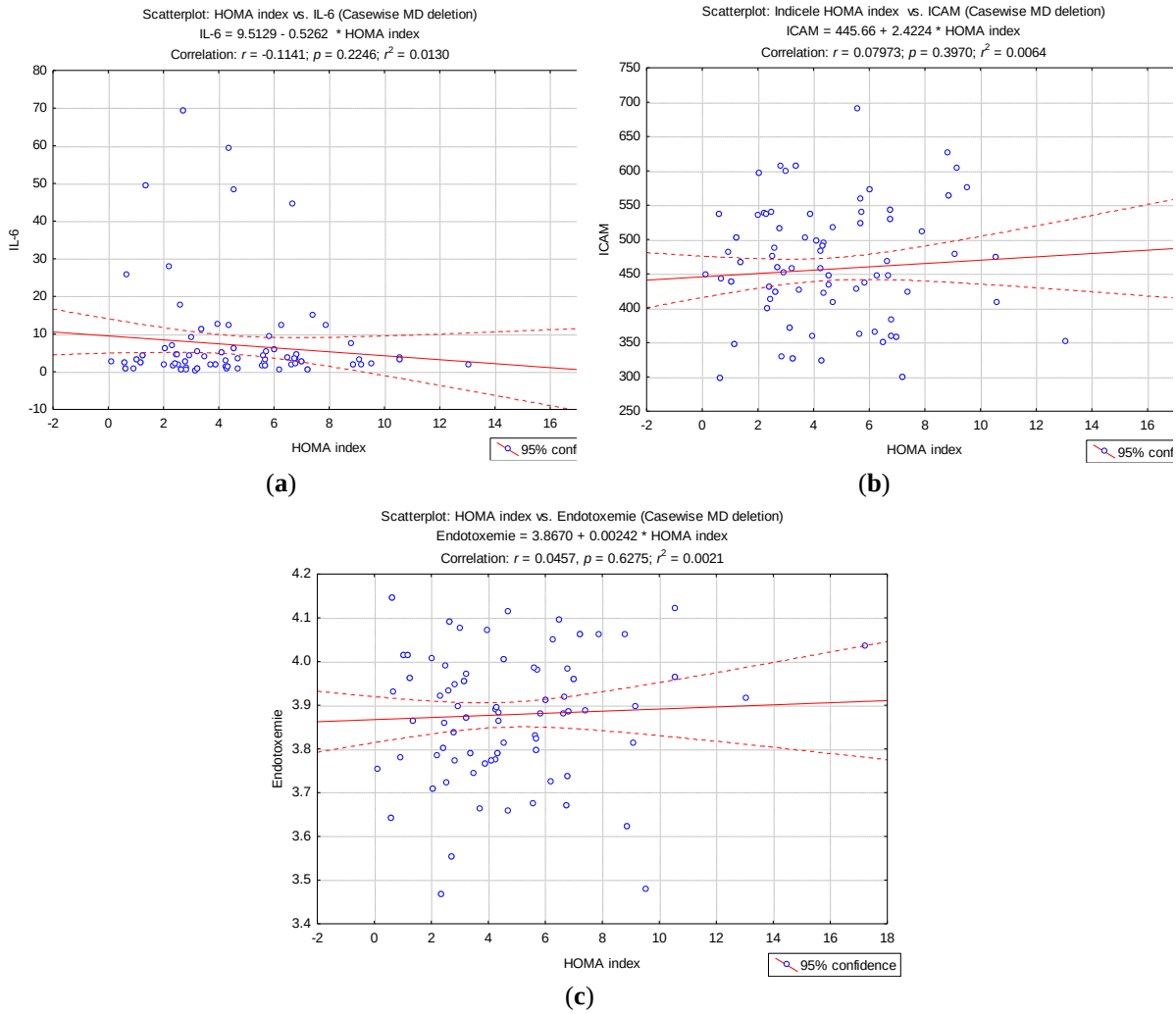


Figure 23. *The regression line in the correlation of the HOMA index and* ***(a)*** *IL-6 values,* ***(b)*** *ICAM and* ***(c)*** *endotoxemia.*

II.1.3.4. Discussions

- ***Waist Circumference a Clinical Criterion for Prediction of Cardiovascular Complications***

Cardiovascular morbidity and mortality in adult life can be influenced by overweight and obesity in childhood. "Obesity cardiomyopathy" is defined as structural and functional cardiac changes specific for obese patients (Abaci A., Tascilar M.E., Saritas T. et al., 2009). Overweight and obese adults can suffer heart failure ten years faster than subjects with normal BMI (Schwimmer J.B., Burwinkle T.M., Varni, J.W, 2003). Janssen and colleagues concluded that BMI and WC indicate an increased cardiovascular risk for children and adolescents who have visceral obesity, despite their origin or ethnicity (Csige I., Ujvárosy D., Szabó Z. et al., 2018). Studies showed that WC provides an identical measurement of visceral adipose tissue also for adults and is used to identify the increased risk of developing cardiovascular disease or type 2 DM (Harrington D.M., Staiano A.E., Broyles S.T. et al., 2013). Flodmark and colleagues showed that WC correlates to atherogenic biochemical profile for obese children (Flodmark C.E., Sveger T., Nilsson-Ehle P., 1994). Also, WC can identify in advance the presence of

metabolic syndrome and predict the cardiometabolic risk (Maffeis C., Banzato C., Talamini G., 2008). Chen et al. showed that increased WC could be an indicator of high BP in preschool male children (Chen B. and Li H.F., 2011). Moreover, childhood obesity is associated with a high risk of adult hypertension.

Visceral obesity can influence the cardiac structure and function; therefore, children with severe obesity may develop abnormal LV geometry (LV hypertrophy or relative wall thickness) (Cozzolino D., Grandone A., Cittadini A., et al., 2015). Many studies have shown that obese children have an increased LVM index (Fang X., Zuo J., Zhou J., et al., 2019. Jing L., Binkley C.M., Suever J.D., 2016). It can be correlated with BMI in children and adolescents with essential hypertension (Lee H., Kong Y.H., Kim K.H., et al., 2015). In the present study, WC above the 90th percentile can be considered a predictive factor for increased LVM index and concentric hypertrophy in both groups: children and adolescents.

Epicardial fat deposition appears in children with obesity and is associated with visceral adipose tissue deposition (de Simone G., Daniels S.R., Devereux R.B. et al., 1992). Also, in our study, visceral obesity correlated with pathological epicardial fat.

Study limitations

This study has some limitations. Visceral obesity is based only on the waist circumference, without abdominal magnetic resonance imaging or dual-energy X-ray absorptiometry and the impossibility of performing the vascular ultrasound to evaluate intima-media thickness.

- ***Predictive Markers of Early Cardiovascular Impairment and Insulin Resistance***

Identifying obese children at high risk for obesity in adulthood could be prevented by changing their lifestyle (Simmonds M., Llewellyn A., Owen C.G. et al., 2016, Jankowska A., Brzeziński M., Romanowicz-Sołtyszewska A. et al., 2021). Chronic inflammation in the context of obesity is associated with the development of IR and atherosclerosis. Without significant interventions, the progression from obesity to IR and diabetes on the one hand, and to atherosclerosis, hypertension and CVD culminating in early mortality on the other hand, is inevitable (Trandafir L.M., Russu G., Moscalu M. et al., 2020). Knowing and understanding the basic mechanisms associated with obesity-induced inflammation is necessary for therapeutic and even prophylactic pro-grams, preventing irreparable complications. If it is intervened in time, in childhood and adolescence, it is possible to avoid or delay the appearance of these comorbidities (Calcaterra V., Regalbuto C., Porri D. et al., 2020).

One of the crucial aims to prevent pediatric obesity complications is to detect children with higher cardiovascular risk and IR at an early stage (Varda N.M., Medved M., Ojsteršek L, 2020). Among clinical markers, BMI percentile and WC are frequently used in practice but with certain limitations (Trandafir L.M.; Baciu G., Leon Constantin et al., 2018). Although BMI is used as a measure of obesity, it is not sensitive enough to detect early fat accumulation and early CVD risk (Varda N.M., Medved M., Ojsteršek L., 2020, Trandafir L.M.; Baciu G., Leon Constantin et al., 2018, Simmonds M., Burch J., Llewellyn A. et al, 2015).

The role of low-grade inflammation as a link between obesity, IR and endothelial dysfunction is increasingly being discussed in the literature. Low-grade in-flammation was assessed in our study by measuring both IL-6 and ICAM. Thus, we observed that IL- 6 was significantly correlated with blood glucose and BMI. The cor-relation between an elevated level

of IL-6 and the occurrence of metabolic or cardiac comorbidities in adulthood has been demonstrated. Low-grade inflammation present in obese children is associated with changes in BP, especially systolic. It is known that BP exerts a pro-inflammatory effect on the arterial wall. IL-6 promotes the proliferation of vascular smooth muscle tissue, an early component of hypertension and atherosclerosis (Syrenicz A., Garanty-Bogacka B., Syrenicz M. et al. 2006). The presence of elevated values of IL -6 is associated with inflammation (Todendi P.F., Klinger E.I., Ferreira M.B. et al., 2015).

In our study, ICAM correlates significantly with serum triglycerides, blood glucose and BMI percentile; the BMI percentile has significant predictive power over ICAM. We did not find a positive correlation between the value of IL-6 and ICAM in the analyzed 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 N.A., Elhosary A.A., Elnashar M.A. et al., 2017).

Along with soluble adhesion molecules such as E-selectin and vascular cell adhesion molecule 1 (VCAM-1), ICAM-1 represent a molecular marker of endothelial dysfunction. Increasing the levels of circulating adhesion molecules in obese patients plays an important role in the development of atherosclerosis (Nirmalkar K., Murugesan S., Pizano-Zárate M.L. et al., 2018). sICAM-1 appears to reflect the extent of atherosclerotic lesions and is likely to be a predictive factor for future cardiovascular events in adulthood (Elnashar N.A., Elhosary A.A., Elnashar M.A. et al., 2017).

Researchers in Poland looked for the relationship between low-grade inflammation and high blood pressure in obese children and adolescents. They studied 281 overweight children aged between 6 and 18. Samples were collected to identify serum concentrations of C-reactive protein, IL-6, IL-1β, ICAM-1, VCAM-1, glucose and insulin. A significant correlation was found between CRP, IL-6, IL-1β and ICAM-1, with mean systolic blood pressure within 24 h. Inflammatory markers, CRP and IL-6 were correlated with mean diastolic BP at 24 h. The researchers found no relation between the ICAM-1 cell adhesion marker and the blood pressure of the patients examined but concluded that low-grade inflammation may play a role in modulating blood pressure relatively early in life (Syrenicz A., Garanty-Bogacka B., Syrenicz M. et al. 2006).

Recently, researchers in Saudi Arabia studied markers of inflammation in obese children in the pre-pubertal period, trying to find a correlation between them and the metabolic syndrome. Weight, height, body mass index, systolic and diastolic pressure, blood glucose, C-reactive protein, IL-6 and sICAM-1 were analyzed in two groups of 25 obese and normal-weight children. Elevated levels of insulin, CRP, IL-6 and sICAM-1 have been observed in obese children. The glucose level was not shown to be altered in any of the groups. Furthermore, a correlation was found between sICAM-1 and the level of insulin, CRP and IL-6. The scientists concluded that obese patients in the pre-pubertal patients show changes that indicate insulin resistance, endothelial dysfunction and the presence of an inflammatory condition, and all this may increase the risk of developing cardiovascular disease and type 2 DM in adulthood (Elnashar N.A., Elhosary A.A., Elnashar M.A. et al, 2017).

In our study, endotoxemia has been significantly correlated with BMI. Metabolic endotoxemia is defined by the slight increase in plasma levels of endotoxins (lipopolysaccharides) from the intestine and having a pro-inflammatory role. The presence of

lipopolysaccharides leads to increased lipid absorption and development of obesity, correlated with the value of BMI (Eworo E.R., Egbe E.R., Okhormhe Z.A., 2020). An increase in endotoxins of 0.5–2 times may be a good indicator for metabolic endotoxemia (Clemente-Postigo M., Oliva-Olivera W., Coin-Aragüez L., 2019). An intervention on the intestinal microbiota through diet and therapeutic measures may be important in reducing inflammation and endothelial dysfunction (Nirmalkar K., Murugesan S., Pizano-Zárate M.L. et al., 2018).

New studies look for a link between endotoxemia and obesity, diabetes and metabolic syndrome, as well as the link between the value of endotoxemia and other in-creased markers.

Researchers in Alexandria, Egypt studied endotoxemia in obese children and adolescents and its possible relationship with insulin, lipid profile and C-reactive protein. They studied 30 obese children and adolescents, aged between 5 and 18 years, and compared the results with those obtained from the control group, i.e., 20 patients with a normal weight. Lipid profile, liver function, endotoxin, C-reactive protein, glycemia and IR were analysed. It was identified that endotoxin and CRP value were significantly higher in the obese group compared to the other group. The researchers also found that there is a positive correlation between serum endotoxin and BMI, WC, TG, TC and insulin. They concluded that endotoxin may play a role in cardiometabolic risk factors associated with obesity in children and adolescents (Omar O.M., Meheissen M.A., Zaki B.M. et al, 2020).

In our study, insulin value was investigated among children and adolescents, trying to find a correlation with excess weight. We found that the values of insulin are correlated significantly with TG and BMI percentile, but only the BMI percentile has predictive power over insulin.

The HOMA index has significantly correlations only with BMI percentile, which is a significant predictive factor for the value of the HOMA index. This correlation con-firms that obesity is a major risk factor for the development of insulin resistance. This aspect was also validated in the study of Elnashar et al. (Elnashar N.A., Elhosary A.A., Elnashar M.A. et al, 2017), who showed that insulin and the HOMA index have a significant increase in obese children. Positive correlations were fund between insulin and HOMA and BMI percentile, systolic BP, LDL cholesterol and TG as metabolic parameters and between insulin and HOMA and CRP, IL-6 and ICAM 1 as an inflammatory parameter. According to this, in our study, we found a significant correlation between cardiovascular risk factors and insulin resistance defined by HOMA. Some studies mention that it is necessary to consider the differences between sexes in the pathophysiology of obesity.

Starting from the obtained results, we want to carry out in the future a complex analysis regarding the changes of inflammation and insulin resistance markers that appear in the case of cardiovascular complications in obese children and adolescents.

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 E.C., Delevatti R.S., Prado A.K. et al, 2016), who did not find any correlation between BMI percentile or weight and cortisol (either salivary or urinary/24 h), and no correlation between cortisol and the values of TG, HDLc and BP.

The limitations of our study were: the relatively small number of the patients in the both groups and the impossibility of evaluating fat mass and fat free mass through densitometry or

dual-energy X-ray absorptiometry. Although BMI is widely used as a surrogate measure of adiposity, it is a measure of excess weight, rather than excess body fat (Freedman D.S., Wang J., Maynard L.M. et al., 2005). We will evaluate in future studies the parameters related to the composition of body mass.

However, in a meta-analysis of four studies, overweight and obese children who became normal in adulthood were no different in terms of more cardiovascular disease risk parameters than those patients who were never obese (Juonala M., Magnussen C.G., Berenson G.S. et al., 2011). Thus, early nutritional interventions instituted in obese children that lead to weight loss will contribute to the health of future adults.

II.1.3.5. Conclusions

- ***Waist Circumference a Clinical Criterion for Prediction of Cardiovascular Complications***

The presence of overweight and obesity in childhood is associated with cardiovascular dysfunction. Visceral obesity is a significant predictive factor for the appearance of vascular impairment (pre-hypertension and hypertension) more for adolescents than for children. WC above the 90th percentile for both groups is a predictive factor for increased LVM index with concentric hypertrophy.

- ***Predictive Markers of Early Cardiovascular Impairment and Insulin Resistance***

An adequate understanding of the inflammatory processes characteristic of obesity constitutes a crucial factor for the prevention of the disease and its complications in obese pediatric patients. Inflammatory markers, IL-6, ICAM 1 and endotoxemia, show significantly higher values in pediatric obese patients, leading to chronic and systemic inflammation. The results of our study indicated that these markers can be considered significant predictors of cardiometabolic diseases in these patients. Of particular interest is the link between low-grade inflammation, IR and endothelial dysfunction and obesity. The significant correlation between the HOMA index and the BMI percentile confirms that obesity is a major risk factor for the development of insulin resistance. It can clearly be considered that the BMI percentile has significant predictive power for metabolic markers of IR. However, our study highlights the need for detailed research in the dynamics of obese pediatric patients by age group.

II.1.4. CHILDHOOD OBESITY: MEDICAL AND PSYCHOSOCIAL CONSEQUENCES

II.1.4.1. Introduction

Global obesity rates have increased exponentially in recent decades. Children are becoming obese younger, morbid obesity is increasing and the full health implications are only beginning to be seen. Children and teenagers with obesity are more likely to develop several potentially serious health problems (as presented in Fig. 17), including:

Cardiovascular disease (CVD)

Obesity makes you more likely to have high blood pressure and abnormal cholesterol levels, which are risk factors for heart disease and strokes. A recent study of almost 2.3 million individuals followed up for over 40 years found the risks of CVD mortality were 2- to 3-fold higher if their BMI as adolescents had been in the overweight (hazard ratio, 2.25; 95% confidence interval, 1.96–2.58) or obese (hazard ratio, 3.46; 95% confidence interval, 2.93–4.10) category compared with youth with normal weight (de Ferranti S.D., Steinberger J., Ameduri R. et al., 2019).

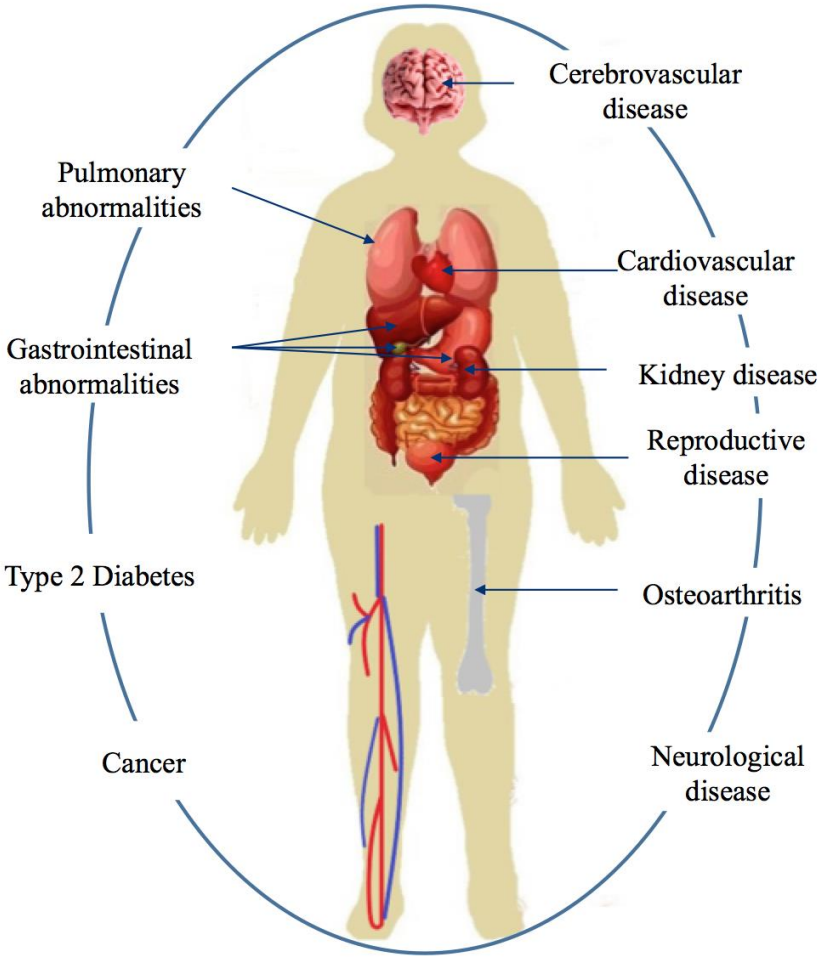


Figure 17. *Medical complications of obesity*

Data available shows a strong association between obesity and hypertension. In one large cohort study of 82,473 participants, BMI was positively associated with hypertension at age 18 and midlife. There was also marked increase in risk of hypertension with weight gain (Huang Z., Willett W.C., Manson J.E. et al., 1998) Obese children are approximately three times likelier to have hypertension than non-obese children (Sorof J. and Daniels S., 2002). In adults, there is a nearly linear relationship between BMI and blood pressure (BP) and weight loss reduces BP in most hypertensive individuals (do Carmo J.M., da Silva A.A., Wang Z. et al., 2016).

In the Framingham study, the relative risk of hypertension in overweight men and women were 1.46 and 1.75, respectively, after adjusting for age (Wilson P.W., D'Agostino R.B., Sullivan L. et al., 2002). In the same study, reduction of weight in obese women at age 18

reduced the risk of hypertension. One study found that for every 4 kg/m^2 increase in BMI there is a 26% increase in odds for *coronary heart disease* (CHD) (Nordestgaard B.G., Palmer T.M., Benn M. et al. 2012). Data from the NHANES study including death information for 2.3 million American adults showed that obesity was associated with significantly increased mortality from both CHD and other forms of CVD (Flegal K.M., Graubard B.I., Williamson D.F. et al., 2007). Although BMI may also affect CHD risk through intermediate factors such as hypertension, dyslipidaemia and diabetes, recent studies have shown obesity is an independent risk factor (McPherson R., 2015).

Obesity has been shown to affect the heart as early as in childhood, with obese children having significantly higher left ventricular mass (Maggio A.B.R., Aggoun Y., Marchand L.M. et al., 2008). The Framingham Heart Study, which followed 6000 adult subjects without a history of *heart failure* for a mean of 14 years, found that the risk of heart failure was doubled in obesity (Kenchaiah S., Evans J.C., Levy D. et al., 2002). After adjusting for established risk factors, the risk of heart failure increased 5% in men and 7% in women for each extra 1kg/m^2 in BMI. A review of 28 studies found that both overweight and obesity are associated with increased risk of heart failure (Aune D., Sen A., Norat T. et al., 2016).

As noted above, obesity is linked to hypertension, coronary artery disease, diabetes mellitus, left ventricular hypertrophy, left atrial enlargement and CHF. Hypertension, left atrial enlargement and congestive heart failure are all strongly linked to *atrial fibrillation* (AF) (Go A.S., Hylek E.M., Phillips K.A. et al., 2001). Despite the close relationship between obesity and several of the risk factors for AF, a clear relationship between AF and obesity has only relatively recently been established. Previous epidemiologic studies produced conflicting results as to whether AF is linked to obesity. This may be due to short-term follow-up, failure to account for interim cardiovascular events and/or lack of echocardiographic data (Benjamin E.J., Levy D., Vaziri S.M. et al., 1994). Data from the Framingham Heart Study (Wang T.J., Parise H., Levy D. et al., 2004) show a correlation between the risk of developing AF and BMI. In multivariate analysis, adjusting for interim myocardial infarction or heart failure, every increase of 1 point in BMI was associated with a 4% increase in the risk of AF (Malnick S.D.H., Knobler H., 2006). In addition, there was a gradual increase in left atrial size as BMI increased. The relationship between BMI and AF was not significant after adjusting for left atrial diameter, suggesting a physiological link between obesity and left atrial diameter. In addition there is an association between obstructive sleep apnoea and AF (Gami A.S., Pressman G., Caples S.M. et al., 2004), and as will be discussed below, obesity and obstructive sleep apnoea are closely linked.

In older populations, hypertension and obesity continue to relate in a predictable manner as has been shown in the Honolulu Heart Program and Japanese data survey (Masaki K.H., Curb J.D., Chiu D., et al.,1997. Matsumura K., Ansai T., Awano S. et al., 2001).

Dyslipidaemia, manifested by reduced high-density lipoprotein (HDL) and increased triglycerides, low-density lipoprotein (LDL) cholesterol, very low-density lipoprotein cholesterol (VLDLc) is associated with obesity (Masaki K.H., Curb J.D., Chiu D. et al., 1997. Matsumura K., Ansai T., Awano S. et al. 2001. Despres J.P., 1991. Grundy S.M. and Barnett J.P. 1990). The prevalence of dyslipidemia among obese children was 46–50.4% (Valerio, G., Maffeis, C., Saggese, G. et al. 2018). The underlying mechanism is largely due to insulin resistance. VLDL in plasma is dependent on the rate of hepatic synthesis and catabolism by

lipoprotein lipase, an enzyme, which is also involved in formation of HDL (Pouliot MC, Despres J.P., Moorjani S., 1991). In obesity, insulin resistance is associated with increased hepatic synthesis of VLDL and impaired lipoprotein lipase (Pouliot M.C., Despres J.P., Moorjani S. et al., 1991). It is well known that serum levels of lipids and lipoproteins vary depending on age, gender and race, normal values in children under 19 years being different compared to those of adults. In the absence of national reference values, the diagnosis of dyslipidemia is based on the criteria proposed by the expert panel on integrated guidelines for cardiovascular health and risk reduction in children and adolescents (Valerio G., Maffeis C., Saggese G. et al. 2018)

Table XVIII shows the abnormal values of the lipid levels as proposed by the Expert Panel (Okusanya O.O., Bhavnani S.M., Hammel J. et al., 2009) are summarized. Recent studies have shown that the TG/HDL-C ratio is associated with insulin resistance and early organ damage (heart, liver, and carotid) (Campagna F. V., Martino F., Bifolco M., et al., 2014). The Tg/HDL-C > 2.2 can be considered as a marker of atherogenic dyslipidemia and an altered cardiometabolic risk profile in obese italian children. (Aissaoui A., Chami M., Hussein M., et al., 2011. Eastman S.J., Lukason M.J., Tousignant J.D., et al., 1997) (LOE V-A). Children with TG ≥ 500 mg/dL or LDL-Cholesterol persistently ≥ 160 mg/dL need lipid specialist consultation (Okusanya O.O., Bhavnani S.M., Hammel J., et al., 2009).

Table XVIII. The values of the lipid levels

Cathegory	Acceptable	Borderline-high	High
Total cholesterol (mg/dl)	< 170	170–199	≥200
LDL-cholesterol (mg/dl)	< 110	110–129	≥130
Non HDL-cholesterol (mg/dl)	< 120	120–144	≥145
Triglycerides (mg/dl)			
0–9 years	< 75	75–99	≥100
10–19 years	< 90	90–129	≥130
	Acceptable	Borderline-low	Low
HDL-cholesterol (mg/dl)	> 45	40–45	< 40

In the American Heart Association (AHA) scientific statement published in 2019, the expert group presents recommendations for clinical approach regarding the assessment and risk reduction of select pediatric populations at high risk for premature CVD, including acquired arteriosclerosis or atherosclerosis. According to this scientific statement, the Expert Panel recommend screen yearly for lipid disorders with nonfasting non-HDL, followed by fasting lipid profile if initial TC >200, HDL <45, or non-HDL >145 mg/dL. If LDL-C is abnormal, consider diagnostic evaluation and initiate therapeutic lifestyle change and statin therapy based on risk category. If TG is abnormal, provide therapeutic lifestyle change counseling and repeat within 1–2 week. If still abnormal, obtain diagnostic evaluation and treat based on TG level. If TG value varies between 130–400 mg/dL and non-HDL <145 mg/dL experts recommended to treat with therapeutic lifestyle change modification; reassess in 3 month and then periodically.

If TG value over 400–999 mg/dL or TG 130–400 mg/dL and non-HDL ≥145 mg/dL recommandation is to treat based on risk category. If TG value is over 1000 mg/dL, confirmed on repeat testing and initiate therapeutic lifestyle change and Omega 3 fatty acids or pharmacotherapy simultaneously (de Ferranti S.D., Steinberger J., Ameduri R. et al., 2019).

In obese children the screening of dyslipidemia is recommended because the association of obesity/hyperlipidemia (expecially hypertriglyceridemia) is predictive of fatal and non-fatal cardiovascular events in adult life. (Morrison J.A., Glueck C.J., Woo J.G. et al., 2012). It has been shown that there is a direct correlation between obesity and aortic and coronary fatty streaks or other atherosclerotic lesions. Visceral obesity is widely considered an independent cardiovascular risk factor yet is also associated with other CVD risk factors, including dyslipidemia (most often manifested as high levels of triglycerides and low levels of HDL cholesterol). (de Ferranti S.D., Steinberger J., Ameduri R. et al. 2019, McGill H.C.Jr. McMahan C.A., Herderick E.E., 2001). According to the Consensus position statement of the Italian Society for Pediatric Endocrinology and Diabetology and the Italian Society of Pediatrics is reccomended performing the lipidogram every three years, if the results are normal, and more frequently if rapid increase in weight or development of other cardiometabolic comorbidities occurs (Valerio G., Maffeis C., Saggese G. et al., 2018).

Type 2 diabetes mellitus (Type 2 DM)

Obesity can affect the way the body uses insulin to control blood sugar levels, therefore, this raises the risk of insulin resistance and diabetes.

There is a strong association between obesity and type 2 DM in both genders and all ethnic groups. The underlying mechanism is thought to be due to insulin resistance. However, there is currently limited data accurately quantifying insulin resistance using the standard hyperinsulinemic euglycemic clamp (Keskin M., Kurtoglu S., Kendirci M. et al., 2005), largely because the invasive nature of the procedure makes it unsuitable for general epidemiological studies. Currently over 34.2 million people have diabetes, (10.5% of the US population) (Centers for Disease Control and Prevention, 2020) and this is projected to rise to 21–33% by 2050 (Boyle J.P., Thompson T.J., Gregg E.W. et al., 2010). Most patients with type 2 DM are obese and the global epidemic of obesity largely explains the explosion in cases of T2DM over the past two decadesn (Eckel R.H., Kahn S.E., Ferrannini E. et al., 2011). The risk of type 2 DM rises with increasing body weight; a study of over 21 000 adults in the National Health and Nutrition Examination Survey (NHANES) found the risk rising from 8% in normal weight people to 43% in individuals with morbid obesity (Nguyen N.T., Nguyen X-M.T., Lane J. et al., 2011). Data from the Nurses' Health Study showed an age-adjusted relative risk of 40 for diabetes in women with BMI of 531 kg/m^2, compared with women with a BMI <22kg/m^2 (Colditz G.A., Willett W.C., Rotnitzky A. et al., 1995). A similar risk was shown for men in the Health Professionals Follow-up Study: a BMI of 535 kg/m^2 was associated with an age-adjusted relative risk for diabetes of 60.9, compared with a BMI of <23kg/m^2 (Chan J.M., Rimm E.B., Colditz G.A. et al., 1994).

Cerebrovascular Disease

Obesity is linked to an increased risk of stroke in both men and women. Currently available evidence shows that the risk of *haemorrhagic and ischaemic stroke*, in relation to obesity, is increased in men. In women this relation is true with ischaemic stroke but not haemorrhage stroke. In the Korean prospective study involving 234,863 men who were

followed up for 9 years, a significant positive association was found between BMI and the risk of ischemic stroke whereas, with haemorrhagic stroke, a J-shaped association was found showing that the risk increased more than that of ischaemic stroke at the upper and lower extremes of BMI (Song Y.M., Sung J., Davey S, G. et al., 2004). In a prospective study of 39,053 participants (all women) followed up for an average of ten years, 432 strokes occurred. Three hundred and seven were ischaemic, 81 hemorrhagic and 4 undefined. In obese subjects (BMI > 30kg/m^2), the hazard ratios (95% CI) for total stroke, ischaemic stroke and hemorrhagic stroke were 1.5 (1.16 to 1.94), 1.72 (1.30 to 2.28) and 0.82 (0.43 to 1.58), respectively. This was in comparison with the group of women with BMI less than 25kg/m^2. (Kurth T., Gaziano J.M., Rexrode K.M. et al., 2005)

Neurological disease

Vascular risk factors such as hypertension, dyslipidaemia and diabetes are all associated with increased risk of *dementia and Alzheimer's disease*. Although a raised BMI contributes to each of these factors, the independent relationship between obesity and dementia is a little more complicated. A review article found that five out of nine studies reported an independent association between high BMI and risk of dementia (Kloppenborg R.P., van den Berg E., Kappelle L.J., 2008). It appears that being obese in midlife is what conveys the risk; studies measuring BMI at midlife had a more consistent association, while the four studies that did not find a link all measured BMI after the age of 75 years (Kinlen D., Cody D., O'Shea D., 2018).

Cancer

It is estimated that obesity accounts for 20% of all cancer cases. The WHO International Agency for Research on Cancer has estimated that overweight and inactivity account for from a quarter to a third of all cancers of the breast, colon, endometrium, kidney and esophagus (Vainio H. and Bianchini F., 2002). Also, obesity may increase the risk of cancer of the uterus, cervix, ovary, rectum, liver, gallbladder, gastric, pancreas and prostate, as well as leukaemia (Segula D., 2014).

One study found obese women with breast cancer were found to be 46% more likely to develop distant metastases and 38% more likely to die than lean counterparts (Ewertz M., Jensen M.-B., Gunnarsdottir K.A. et al., 2011). Chemotherapy and radiotherapy dosing is more difficult with up to 40% of obese patients receiving limited chemotherapy doses that are not based on their body weight (Griggs J.J., Mangu P.B., Anderson H. et al., 2012).

Obesity also increases the likelihood of dying from cancer. A 16-year prospective study of 4,900,000 men and women in the US found a relative risk of death from cancer of 1.5 for men and 1.6 for women in the group with BMI 440 kg/m^2 *vs.* BMI 18.5–24.9 kg/m^2 (Calle E.E., Rodriguez C., Walker-Thurmond K. et al., 2003) For both men and women, increasing BMI was associated with higher death rates due to cancers of the esophagus, colon and rectum, liver, gallbladder, pancreas, kidney, non-Hodgkin's lymphoma and multiple myeloma. Men were also at increased risk for death from stomach and prostate cancer, while women were at increased risk of death from cancers of the breast, cervix, uterus and ovary. On the basis of these data, the authors estimated that overweight and obesity in the USA could account for 14% of all cancer deaths in men and 20% in women.

In a systematic review and meta-analysis from the Comparative Risk Assessment Project evaluating data on 7 million deaths from cancer, 2.43 million were attributable to potentially modifiable risk factors, including overweight and obesity. For every risk factor, they

calculated the population attributable fraction (PAF), estimating the proportional reduction in cancer death if the risk factor was reduced. The corresponding PAF for overweight and obesity was: 11% for colon and rectum cancers; 5% for breast cancer; 40% for uterine cancer (Danaei G., Vander Hoorn S., Lopez A.D., et al., 2005).

Gastrointestinal abnormalities

Obesity increases the likelihood that will develop heartburn, gallbladder disease and liver problems.

Non-alcoholic fatty liver disease (NAFLD) is now the commonest cause of chronic liver disease worldwide, estimated to be present in 20–35% of adults in the developed world (Moore J.B., 2010). One-third of these cases progress to non-alcoholic steatohepatitis (NASH), characterized by liver inflammation and injury, which can lead to cirrhosis and hepatocellular carcinoma (Dietrich P. and Hellerbrand C., 2014. Vernon G., Baranova A., Younossi Z.M. 2011). NAFLD is considered the hepatic manifestation of metabolic syndrome and its risk is strongly correlated with BMI. One study found rates of steatosis to be 15% in non-obese persons, 65% in people with Class I or II obesity and 85% in Class III obesity (Fabbrini E., Sullivan S., Klein S., 2010).

Obesity is also a risk factor for gallbladder disease. A meta- analysis of 17 prospective studies covering nearly 2 million participants found a relative risk of 1.63 for a 5-unit increment in BMI (Aune D., Norat T., Vatten L.J., 2015). The risk of gallbladder disease doubled from lower to upper limit of the normal BMI range, suggesting even moderate weight gain increases risk.

Obesity is associated with greater risk of pancreatitis (Bonfrate L., Wang D.Q.H., Garruti G., et al., 2014) and is a poor prognostic factor in the disease. It is also considered a major reason for the 2-fold increase in incidence of gastroesophageal reflux disease and its associated conditions Barrett's esophagus and esophageal adenocarcinoma (Lee Y.Y. and McColl K.E.L., 2015).

Another gastrointestinal condition that has been studied in relation to obesity is cholelithiasis. Data from the Nurses' study showed that females with BMI of more than 45 kg/m^2 had a seven-fold increase in risk of gallstone disease compared to those with BMI of less than 24 kg/m^2 (Stampfer M.J., Maclure K.M., Colditz G.A., et al., 1992). Men have had similar results (Willett W.C., Dietz W.H., Colditz G.A. 1999).

The association between functional gastrointestinal disorders (FGIDs) in paediatric patients and overweight and obesity is well known. The frequent GI disorders associated with childhood obesity are gastroesophageal reflux disease (GERD), functional constipation, functional abdominal pain (FAP), and irritable bowel syndrome (IBS). FGIDs are often associated with psychological comorbidities (depression, anxiety, and social phobia) and are negatively impact the quality of life of obese children (Phatak U.P., Pashankar D.S., 2014).

As one of the most common chronic disorders in children and adolescents, constipation represents a significant health problem. Chronic constipation is a disorder that can negatively impact the quality of life, resulting in a major economic and social burden. Among the pediatric population, constipation is a frequent disorder with a growing incidence ranging from 3% to 25% (Liem O., Harman J., Benninga M., 2009). Constipation is also a condition based on clinical symptomatology, with its definition being particularly subjective (Longstreth G. F., Thompson W. G., Chey W. D, 2006) In this regard, there is often a lack of understanding

between the physician and the patients' perception in terms of defining constipation (Talley N. J., 2004). The Rome III criteria consider the frequency and consistency of stools, as well as the secondary disorders associated with constipation (retentive behavior, pain).

The Definition of Functional Constipation according to the Rome III Criteria (Afzal N. A., Tighe M. P., Thomson M. A., 2011): children over the age of four, at least two of the following events occur, once a week or more frequently, for a duration of at least two months:

- less than 3 bowel movements per week
- at least 1 episode of fecal incontinence per week
- stool retention position or behaviour
- difficult or painful passing of stool
- the presence of a large fecal mass identified upon rectal examination or abdomen palpation
- the existence of large stools that can obstruct the toilet.

Kidney disease

The epidemic of obesity in the developed world has been associated with an increase in the prevalence of chronic kidney disease. It is however, unclear whether obesity is a risk factor independent of diabetes and hypertension (Wiggins K.J., Johnson D.W., 2005).

A study following up over 8 million person-years found that, compared with lean people, the relative risk for End-Stage Renal Failure (ESRF) was 1.87 for overweight individuals, 3.57 for those with class I obesity, 6.12 for those with class II obesity, and 7.07 for those with class III obesity(Hsu C.Y., McCulloch C.E.., Iribarren C, et al., 2006). After adjusting for other risk factors, higher BMI remained an independent predictor of ESRF.

Obesity is also associated with greater risk of kidney stones (Semins M.J., Shore A.D., Makary M.A., et al., 2010) and urinary incontinence in women (Al-Mukhtar O. J., Akervall S., Milsom I., et al., 2017), while obesity-related glomerulopathy has increased in prevalence in parallel with obesity (D'Agati V.D., Chagnac A., de Vries A.P.J., et al., 2016).

Reproductive disease

Obesity may cause infertility and irregular periods in women. Obesity also can cause erectile dysfunction in men. Obesity is now estimated to be responsible for 6% of primary infertility (Green B.B., Weiss N.S., Darling J.R., 1988).

In men, there is a link between impotence and increasing infertility, with abdominal obesity a particular risk (Esposito K., Giugliano F., Di Palo C., et al., 2004). Obesity is associated with reduced sperm count (Sermondade N., Faure C., Fezeu L., et al., 2012) and increased rates of erectile dysfunction (Shamloul R. and Ghanem H., 2013).

In women, it also leads to reduced fertility, poorer outcomes after fertility treatment and more pregnancy loss. Polycystic Ovarian Syndrome (PCOS), characterized by anovulation, hyperandrogenism and a polycystic ovary is the primary cause of female infertility and increases the rate of pregnancy complications (Moran L.J., Norman R.J., Teede H.J., 2015). The risk of PCOS is slightly increased with obesity and obese women with PCOS often have a more serious phenotype (Yildiz B.O., Knochenhauer E.S., Azziz R.,2008).

Obesity during pregnancy is associated with an increased risk of complications, including gestational diabetes, pre-eclampsia, and delivery complications such as macrosomia, shoulder dystocia and higher rates of caesarean sections and infections. Maternal obesity may

also be an independent risk factor for neural tube defects and fetal mortality. This subject has been reviewed recently (Dietl J., 2005).

Osteoarthritis

Obesity increases the stress placed on weight-bearing joints, in addition to promoting inflammation within the body. These factors may lead to complications such as osteoarthritis (OA).

In the Framingham cohort study, data from 1420 participants indicated that obesity was an important independent risk factor for OA after adjusting for age, physical activity and the levels of uric acid (Felson D.T., Zhang Y., Anthony J.M., 1992). In a study of over 1000 women, the age-adjusted odds ratio of unilateral and bilateral osteoarthritis of the knee, as determined by X-ray, was 6.2 for BMI <23.4 kg/m^2 and 18 for BMI 426.4 kg/m^2. When BMI <23.4 kg/m^2 was compared to BMI 23.4– 26.4 kg/m^2, the odds ratios for osteoarthritis were increased: 2.9 fold for the knee, 1.7 fold for carpometacarpal joint, 1.5 fold for the distal interphalangeal joint, and 1.2 fold for the proximal interphalangeal joint (Hart D.J. and Spector T.D., 1993). A co-twin control study noted that each one kg increase in weight was associated with an increased risk of radiographic features of osteoarthritis at the knee and carpometacarpal joint (Cicuttini F.M., Baker J.R., Spector T.D.,1996).

Pulmonary abnormalities

Obesity is a major risk factor for *obstructive sleep apnea* (OSA). OSA adversely affects multiple systems and is associated with hypertension, insulin resistance, liver dysfunction, systemic inflammation and dyslipidaemia (Romero-Corral A., Caples S.M., Lopez-Jimenez F., et al., 2010). In children, it can lead to failure to thrive, behavioural problems, decreased intellectual function and a higher risk of cardiovascular morbidity (Tauman R. and Gozal D., 2006). Obesity has long been known to be a major pathogenic factor in OSA in adults. A study of 4000 US adults found prevalence was 12% in obese *vs.* 3% in lean subjects (Li C., Ford E.S., Zhao G., 2010).

Asthma is another condition that may occur as a complication of obesity. There is evidence that obesity increases the risk of asthma. In one prospective multicentre study, the prevalence of asthma was observed to increase in obese patients. Seventy five per cent that presented with an asthmatic emergency were either obese or overweight (Guerra S., Wright A.L., Morgan W.J., et al., 2004). Further prospective studies have shown that obesity predicts asthma (Aaron S.D., Fergusson D., Dent R., et al., 2004). The mechanism linking obesity and asthma includes increased airway hyper-responsiveness, decreased functional and tidal volumes, chronic systemic inflammation driven by increased inflammatory cytokines and chemokines, adipocytes derived factors leptin, adiponectin and plasminogen activator inhibitor (Shore S.A. and Fredberg J.J., 2005).

The relation between malnutrition and pulmonary death in patients with CF has resulted in intensive nutritional intervention over the last few decades, leading to a significant decline in underweight and the emergence of overweight/obesity as a potential new problem.

Psychosocial implications of childhood obesity

Rates of childhood obesity are increasing at alarming rates worldwide. This is especially alarming as obesity is associated with many physical and psychological consequences. A great number of studies indicate that obese children have an impaired psychological well-being, such as depression, self-esteem, and quality of life compared to their non-overweight peers.

Although it seems obvious that obese children and adolescents would likely be at higher risk for psychological problems, the mediating factors in the relationship between psychological problems and obesity are still not well established. Obesity is a complex disorder with an equally complex etiology and is thus associated with complex behaviors and outcomes that make it difficult to study in children. It has been indicated that family-based lifestyle interventions can improve psychological well-being in obese children; however, not all children profit from these interventions. Interventions aimed at improving treatment results need further investigation.

Personal contributions related to obesity associations and complications

Articles:

1. **Trandafir LM**, Anton-Paduraru DT, Miron I, Indrei LL Psychosocial Implications of Childhood Obesity, Review of Research and Social Intervention, 2015, 49: 205-215. (IF=0.424)
2. Russu G, Frasinariu OE, **Trandafir L** Cardiovascular suffering in childhood obesity, Romanian Journal of Pediatrics, 2017, 66(1): 18-23 (EBSCO)
3. Temneanu OR, **Trandafir LM**, Purcarea MR. Type 2 diabetes mellitus in children and adolescents: a relatively new clinical problem within pediatric practice, Journal of Medicine and Life, 2016, 9(3): 235-239 (Pubmed)

Proceedings ISI:

1.**Trandafir LM**, Frasinariu OE, Corciova C, Boiculese LV, Moscalu M. Prevention of Cardiovascular Risk Factors in Childhood Obesity, 2017 IEEE International Conference on E-Health and Bioengineering Conference (EHB), 2017, 717-720 (Web of Science Core Collection)

2.**Trandafir LM**, Frasinariu OE, Subotnicu M, Miron IC. Gastrointestinal Disorders in Childhood Obesity, NEUROGASTRO 2017 - Meeting of the Romanian Society of Neuro-gastroenterology with Rome IV Regional Central East European Meeting, 2017, 142-146 (Web of Science Core Collection)

Objectives

- ***Childhood Obesity – Risk Factor for Cardiovascular Disease***

Because obesity is an independent and important modifiable risk factor for cardiovascular disease, we conducted this review to highlight the importance and need of programs for early detection, diagnosis, treatment and prevention of childhood obesity in order to decrease the incidence of the cardiovascular pathology in adults.

- ***Psychosocial Implications of Childhood Obesity***

In this review paper we aimed to describe the main psychosocial dimensions of child-hood obesity: stigmatization, altered cognitive performance, low self-esteem and respect for one's body, emotional disorders. Stigmatization, victimization and teasing are related to social non-acceptance and discrimination of overweight children and adolescents. The quality of life

of obese children is lower due to their poorer physical and mental health, or to their deficient social functioning and poorer school performance.

II.1.4.2. Materials and methods

- ***Childhood Obesity – Risk Factor for Cardiovascular Disease***

We revised the current literature concerning child obesity as risk factor of cardiovascular disease and metabolic syndrome. Identifying children who are at high risk of dyslipidemia, hypertension, cardiovascular diseases, and severe adult obesity will allow the early establishment of weight control strategies. To write this review we used the following keywords: obesity, visceral adiposity, child, adolescent, hypertension, dyslipidemia, myocardial dysfunction, cardiovascular disease. We read over a hundred articles and finally we selected fifty bibliographic references for writing this review.

- ***Psychosocial Implications of Childhood Obesity***

In this review we discussed the physiological consequences of child obesity which included stigmatization, low self-esteem, and respect for one's body, altered cognitive performance, or emotional disorders. All these obesity complications were discussed based on literature data.

II.1.4.3. Results and Discussions

- ***Childhood obesity – Risk Factor for Cardiovascular Disease***

Obesity is one of the diseases encountered in adults but with fetal determination, with proven genetic transmission, or acquired by epigenetic resetations. Under environmental factors named epigenetic risk factors, the genes function suffers reversible changes without changing of the DNA sequences by DNA methylation, physical changes in the chromatin structure, and the action of some non-codant RNA molecules. The result is a genic modulation with impact on the phenotype of obesity, as a response reaction to environmental factors: nutritional factors, infections, oncogenic factors, stress toxics. The action of these epigenetic factors involved in the prenatal genetic determinism of obesity has been presented in detail in subchapter II.1.3.

Vascular remodeling

Obesity is known as independent predictor and major, but modifiable, risk factor for ATS and acute coronary heart disease. Obesity acts on the blood vessels by generalized endothelial dysfunction, accelerates the proliferation, migration and arterial smooth muscle cell hypertrophy and the recruitment of macrophages in the vascular wall. Generalized endothelial dysfunction and subsequent vasodilatation are regarded as the initial step in the pathogenesis of ATS, followed by stocking of LDLc. Dyslipidemia associated with obesity, insulin resistance, adipocitokines released by the adipose tissue accelerate further the process of ATS (Lastra G., Manrique C., Whaley-Connel A., 2005).

It was reported increased value of carotid intima media thickness (IMT) assessed by Doppler vascular ultrasound in obese children and adolescents compared to children of the same age with normal BMI (Cote A.T., Harris K.C., Panagiotopoulos C. et al., 2013). The concept of vascular age was im- posed through clinical use of this parameter for IMT evaluation on the percentiles scale for adult population of the same breed and sex (Stein J.H, 2004).

Arterial stiffness is associated with increased cardiovascular risk in adult. A vascular system with normal elasticity requests less work from the heart, increases coronary artery perfusion and is associated with a reduction in the rate of ATS production. In children with obesity occur changes of some biophysical parameters of the aorta, reflecting the decreased elasticity of this artery (Pac F.A., Guray Y., Polat T.B., 2010). Autonomic nervous system dysfunction associated with obesity in children reduces the vagal variability of the heart rate (Vanderlei L.C., Pastre C.M., Freitas Júnior I.F., et al., 2010), and the baroreceptors sensitivity, with essential role in regulating BP (Lazarova Z., Tonhajzerova I., Trunkvalterova Z. et al. 2009).

Hypertension and the stage of pre-hypertension are frequent and early complications in children with obesity, the frequency increasing in the last decade to 4% and 10% respectively (Jain S., 2016). Hypertension in children is an important predictor for adult hypertension, but it is underdiagnosed. Identification and treatment of hypertensive youth encounters many difficulties because BP values range from one time to another depending on many physiological parameters and factors in the environment. Therefore, BP determination in outpatient assessment allows a real evaluation, allows risk stratification and prediction of cardiovascular prognosis (Urbina E., Alpert B., Flynn J. et al., 2008).

Three pathophysiological mechanisms were identified explaining the occurrence of hypertension in obese children: autonomic nervous system dysfunction, insulin resistance and alteration of the structure and function of vessels.

Adipose tissue in obesity is composed of mature adipocytes, preadipocytes, endothelial cells, and macrophages and presents specific features. Physiologically, mature adipocytes are active endocrine and paracrine cells, secreting a great number of mediators involved in various metabolic processes. The best known is leptin, the hormone that acts as a regulating feedback factor which suppresses the hunger sensation at hypothalamic level. In the obese people, the circulating leptin is increased, in conjunction with adiposity. It was demonstrated the link between leptin and HB: leptin adjust the activity of the Na, K-ATPase modifying the renal sodium exchange, activates the renin-angiotensin-aldosterone axis and the sympathetic nervous system, appears to be linked to insulin resistance and acts together with other proinflammatory cytokines, inducing vascular oxidative stress and HBP (Leiter A. L., Fitchett D. H., Gilbert R.E., 2011).

Excessive adipose tissue is composed of hypertrophied adipocytes and macrophages and becomes dysfunctional, secreting proinflammatory adipokine cells: interleukin-6, α tumor necrosis factor, the plasminogen activating inhibitor and C-reactive protein. Adiponectin, protein secreted in abundance by the adipose tissue, is an important stimulator of nitric oxide synthase activity and confers protection against oxidative stress and insulin resistance. Circulating levels of adiponectin are decreased in obesity because the production is suppressed by proinflammatory adipokines. Taken together, these phenomena modify glucose and lipids metabolism, causing vascular endothelial dysfunction and accelerating processes of atherosclerosis at the lev- el of the vascular wall, thus increasing the cardio- metabolic risk (Bakris G.L. and Ritz E., 2009).

The treatment of pre-hypertension and stage 1 hypertension in obese children consists of lifestyle changes and reducing body weight. The drugs are recommended only starting with stage 2 hypertension (Cai L.,Wu Y.,Wilson R., 2014) The beneficial effect of the decrease in

body weight to reduce BP values was demonstrated. Thus, a meta-analysis of integrative studies regarding obesity and prevention of its complications has shown the effectiveness of the measures aimed at both diet and physical exercise to reduce BMI and normalize BP (Dorresteijn J.A., Visseren F.L., Spiering W., 2012).

The Impact of Obesity on the Cardiac Disease

Obesity is associated with increased metabolic demands caused by increased quantity of adipose tissue; increased preload growth determined by an increase in the blood volume is associated with increased afterload determined by arterial stiffness and resistance. Consequently, excentric and concentric hypertrophy occurs in the left ventricle (VS), correlated with the degree and duration of association with obesity and HBP (Iacobellis G., Ribaudo M.C., Assael F., et al., 2003). It is proven that the dimensions of the left atrium and left ventricle (LV), and also the LV mass are bigger in obese children than in children with normal BMI.

Epicardic fat tissue stored around the heart between pericardium and the outer wall of the myocardium is proposed as a cardiovascular risk factor (Abaci A., Tascilar M.E., Saritas T., 2009). Echocardiographic assessment of epicardic adipose tissue showed a positive correlation with visceral adipose tissue depots. There is a small amount of adipose epicardic tissue in subjects with normal BMI, but the limit value considered normal has not yet been established. However, a recent study has suggested the figure of 4.1 mm as reference value in children (Wong C. and Marwick T.H., 2007).

Epicardic adipose deposit size correlates with insulin resistance, coronary artery disease, carotid wall thickness and arterial stiffness in adult. Measurement of epicardic tissue may be useful for assessing the cardiovascular risk in children.

Macroscopic changes of the heart in obese children are increased cardiac weight, hypertrophy of LV, RV, and ventricular dilation. Microscopically, it is observed myocytes hypertrophy and myocardial fibrosis, followed by apoptosis and necrosis of the myocytes, capillaries rarefaction and inflammatory infiltrate (Chiu H.C., Kovacs A., Ford D.A., 2001).

The concept of lipotoxicity in obese cardiomyopathy is based on experimental discovery of intra and intercellular fat deposit, causing degradation of myocytes, apoptosis, atrophy and heart dysfunction (Szczepaniak L.S., Dobbins R.L., Metzger G.J., 2003). Similarly, studies in humans have shown in- creased content of triglyceride at myocardial level (Wong C.Y., O'Moore-Sullivan T., Leano R., 2004).

The first change of heart function that appears in obesity is diastolic dysfunction, in the form of disturbed relaxation and/or compliance of the myocardium. It occurs independently or in combination with other changes characteristic to the obese cardiomyopathy, such as LV hypertrophy. Moreover, the severity of the diastolic dysfunction is directly proportional to the duration of obesity (Pergola G. D., Nardecchia A., Giagulli V.A. et al., 2013).

LV hypertrophy determines also subendocardial ischemia, a phenomenon accentuated by the presence of epicardial fat which, by his size, restricts the normal mechanics of heart (Border W.L., Michelfelder E.C., Glascock J.B. et al., 2003).

Gradually, systolic dysfunction installs correlated with the severity of obesity, as demonstrated by the modern techniques of echocardiography (Montani J.-P., Carroll J.F., Dwyer T.M. et al., 2004).

Visceral adiposity is independently associated with the risk of cardiovascular disease. Fatty tissue is more and more acknowledged as a metabolically active organ. When the storage

capacity of fat tissue achieves the maximum, excess fat can be stored in the ectopic tissue, such as skeletal muscle, heart, vessel, and kidney, causing an f insulin-resistant status. This results in lipolysis with excessive release of free fatty acids and other peptides derived from adipocytes, such as leptin, rezistine, α tumor necrosis factor, adiponectin, plasminogen activation inhibitor and interleukin 6 (Cancello R.,Henegar C.,Viguerie N. et al., 2005).

Adipose tissue is infiltrated with macrophages. Most inflammatory cytokines released from adipose tissue are derived from macrophages which infiltrate functional dysfunctional adipose tissue. Cytokine's environment maintains the macrophages recruitment. Cancello and collab have shown improvement of the inflammatory status by reducing macrophage infiltration from the adipose tissue and improvement of insulin sensitivity in obese sub- jects who have lost weight (Kirk S., Scott B.J., Daniels S.R., 2005).

Cardiovascular effect of obesity treatment

Current guidelines recommend lifestyle changes, diet, reducing sedentary activities and physical exercises program for prevention and treatment of childhood obesity. BMI is used as a marker of treatment success. More aggressive measures, such as drug treatment and bariatric surgery are reserved for the adolescents with severe obesity who have failed conventional therapeutic measures (Cote A.T., Harris K.C., Panagiotopoulos C. et al., 2013). Physical exercise has a favourable effect on BMI and adiposity dimensions in short term (10 weeks) and medium term (4 months). Many studies have demonstrated improvement in cardiac and vascular function after 6 months of dietary measures and physical exercise, with significant improvement of diastolic dysfunction (Gaborit B., Jacquier A., Kober F. et al., 2012). In adolescents with morbid obesity, bariatric surgery improved cardiac geometry, corrected diastolic function and de- creased epicardial fat; the assessment was made after 10 ± 3 months postoperatively Huang et al have noted an improvement in the status of adhesion molecules (selectine E), parameters of fibrinolysis (the plasminogen activation inhibitor), in the amount of triglycerides and glucose homeostasis in patients who have obtained a reduction in BMI after six months of life style modification (Huang F., del-Río-Navarro B. E., de Castro G. T. M. et al., 2011). These improvements have been accompanied by improving cardiometabolic risk factors, adiposity, and bio- markers.

- ***Psychosocial Implications of Childhood Obesity***

Childhood obesity is more than a physical problem. The psychosocial consequences of obesity in children are less clear than physiological consequences. Obese children often experience stigmatization, bullying, discrimination, and peer victimization, low self-esteem altered cognitive performance, or emotional disorders that are further discussed based on literature data (Zametkin A.J., Zoon C.K., Klein H.W. et al., 2004)

Social stigmatization of obese children

Discrimination is related to stigmatization (Lobstein T., Baur L., Uauy R., 2004) because obesity is one of the most stigmatising conditions. In a study conducted by Puhl and Latner (2005) (Puhl R.M. and Latner J.D., 2005), prejudice against obese children develop since as three years of age in children because they consider their obese classmates as being lazy, ugly, and unhappy. On the other hand, school-aged children associate normal body weight with intelligence, happiness, and popularity. In other study, 96% of the 50 obese girls reported that they had weight-based stigmatizing experiences, like jokes or name calling from their

classmates at school, especially junior high school. Additionally, some studies showed that some obese children share negative opinions and attitudes on obesity.

Unfortunately, obese children do not get support from their classmates. Moreover, some obese children are severely stigmatized by their classmates, and this could lead to suicide after rejection and marginalization. So, special programs to support and provide counselling for the obese children are needed. The psychological stress can cause low self-esteem, which can hinder academic or social functioning, and most of them persist into adulthood (Swartz M.B. and Puhl R., 2003). The long-term impact of stigmatization, of overweight children and adolescents is lower education and socio-economic level, less romantic relationships, and problems in getting married, compared to their normal weight classmates. Also, the stigmatization leads to unhealthy eating habits and bulimia nervosa. A hostile schooling environment for obese adolescents may play an important role in the evolution of the future adult.

Teasing and victimization

Stigmatization also refers to weight-related victimization and teasing of the obese children. When people intend to harm the former, they use physical violence or verbal abuse, or they exclude an individual from social activities, alienating him from his friends. Generally, verbal abuse involves the victim's physical appearance (Haines J., Neumark-Sztainer D., Hannan P. et al., 2008)., causing psycho-social damage. Calling an obese person with mean and unpleasant names leads to intimidation that was defined as abuse against another person. The main sources of teasing are the individual's classmates, family members or even foreigners (Neumark-Sztainer D., Croll J., Story M. et al., 2002). Janssen et al. argued that obese and overweight children are victims of aggression compared to their normal weight classmates (Janssen I., Craig W.M, Boyce W.F., et al., 2004). Other research consider that obesity has to involve also aggression support. Another study showed that obese children were more susceptible to emotional problems such as low self-esteem or even suicidal thoughts (Eisenberg M.E., Neumark-Sztainer D., Story M., 2003).

Victimization and teasing are considered significant psychological trauma risk factors and stress factors. These factors are triggering excessive emotional eating. Stressed children suffer overeating or emotional eating with a weight-related prejudice, defined as the tendency to unfairly judge based on their overweight. These children are more often abused or humiliated and they tend to aggressive behaviours. Abused children need to be identified and supported, because results will not appear only by changing their lifestyle. Some children cannot follow the therapeutic nutritional programs because they have emotional eating behaviour. Children and adolescents' therapy need to involve strategies against people discrimination.

Impact on self-esteem

In the development process of the children self-esteem plays an extremely important role. Self-esteem includes five dimensions by Harter's model: perceived self-efficacy, scholastic competence, self-concept, and athletic competence, peer social acceptance and physical appearance. In 1981, Bruch has theorized the role of the body weight in self-concept and in the occurrence of eating disorders. It seems like individuals develop ideal identities, their so called-aspirational self. Chart of the Self is focusing on a particular aspect that the individual finds important (Kendzierski D., 2007). Body image is causally linked to self-esteem and is made of a complex construct, which includes perceptions, cognitions, emotions and attitudes

(Cash T.F., 1990). This conceptualization is a subjective image of one's body and also attractiveness. For children and adolescents, the ideal body image is the result of family, social and cultural factors in connection with their self-esteem.

French et al. (1995) (French S.A., Story M., Perry C.L., 1995) concluded that 13 of 25 studies had reported low self-esteem in obese young people. Also, he noted that self-esteem has improved with lose weight after dieting. In children and adolescent's self-esteem varies with gender and the females are more affected than males (Nowicka P., Hoglund P., Birgerstam P. et al., 2009). It seems that self-esteem is inversely proportional to patient's age. In a tender age, obese children do not have negative feelings but when they start going to school, things change in a negative way. Another study revealed that self-esteem was lower in overweight girls because their parents were unhappy with their child's weight (Pierce J.W. and Wardle J., 1997). Poorer self-esteem among obese adolescents is closely related with weight-based teasing from parents especially in the absence of psychological counselling and support (Davison K.K. and Birch L.L., 2002). So, in addition to their fight against their overweight, children have to struggle with low self-esteem, increasing frustrations, low self-worth and low motivation to change their unhealthy behaviours (Wardle J. and Cooke L., 2005). This is why clinicians should employ a positive language with hope and courage with motivational interviewing methods for overweight young people. Also, they should avoid negative verbal or non-verbal communication, and prevent the increasing depreciation of the patient's self-worth.

Feeling of guilt

In addition, obese children feel guilty for their weight. A study conducted on obese children related that they are feeling ashamed of their weight. This is the reason why obese children have fewer friends and are left out from different social activities. Almost 90% of the overweight children are convinced that their classmates will stop harassing them if they will lose weight, and 69% of them believe that they will have more friends if they have a normal weight. Also, this study suggests that obese children blame themselves and they think they are guilty for their overweight.

- *Quality of life*

WHO's defined quality of life like "an individual's perception of their existence given the cultural environment and system of values in which one lives". Unfortunately, BMI fails to assess the physical, social and scholastic areas of obese children. It seems that poorer physical and mental health, deficient social functioning and school performance leads to lower quality of life of obese children. In a study conducted by Schwimmer et al. (Schwimmer J.B., Burwinkle T.M., Varni J.W., 2003) was noted that BMI correlates inversely with physical activity of obese children and adolescents. The authors observed the lower quality of life of these children as compared to normal weight children. Poorer quality of life is associated also with the comorbidities of obese children and adolescents, (dyslipidemia, hyperinsulinemia, bone disorders and sleep apnea). The lower score of the quality of life has been found to the more obese children (Pinhas-Hamiel O., Singer S., Pilpel N. et al., 2006).

- *Mental health*

It has been described a straight causality between obesity and depression, but it is not well understood whether depression is the cause or the consequence of obesity. According to literature data, obesity increases the risk of developing neuropsychiatric disorders in adulthood. Association between nutritional therapy failure and emotional stress, lead to anxiety and

depressive disorders over time. Some prospective studies found that obesity cannot predict depression (Latzer, Y. and Stein D., 2013), but Sjöberg et al. (2005) (Sjöberg R.L., Nilsson, K.W., Leppert J., 2005) have shown that obese school aged girls are the first candidates to depressive behaviours. Therefore, higher BMI was associated with higher risk of developing depression or other mental disorders. Some research made among boys revealed a weak relationship between obesity and depression over time. The childhood depression on the other hand, predicted development of obesity. A longitudinal study reported that adolescent depression could predict obesity appearance in adulthood. It seems that insulin resistance modifies in children with depressive disorders, leading to appetite increase. Depression is accompanied by insomnia, unwillingness to exercises or tiredness that also helps the increase in body weight. Neuroendocrine responses can be stimulated by anxiety and depression. This is the response why the activation of the hypothalamic-pituitary axis by producing an excessive amount of cortisol influences intra-abdominal adiposity, insulin resistance and metabolic syndrome. So, psychological disorders (e.g., depression, anxiety, and eating disorders) impede children to control their consumption of food, exercising for maintaining a healthy weight. It is well known that overweight and obese adolescents have suicidal ideation (Ackard D.M., Neumark-Sztainer D., Story M., 2003) because stigmatization and weight-based teasing are important risk factors related to the idea of suicide (Neumark-Sztainer D., Story M., Faibisch L., 1998).

- *Social relations*

As compared to their normal weight peers, loneliness is very common among obese children (Zeller M.H., Reiter-Purtill J., Ramey C., 2008). Phillips and Hill study (1998) (Phillips R.G. and Hill A.J., 1998) reported that overweight and obese pre-adolescent children had as many friends as their normal weight peers, but the study made by Zeller et al. (2008) reported the contrary. Furthermore, obese children are less accepted in many groups of children. Overweight and obese children have a predisposition to pro-social behaviours because they are being helpful and kind to others. Two studies showed that obese adolescent girls had less romantic relationships (Sobal J., Nicolopoulos V., Lee J., 1995).

- *Academic performance*

Conflicting results of the studies are described in the literature. Warschburger (2005) (Warschburger P., 2005) revealed that academic achievement difficulties in obese adolescents, but many studies didn't establish a link between BMI and academic performance. However, statistical data show that absenteeism is higher among obese students (Geier A.B., Foster G.D., Womble J., et al., 2007). Wadden et al. (1984) (Wadden T.A., Foster G.D., Brownell K.D., 1984) argued "society does not tolerate obesity, especially during childhood". This is why children may develop harmful psychosocial sequel like depression, social isolation, teasing, and discrimination, behavioural problems, lower self-respect, body image dissatisfaction. Because of the psychological this victim of stigmatization, may develop mental disorders later in life.

II.1.4.4. Conclusions

- ***Childhood Obesity – Risk Factor for Cardiovascular Disease***

Obesity, fetal scheduled disease, with onset anytime along childhood under the influence of a context of risk factors, is accompanied (in proportion to the severity) by

cardiovascular impairment. The child and adolescent obesity are associated with cardiovascular risk on short and medium term, which includes the hemodynamic changes and structural and functional changes at the level of the heart and vessels. Obesity in childhood accelerates these processes, increasing the risk of cardiovascular disease in adulthood, but determines cardiovascular damage also in the child. Prevention programs, diagnosis, and treatment of obesity in children and adolescents should include early identification of cardiovascular dysfunction and follow-up as correcting BMI. Young age ensures reversibility of cardiovascular side effects of obesity, only with early inclusion of the patient in the recovery program.

- ***Psychosocial Implications of Childhood Obesity***

Childhood obesity prevention must become a public heath priority in all the countries of the European Union because healthy nutrition in children should play an important role in public health. Psychosocial issues can influence the quality of life and the school and social performance of children and adolescents with obesity. This category of children has greater school absenteeism, psychosocial stress, and more behavioural problems. A strategy for minimizing discrimination must be included in the treatment of obese children and adolescents.

II.1.5. CURRENT AND LONG-TERM THERAPEUTIC STRATEGIES IN CHILDHOOD OBESITY AND ASSOCIATED PATHOLOGIES

II.1.5.1. Introduction

Childhood obesity is an important public health problem, considering its association with many comorbid conditions as metabolic syndrome, cardiovascular disease, hepatic steatosis, and sleep apnea (Eklioğlu B.S., Atabek M.E., Akyürek N. et al., 2015). Knowing that obesity is a chronic illness, we find it essential to diagnose and treat it early, in association with psychological counselling conducted by a multidisciplinary team, in order to prevent long-term complications, to reduce morbidity, mortality, and expected costs for the care of obese adults (Valerio G., Maffeis C., Saggese G. et al., 2018). We consider that identification and early intervention of overweight and obesity is critical in preventing or delaying the onset of chronic diseases. Child obesity treatment is imperative to the overall health and wellness of children and adolescents (Fitch A., Fox C., Bauerly K. et al, 2013). Dietary and behavioral changies and physical activity for both children and their families is essential for healthy living and a prerequisite for all overweight and obesity treatments. Therapeutic strategies include diet, physical activity to avoid sedentarism, behavior and lifestyle changes, medication and surgical considerations. Avoidance or reduction of calorie-dense, nutrient-poor foods (i.e., “fast food”) as well as sugar-sweetened beverages, sports drinks, and fruit drinks and juices are essential for weight loss (Gidding S.S., Dennison B.A., Birch L.L., et al., 2006, Matson K.L. and Fallon R.M., 2012).

Complications of pediatric obesity occur during childhood and adolescence increased the risk for morbidity and mortality into adulthood. Dyslipidemia, arterial hypertension, T2DM, nonalcoholic fatty liver disease, polycystic ovaries syndrome, orthopedic and respiratory complications reresent the most important complications. Therefore, treatment of comorbid conditions associated with obesity is an important goal of therapeutic protocol. Effective weight

reduction is one of the key elements in the treatment of comorbidities. Bariatric surgery and laparoscopic sleeve gastrectomy have been used in adolescence with morbid obesity. (Maffeis C., Licenziati M.R., Vania A. et al., 2016., Trandafir L.M., Ioniuc I., Miron I., 2017)

The opportunity to carry out my activity in the IIIrd Pediatric Clinics for the past sixteen years, which also includes the Department of Nutritional Diseases and Diabetes (and the Pediatric Gastroenterology Department until 2009), as well as in the Center for Diagnosis, Treatment, and Monitoring of children with obesity since 2016, allowed me to deal on a daily basis with children suffering from obesity. Thus, we observed that among the complications of obesity, hepatic steatosis, identified using ultrasound, with or without hepatic cytolysis, is relatively common in adolescents with obesity and a high BMI. Another prevalent complication in these patients is dyslipidemia. The basic therapy for all these patients is to change their lifestyle, which implies adopting a healthy diet and exercise regularly. Concerns about applying a current therapeutic strategy led me to study the current literature. Thus, I also published two articles on nutritional therapy in children with hepatic steatosis and the management of dyslipidemia.

All these concerns about the comorbidities of obesity in childhood and adolescence can be found in the articles presented below.

Personal contributions related to therapeutic strategies in childhood obesity and associated pathologies

1. Trandafir LM, Baciu G, Constantin MML, Mastaleru A, Temneanu OR, Mihai B, Novac O, Frasinariu OE, Ivan A, Tudorachi NB, Al Hiary R, Moscalu M. Predictive Biological Markers in Post-therapeutic Evolution in Obese Patients, *Rev Chim* (Bucharest). 2018; 69(11): 3048-3051. (IF= 1.22)

2. Trandafir LM, Frasinariu OE, Chiriac MI, Miron I. Management of dyslipidemia in childhood may prevent cardiovascular events in adults, *The Medical-Surgical Journal*. 2017; 121(2): 313-320. (Web of Science Core Collection)

3.Trandafir LM, Frasinariu OE. Nutrition Recommendations in Hepatic Steatosis in Children, Proceedings of the 4th Congress of the Romanian Society for Minimal Invasive Surgery in Ginecology / Annual Days of the National Institute for Mother and Child Health Alessandrescu-Rusescu. 2019; 648-653. (Web of Science Core Collection).

The scientific interest related to therapeutic strategies in childhood obesity and associated pathologies was synthesized in the following articles:

1.Trandafir LM, Baciu G, Constantin MML, Mastaleru A, Temneanu OR, Mihai B, Novac O, Frasinariu OE, Ivan A, Tudorachi NB, Al Hiary R, Moscalu M. Predictive Biological Markers in Post-therapeutic Evolution in Obese Patients, Revista de Chimie, 2018, 69(11): 3048-3051. (IF= 1.22)

2. Trandafir LM, Frasinariu OE. Nutrition Recommendations in Hepatic Steatosis in Children, Proceedings of the 4th Congress of the Romanian Society for Minimal Invasive Surgery in

Ginecology / Annual Days of the National Institute for Mother and Child Health Alessandrescu-Rusescu. 2019; 648-653. (Web of Science Core Collection).

Objectives

- ***Treatment and Monitorization of Obese Children***

Child obesity needs a complex program that included diet and physical activities changes and psychological counselling. The main objective of our study was to observe the influences of this program on the health obese patients after 6 months of multidisciplinary treatment.

- ***Nutritional Recommendations in Hepatic Steatosis in Children***

The growing incidence of obesity has increased the number of patients with fatty liver. Thus, NAFLD has become the most common chronic liver disease in both adults and children. Common since childhood, NAFLD is a major cause of liver morbidity and mortality, especially in adulthood. Best therapeutic practices in management of pediatric NAFLD are not clearly defined (Vos M. B., Abrams S. H., Barlow S. E. et al., 2017). Therefore, the purpose of our article was to identify appropriate treatments for children and adolescents with NAFLD as an important priority for healthcare systems around the world.

II.1.5.2. Material and methods

- ***Treatment and monitorization of obese children***

We conducted a retrospective study in which we included a sample of 69 children and adolescent with obesity, boys and girls, aged 12 to 18 years, followed-up at Saint Mary Children's Hospital and Regional Center of Diagnosis, Counselling and Monitoring of Overweight and Obese Children from "Grigore T. Popa" University of Medicine and Pharmacy Iasi, Romania. These patients with obesity (body mass index - BMI percentiles between 95 - 97^{th}) and severe obesity (BMI percentiles > 97^{th}) were separated into two groups: first group (38 patients) followed only a hypocaloric diet and the second group (31 patients) followed a hypocaloric diet with kinetotherapy and psycological counselling (Kuczmarski R.J., Ogden C.L., Grummer-Strawn L.M. et al., 2000).

Every participant of the study had a first examination at the beginning of the study and after 6 months of program. The parameters observed at the first consult were psysical parameters (height, weight, BMI and waist circumference) and blood parameters (tryglycerides (TG), total cholsterol (TC) and high-density lipoprotein (HDL) cholesterol).

The abdominal fat was estimated with the waist circumference and its values were compared to the percentiles for age and sex (Matsushita R., Isojima T., Takaya R. et al. 2015).

Cholesterol and tryglycerides values were compared to the percentiles for sex and age. An increased value of tryglycerides over the 95^{th} percentile for age and sex is defined as hypertriglyceridemia: ≥ 125 mg/dL for boys age 10-14, ≥ 148 mg/dl for boys age 15 – 19, ≥ 131 mg/dL for girls age 10 – 14, ≥ 124 mg/dL for girls 15 – 19. Every child included in the study received a hypocaloric diet specific for age and eating habits. Furthermore, for every participant physiotherapist conceived an exercise especially for them.

In our study no patient with known conditions (cardiovascular, neurological or renal diseases or diabetes), with chronic treatment or eating disorders was included.

We made the statistical analysis in SPPS 24. We expound the statistical results using the significant value p=0.05 as reference, which is associated with a confidence level of 95%. We used continuous type variables as average ± standard deviation. We apply specific statistical parameters ((F-tests, t-test, ANOVA) and non-parametric (Yates Chi-square) to compare the parameters in our study groups.

- ***Nutritional Recommendations in Hepatic Steatosis in Children***

We revised the current literature concerning nutritional recommendations in hepatic steatosis in children. There are relatively few guidelines about pediatric NAFLD and experts believe that its management is a challenge for general pediatric practitioners, subspecialists and for health systems.

II.1.5.3. Results

- ***Treatment and Monitorization of Obese Children***

In this study, we had a sample of pediatric patients with obesity (children and teens), which we divided into 2 groups. The first group received just a hypocaloric diet and the second group besides the hypocaloric diet also followed a physical exercise schedule established by a specialized team. After 6 months, we compared the two groups and found that the association of diet and physical activity has a stronger impact on decreasing weight, BMI, WC, as well as the biological values (TC, TG) than the hypocaloric diet alone.

Group I: We included in the first group children with a value of BMI of 30.4 ± 7.8. We compared their BMI with the percentiles for age and sex and we saw that 39.5% of these patients were between 95 - 97 th percentiles (obesity) and 60.5% had a BMI > 97th percentiles (severe obesity). We analysed further the average age of the patients (12.8 ± 1.9 years) and for sex: 12.6 ± 1.9 (for girls) and 13 ± 1.9 (for boys). The children from this group received a hypocaloric diet for 6 months, time after we observed that even though 26.3% of them had a BMI < 90% percentile, 73.7% of them had still a waist circumference (WC) higher than 90th percentiles.

Group II: We included in the second group, children with a value of BMI of 29.1 ± 3.4. Among these patients, 45.2 % had a BMI on 95 - 97 th percentiles (obesity) and 54.8 % had a BMI > 97th percentiles (severe obesity). We observed that the avarage age was 13.7 ± 2.4 years: 13.5 ± 2.9 years (for girls) and 13.8 ± 2.2 years (for boys). In contrast to the first group, these children recieved in addition to the hypocaloric diet, kinetotheray. We analysed the results after 6 months. Even though 48.4% of the children had a value of BMI < 90% percentile, 51.6 % of the cases still had a high value of the waist circumference (WC> 90th percentiles).

Comparing the two groups, we saw better results in the second one. After 6 months, among the second group were 6.5 % cases with a value of BMI > 97% percentiles, compared to 13,2% in the first group (Table XXI).

The values of WC, TG and TC were also significally more reduced in the cases included in group II, compared to those included in group I (Table XXI).

Table XXI. Clinical-biological parameters initially and after 6 months

Group I (N=38) female (n=15) male (n=23)		First visit	6 months	p-value‡
BMI (>97)	All	23(60.5%)	5(13.2%)	
Male		7(46.7%)	(3)13.1%	0.024
Female		16(69.6%)	(2)13.3%	0.015
WC (>90)	All	38(100%)	28(73.7%)	
Male		(23)100%	19(82.6%)	0.026*
Female		(15)100%	9(60%)	0.007*
TG (tryglicerides) (>95)	All	9(23.7%)	6(15.8%)	
Male		5(21.7%)	3(13.1%)	0.035*
Female		4(26.7%)	3(20%)	0.051
Total cholesterol (>95)	All	5(13.16%)	2(5.3%)	
Male		1(4.35%)	0(0%)	0.001*
Female		4(26.7%)	2(13.3%)	0.028*
Group II (N=31) female (n=11) male (n=20)		first visit†	6 months†	p-value‡
BMI (>97)	All	17(54.9%)	2(6.5%)	
Male		13(65%)	(1)5%	<0.01*
Female		4(36.4%)	(1)9.1%	<0.01*
WC (>90)	All	31(100%)	16(51.6%)	
Male		(20)100%	11(55%)	0.013*
Female		(11)100%	5(45.5%)	0.002*
TG (tryglicerides) (>95)	All	9(29)	1(3.2%)	
Male		6(30%)	0(0%)	<0.01*
Female		3(27.3%)	1(9.09%)	<0.01*
Total cholesterol (>95)	All	7(22.6%)	2(6.5%)	
Male		4(20%)	2(10%)	0.03
Female		3(27.3%)	0(0%)	<0.01*

† Values were expressed as number (percent%); (*) Marked effects are significant at p <0.05; ‡ Yates Chi-square test or Fisher's exact test.

After 6 months in group II were 51.6 % patients with WC higher than 90 percentiles compared to 73.7 % in the first group.

Our study showed better results in the group II. After 6 months of therapy, we remarked an improvement of the percentage of patients with medium cardiovascular risk in group II (from 19.4 % to 3.23 % - p = 0.0092) compared to group 1 (from 7.9 % to 5.3 % - p = 0.068) (Table XXII).

Table XXII. Cardiovascular risk assessment

Group I (N=38)		First visit	6 months	p-value‡	p-value**
Cardiovascular risk	Absent	27(71.1%)	35(92.1%)	0.019*	
Law		6(15.8%)	1(2.6%)	0.036*	
Medium		3(7.9%)	2(5.3%)	0.068	
High		2(5.3%)	-	0.001*	
Group II (N=31)		first visit	6 months	p-value‡	<0.001*
Cardiovascular risk	Absent	18(58.1%)	29(93.6%)	0.0041*	
Law		6(19.4%)	1(3.23%)	0.0068*	
Medium		6(19.4%)	1(3.23%)	0.0092*	
High		1(3.23%)	-	<0.01*	

† Values were expressed as number (percen %); (*) Marked effects are significant at p <0.05;‡ Yates Chi-square test or Fisher's exact test. (**) Mark the difference between groups (I vs. II);

We observed in both groups that WC was correlated with a high risk of cardiovascular disease. Our study established a significant association between WC (r = 0.71, p = 0.003) and BMI (r = 0.65, p = 0.002) and the cardiovascular risk. A lower WC in group II was associated with a lower TG (r = 0.084, p <0.001), compared to group I (r = 0.31, p = 0.035).

Analysing the two groups after 6 months, we saw a significantly improvement of the TC levels in patients who exercises in addition to following the hypocaloric diet (p=0.0053). We observed a significant difference in the WC (< 90th percentiles - p < 0.013), weight and BMI in the group 2 compared to group 1 (p = 0.042). Furthermore, we found a higher correlation between the reduced TG values with reduced WC values in the group II compared to group I (r = 0.824, p = 0.003).

Also, we found a significant/strong association (r = 0.703, p = 0.002) between the lower TG values and the lower WC values in group II, compared to a moderat association (r = 0.358, p = 0.037) of these parameters in the group I, as well as for TC. We found that the decrease of WC and TG values was significant in group II (r = 0.669, p = 0.0132).

After our statistical analysis, we established predictive factors for the outcome of obese patients (Table XXIII).

Tabel XXIII. The predictive factors regarding the evolution of obese patients

Multiple Logistic Regression	p-value	Exp(B)	95% CI for Exp(B)	
Positive Evolution vs.		OR	Lower	Upper
Type of therapy	<0.001	5.981	2.187	9.453
TG (tryglicerides)	0.001	3.129	1.907	7.882
Total Cholesterol	0.027	2.874	1.882	8.158
WC (>90)	0.003	2.172	1.855	5.619
BMI (>97)	0.024	1.915	1.682	6.998
OR - Odd ratio; CI – confidence interval				

- ***Nutritional Recommendations in Hepatic Steatosis in Children***

The onset of NAFLD in childhood leads to development of advanced liver disease in young adults and the early onset of comorbidities, such as metabolic syndrome (MS) and cardiovascular disease. Some authors consider NAFLD the liver manifestation of metabolic syndrome and a group of metabolic disorders, including excessive increase in central adiposity, insulin resistance, dyslipidemia, and high blood pressure (Crespo M., Lappe S., Feldstein A.E. et al., 2016. Paschos P. and Paletas K., 2009). Because NAFLD's molecular pathogenesis is still incompletely elucidated, current therapeutic approaches consist of strategies designed to reduce the incidence of risk factors: obesity, dyslipidaemia, insulin resistance.

The first line in prevention and treatment is to respect a proper diet and increase physical activity. Thus, improvement in liver parameters and histological aspects was correlated with weight loss. Decrease in ALT level is commonly used as a biological marker of improvement in histology of NAFLD (Vos M.B., Abrams S. H., Barlow S. E. et al., 2017). Analysis of the recently published literature showed that improvement in liver histology is rare in children following diet as monotherapy. Recent studies have shown that reducing fructose in the diet and supplementing with vitamin E, omega-3 fatty acids and prebiotics / probiotics improve NAFLD evolution. It is therefore difficult to establish if a proper diet associated with nutritional supplements is sufficient to prevent early signs of NAFLD in the childhood and to stop its progression.

II.1.5.4. Discussions

- ***Treatment and Monitorization of Obese children***

Analysing the results in our study, we found that for a positive evolution of obese pediatric patients the physical exercise is a strong prognostic factor (OR = 5.9, $p < 0.01$). Other strong prognostic factors were TC and TG values. We saw that their values were proportional with the value of weight. Reduced weight is correlated with lower TC and TG values. However, we found that BMI value has a lower influence on the outcome of these patients. Hence, we saw of greater importance the role of physical exercise compared to the initial nutrition status. After 6 months, we could see that combination between diet and physical activity is essential in reducing the cardiovascular risk in the paediatric patients with obesity.

However, our study had some limitations such as the short follow-up period, the impossibility to evaluate the exact physical exercise of every patient and the lack of physical activity besides the specialized kinetotherapy.

The specialty literature established a strong connection between the biological high values of TC and TG and low values of HDL cholesterol (Huang R.C., Prescott S.L., Godfrey K.M. et al., 2015) and the increased risk of developing cardiovascular disease, obesity, and abdominal adiposity in paediatric patients. Hence, these biological parameters are strong predictor factors in the outcome of these patients.

Waist circumference (WC) measuring the visceral fat represents a separate risk factor for developing diabetogenic and atherogenic disturbances in adolescents (Do K., Brown R.E., Wharton S. et al., 2018). In our research, we saw a high percentage of obese paediatric patients with dyslipidemia. Moreover, we evaluated the cardiovascular risk using the TC levels. We saw that 13, 16 % cases from group 1 and 22, 16 % cases from group 2 had a high risk and an early intervention was needed in these cases. Furthermore, we found an association between the weight > 95 percentiles and high level of TG, correlated to a high cardiovascular risk (Macdonald I.A., 2016).

However, the role of diet changes in the obese patients should not be neglected. We found that a hypocaloric diet influences the lipid level changes in the obese children. Hence, the diet therapy should be promoted in these cases (Lakshman R., Elks C.E., Ong K.K., 2012).

Furthermore, we analysed the amount of physical activity that the patients included in our study have done. We discovered that the period of physical activity is of great importance in reducing the TC levels and therefore the cardiovascular risk (Lean M.E.J, Astrup A., Roberts S.B., 2018). The patients with obesity should practice physical activity circa 30 minutes, 5 times per week (at least 150 minutes per week). The impact of physical exercise on reducing weight and lipid values is proportional with the intensity (Colberg S.R., Sigal R.J., Yardley J.E. et al., 2016).

- ***Nutritional Recommendations in Hepatic Steatosis in Children***

The most commonly accepted goal of treatment is regression of NAFLD, defined as decrease in steatosis, inflammation, and/or fibrosis. An additional goal of therapeutic strategies in NAFLD patients is to decrease excess adiposity in order to improve dyslipidemia, insulin resistance, high blood pressure, and visceral adiposity. In children, the NAFLD comorbidities (diabetes, CVD, and hypertension) are important considerations of treatment to improve future clinical outcomes (Vos M.B., Abrams S. H., Barlow S. E., et al., 2017).

Changing lifestyle through an adequate diet and age-appropriate physical activity is the first line in pediatric patient with NAFLD management. In the children and adolescents, the diet should be balanced to the individual calorie requirement to ensure normal body growth, but also take into account the patient's preferences.

Recomandations for optimal dietary nutrition in NAFLD include reducing fat and carbohydrates, avoiding high fructose beverages and supplementing with polyunsaturated fatty acids and fiber. At this time, the available data do not support a specific diet over others for the treatment of NAFLD (eg, low glycemic index vs low fat) (Vos M.B., Abrams S. H., Barlow S. E. et al., 2017).

A hypolipidic diet with low glycemic index (predominantly complex carbohydrates - 50-60%, proteins - 15-20%, fats -23-30%, of which two thirds unsaturated and a third saturated)

is recommended with minimal physical activity daily. Restriction of sugars and foods rich in fructose and trans fatty acids as well as high consumption of fruit and vegetables is also recommended. Regarding the Mediterranean diet, due to its role in the prevention of metabolic and cardiovascular diseases, this diet was proposed as a dietetic therapeutic option for children with obesity and NAFLD, but additional studies are needed to confirm this observation (Corte D. C., Mosca A., Vania A. et al., 2017). Aerobic exercise intervention has been shown to decrease levels of hepatic triglycerides, visceral adiposity and insulin resistance and may benefit overweight and obese children with NAFLD (van der Heijden G.J., Wang Z.J., Chu Z.D. et al., 2010).

First behavioral interventions of NAFLD treatment in children have had disappointing results and new pharmacological, non-pharmacological or surgical therapeutic options are needed. Nutritional supplements have been proposed as therapeutic options for improving NAFLD in children and preventing progression to hepatic fibrosis.

The monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) intake are widely recommended. Omega-3 and omega-6 polyunsaturated fatty acids (PUFAs) are recognized as the essential fatty acids extremely necessary for the body health (Nobili V., Alisi A., Liu Z. et al., 2018). Due to the fact that ω-3 fatty acids are well tolerated in the pediatric population, DHA therapy should be considered for the management of children with NAFLD.

Oxidative stress had a pivotal role in the multihit hypothesis of NAFLD. The pathogenetic role of oxidative stress in NAFLD has led to the evaluation of some molecules with antioxidant effect in the treatment of this liver disease (Nobili V., Manco M., Devito R. et al., 2008). Both, D and E vitamins act as antioxidants mitigating the oxidative stress response. Vitamin E is the most promising pharmacological agent that may slow down the progression of simple steatosis to NASH. Nobili et al., 2006, showed that administration of α-tocopherol and ascorbic acid, combined with a balanced diet and physical exercise, led to improved ALT level and glicemic control. Vitamin D is also involved in attenuation of proinflammatory liver response (Corte D. C., Carpino G., De Vito R. et al., 2016).

Polyphenols with antioxidant and anti-inflammatory properties derived from plants, can be used to decrease liver inflammation. Many experimental and clinical studies have tested the effects of different polyphenols: resveratrol, curcumin, green tea catechins in the peroxidation and healing of liver enzymes and inflammatory biomarker levels. First pilot study about the effects of Resveratrol in adolescents with NAFLD of 13 to 18 years has been completed (ClinicalTrials.govIdentifier: NCT02216552), but the results are not yet available (Aguirre L., Portillo M.P., Hijona E. et al.,2014. Chen S., Zhao X., Ran L. et al., 2015).

Choline and some probiotics modulate intestinal microbiota, improving gut dysbiosis and bile acid homeostasis. Choline, constituent of cell membranes, derived from food intake and endogenous synthesis, is involved in the hepatic synthesis of VLDL cholesterol and intestinal microbial interactions. The use of choline in combination with DHA and vitamin E reduced ALT and blood glucose levels in children with obesity and NAFLD (Guerrerio A.L., Colvin R.M., Schwartz A.K. et al., 2012).

Probiotics reduce liver inflammation, improve gut epithelial barrier function, and improves IR in NAFLD patients. The study led by Famour F et al. revealed the efficacy of the combination of probiotics in NAFLD obese children and adolescents: Lactobacillus acidophilus ATCC B3208, Bifidobacterium lactis DSMZ 32269, Bifidum ATCC SD6576, and rhamnosus

DSMZ 216 which resulted in decreased AST and ALT, improved the NAFLD ultrasonographic aspect and improved lipid profile (Famouri F., Shariat Z., Hashemipour M. et al., 2017). But, additional clinical trials are required to establish the optimum probiotic mix to achieve the best results in adults and children NAFLD.

II.1.5.5. Conclusions

- ***Treatment and Monitorization of Obese Children***

It is important to have a multidisciplinary program including nutritional changes, kinetotherapy and psychological counselling for addressing the childhood obesity. Our study established a great impact of diet associated with physical exercise on the lipid biological parameters. An overweight or obese child has a high chance of developing hypertriglyceridemia and hypercholesterolemia later. Our study confirmed that diet associated with physical activity reduce the principal risk factors of cardiovascular diseases (WC, TC, TG values). Taken into consideration all these results, we find important to develop a program promoting nutritional changes and physical activity in obese children in order to prevent cardio-metabolic illnesses later on, the adult life.

- ***Nutritional Recommendations in Hepatic Steatosis in Children***

In conclusion, multifactorial pathogenesis of NAFLD is a crucial element that should be considered in the design of a multiple therapeutic intervention and in predicting the response to treatment. Lifestyle modifications to improve diet and increase physical activity are recommended as the first-line treatment for all children with NAFLD. The medical therapies should be tailored to the prominent associated metabolic condition of NASH. There are clear comprehensive health benefits to a healthier diet and increasing physical activity and these remain the first line approach to treatment of NAFLD in children.

II.1.6. THE ROLE OF INTERDISCIPLINARITY IN DYSLIPIDEMIA RESEARCH

II.1.6.1. Introduction

The etiology of obesity is multifactorial and recognizes multiple complex pathways that can be encountered from the beginning of life. Prevention is extremely important because childhood obesity is associated with cardiovascular risk factors that can lead to early atherosclerosis and premature CVD. Obesity in first years of life promotes atherosclerotic disease in vascular structures such as the aorta and the coronary arteries (Raj M., 2012). High blood pressure, insulin resistance, dyslipidemia and systemic inflammation are associated with vascular changes in childhood and all these factors contribute to the increased risk of cardiovascular events in adulthood if not treated properly as early as possible. Lifestyle changes are essential in the early treatment of childhood obesity. The effects of short-term change have been shown to be beneficial in the short term by ameliorating the consequences of endothelial dysfunction and insulin resistance in obese children. However, the long-term effects on cardiovascular risk factors remain unknown. The concentration of research through both experimental and clinical studies in these fields will contribute to the treatment of childhood

obesity and the risk factors associated with CVD in order to prevent the development of CVD in adulthood (McPhee P.G., Singh S., Morrison K. M., 2020).

Atherosclerotic cardiovascular disease represents nowadays a leading cause of death worldwide (Laslett L.J., Alagona P. Jr., Clark B.A. et al., 2012, De Backer G.G., 2018). Survey's data of obese patients prove that the implementation of guidelines regarding CVD prevention in clinical practice both in child and adult needs improvement (Kotseva K., De Bacquer D., De Backer G., et al., 2016). CVD prevention requires the control of risk factors involved in the etiology of atherosclerotic disease represent by total cholesterol, LDL-cholesterol, glycemia, uric acid, smoking, hypertension, hyperhomocysteinemia, etc. (Yuan C., Lai C.W., Chan L.W. et al., 2014. Tuñón J., Bäck M., Badimón L. et al., 2018. Filip C., Albu E., Zamosteanu N. et al.,2010). Childhood and adolescence dyslipidemia contribute to early atherosclerosis. Atherosclerosis and other coronary artery pathology, termed as arteriosclerosis, can begin in the first years of life, generally accelerated by exposure to factors associated with increased cardiovascular risk (de Ferranti S.D., Steinberger J., Ameduri R. et al., 2019). In 2011 the National Heart, Lung, and Blood Institute Expert Panel published recommendations for cardiovascular health and risk reduction in children and adolescents. The Expert Panel's goal was to develop comprehensive evidence-based recommendations that address all major risk factors of the CVD, to help the multidisciplinary pediatric care team - pediatricians, family physicians, nurses and nurses, nurses registered doctors and dietitians - both in promoting cardiovascular health and in identifying and managing specific risk factors from childhood to young adulthood. The National Heart, Lung, and Blood Institute 2011 guidelines recommend the universal screening for lipid levels in children at 9 to 11 years of age and again at 17 to 21 years of age4) in order to early reducing the risk and severity of CVD in adulthood (Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents, & National Heart, Lung, and Blood Institute, 2011., Lim J.S., 2013.).

In American Heart Association scientific statement published in 2019, the expert group presents recommendations for clinical approach regarding the assessment and risk reduction of select pediatric populations at high risk for premature CVD, including acquired arteriosclerosis or atherosclerosis. According to this scientific statement, the Expert Panel recommend screen yearly for lipid disorders with nonfasting non-HDL, followed by fasting lipid profile if initial TC >200, HDL <45, or non-HDL >145 mg/dL. If LDL-C is abnormal, consider diagnostic evaluation and initiate therapeutic lifestyle change and statin therapy based on risk category.

The treatment of obesity-related dyslipidemia is focusing on the lifestyle change modification including lowering of the dietary glycemic index, including limiting the intake of added sugar to 5% of total calories, as recommended by the AHA and the WHO, and increasing daily physical activity. The magnitude of weight loss necessary to achieve significant improvements in CVD risk factors among child and dolescent with obesity has not been fully determined; a BMI reduction of 5% to 10% or 0.25 to 0.5 in BMI standard deviation score could be required. (de Ferranti S.D., Steinberger J., Ameduri R. et al., 2019).

Hypertriglyceridemia represents a prevalent risk factor for CVD among the types of dyslipidemia and plays an important role in the pathogenesis of atherosclerosis. The mechanism by which decreasing the level of plasma triglycerides leads to a reducing the total cardiovascular risk appears to be the apoB (apolipoprotein B) lowering (Ference B.A., Kastelein J.J.P., Ray K.K., 2019). Another mechanism by which triglycerides are involved in the atherosclerotic

process is stimulation of inflammatory cytokines, of fibrinogen, and coagulation factors (Tenenbaum A., Klempfner R., Fisman E.Z., 2014). Even though it is established that triglycerides are involved in atherosclerosis process and in increasing the cardiovascular risk is therefore evident, but certainly additional studies are necessary in order to establish if increased levels of triglycerides cause CVD per se or by association with other known cardiovascular risk factors such as obesity (Simha V., 2020. Packard C.J., Boren J., Taskinen M.R, 2020).

My continued concern for the study of childhood dyslipidemia, especially in obese children, has exceeded the limits of clinical trials. I am convinced that only through multidisciplinarity will we be able to carry out experimental studies with applicability in clinical practice. Thus, I collaborated with colleagues from the preclinical departments regarding the research in dyslipidemia, an incompletely explored territory that requires future studies.

Personal contributions related to the role of interdisciplinarity in dyslipidemia research

1. Cojocaru E, Magdalena Leon-Constantin M, Ungureanu C, Trandafirescu MF, Maștaleru A, **Trandafir LM**, Dumitru Petrariu F, Viola Bădulescu O, Filip N. Hypolipemiant Actions and Possible Cardioprotective Effects of Valine and Leucine: An Experimental Study. Medicina (Kaunas). 2021 Mar 5;57(3):239. doi: 10.3390/medicina57030239. PMID: 33807510. (IF= 1.205)

Objective

In this paper, we focus on our results regarding the study of triglycerides levels, agreeing that hypertriglyceridemia could become an important therapeutic target in the management of atherosclerosis.

II.1.6.2. Materials and Methods

We conducted an experimental study carried out over a period of 60 days which included male Wistar rats weighing 250-280g obtained from the animal farm of the "Grigore T. Popa" University of Medicine and Pharmacy, Iasi. All animal protocols were carried out in accordance with the instructions of the Guide regarding animal care and scientific use, in strict accordance to international ethical regulations (Guidlines on the care and use of animals for scientific purposes, 2004, AVMA guidelines for the euthanasia of animals, 2013)

The rats were divided into 4 study groups as follows:

1.Control group I (n=8): fed with a regular diet composed of agricultural by-products.

2.Group II – C (n=8): received regular diet supplemented with 0.4g/kg/day cholesterol.

3.Group III –C+V (n=8): received regular diet supplemented with 0.4g/kg/day cholesterol and 62.5mg/kg/day valine powder for animal nutrition.

4.Group IV–C+L (n=8): received regular diet supplemented with 0.4g/kg/day cholesterol and 69.985mg/kg/day leucine powder for animal nutrition.

Blood samples were collected from the retroorbital plexus, under anaesthesia of animals with 75 mg/kg of intraperitoneal ketamine, in three moments of the experiment as follows: R0 – 1st day, R1 – 30th day R2 – 60th day. The measurement of triglycerides levels was made

using Diagnosticum Zrt kit bought from Budapest, Hungary as in our previous studies (Fossati P. and Prencipe L.,1982).

The study was conducted in accordance with the 2010/63/EU directive and followed the recommendations of the NIH Guide for the Care and the Use of Laboratory Animals. Prior to the beginning of the study, the protocol received ethical approval from the ethics committee of the University of Medicine and Pharmacy "Grigore T. Popa", Iași, Ethic Committee approval number 15186/2008.

The data were centralized in EXCEL and SPSS databases and processed with the statistical functions that they are suitable for. Statistical confidence intervals with a 95% significance level were used. In order to evaluate the statistically significance between our groups we used ANOVA test (including repeated measures ANOVA). The thresh-old for statistical significance is the maximum level of probability which affords an er-ror. A significance level of 0.05% points to sufficient precision in practice, and 95% probability indicates reliability.

II.1.6.3. Results

Triglycerides were measured in R0, R1 and R2 for each group. Table XXIV and Figure 18 show the levels of triglycerides throughout the experiment, as well as the variance of the measured values series. In the control group, the variance was low, ranging from 3.27 to 3.77 CV (coefficient of variation) %, with R2 results being the most homogenous (3.27 CV%). In groups II, III and IV, the coefficients of variation ranged from 2.45 to 14.82 CV%. In these cases, the most homogenous results were registered in R2 in group II – C (8.04 CV%) and group IV – C+L (4.36 CV%), and in R1 in group III – C+V (2.45 CV%).

Table XXIV. Levels of triglycerides in all groups

Group	R0	R1	R2
Group I: control			
Average mg/dl	23.70	23.88	23.90
SD	0.89	0.90	0.78
CV%	3.76	3.77	3.27
Group II: C			
Average mg/dl	24.19	45.73	60.61
SD	3.44	6.78	4.87
CV%	14.22	14.82	8.04
Group III: C+V			
Average mg/dl	23.91	34.15	39.73
SD	1.31	0.84	1.30
CV%	5.48	2.45	3.27
Group IV: C+L			
Average mg/dl	24.04	39.15	47.56
SD	2.43	2.01	2.07
CV%	10.13	5.14	4.36

The highest individual levels of triglycerides were found in group II corresponding to the rats who received cholesterol only (Fig. 18).

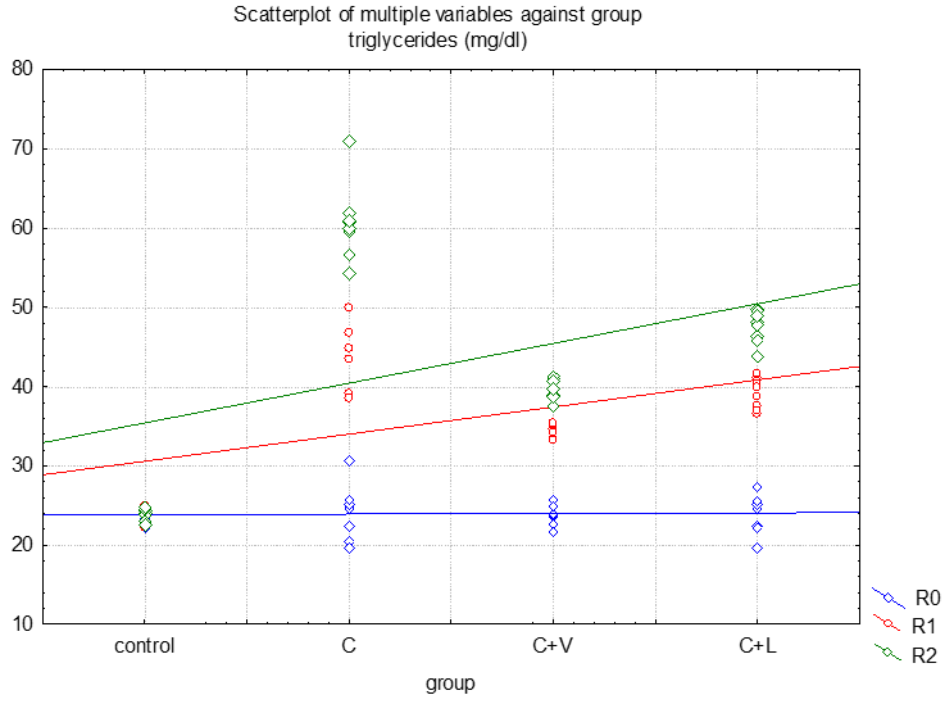


Figure 18. *Individual levels of triglycerides in all groups.*

Table XXV. Multivariate tests using one-way repeated measure analysis of variance

Effect		Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[b]
Time	Pillai's Trace	.985	879.337[a]	2.000	27.000	.000	.985	1758.674	1.000
	Wilks' Lambda	.015	879.337[a]	2.000	27.000	.000	.985	1758.674	1.000
	Hotelling's Trace	65.136	879.337[a]	2.000	27.000	.000	.985	1758.674	1.000
	Roy's Largest Root	65.136	879.337[a]	2.000	27.000	.000	.985	1758.674	1.000
Time * Type	Pillai's Trace	1.058	10.487	6.000	56.000	.000	.529	62.920	1.000
	Wilks' Lambda	.028	44.831[a]	6.000	54.000	.000	.833	268.989	1.000
	Hotelling's Trace	31.694	137.340	6.000	52.000	.000	.941	824.037	1.000
	Roy's Largest Root	31.596	294.898[c]	3.000	28.000	.000	.969	884.694	1.000

a. Exact statistic
b. Computed using alpha = .05
c. The statistic is an upper bound on F that yields a lower bound on the significance level.
d. Design: Intercept + Type
Within Subjects Design: Time

A one-way repeated measure analysis of variance (ANOVA) was conducted to evaluate the null hypothesis that there is no change in the triglycerides values in dif-ferent subgroups that were measured in R0, R1 and R2 (N=32) (Table XXV). The results of the ANOVA indicated a significant time effect, Wilks' Lambda = 0.015, F (2, 27) = 879.33, $p < 0.01$, $\eta 2 = 0.98$. Thus, there is significant evidence to reject the null hypothesis.

Table XXVI. Pairwise comparisons between the studied groups

(I) Time	(J) Time	Mean Difference (I-J)	Std. Error	Sig.[a]	95% Confidence Interval for Difference[a]	
					Lower Bound	Upper Bound
R0	R1	-11.766*	.459	.000	-12.934	-10.597
	R2	-18.986*	.454	.000	-20.143	-17.829
R1	R0	11.766*	.459	.000	10.597	12.934
	R2	-7.220*	.321	.000	-8.038	-6.401
R2	R0	18.986*	.454	.000	17.829	20.143
	R1	7.220*	.321	.000	6.401	8.038

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

a. Adjustment for multiple comparisons: Bonferroni.

Follow up comparisons indicates that each pairwise difference was significant, $p<0.01$. There was a significant increase of values over time (Table XXVI).

In R0 there were no significant differences between the average levels of triglyc-erides in groups II, III and IV and neither between these and control group ($p<0.05$). Compared to the control group, the average levels of triglycerides were significantly higher in all groups in R1 and R2 ($p<0.001$).

Also, in R1 and R2 measurements, the average triglycerides in group II receiving cholesterol only (C) were significantly higher than those in group III receiving valine (C+V) as well as in group IV receiving leucine (C+L) ($p<0.001$; $p<0.05$).

At the end of the experiment (R2), the average triglycerides in group III were significantly lower than in the case of rats who received leucine ($p<0.001$) (Table XXVIII, Figure 19).

Table XXVII. Statistical differences between the average levels of triglycerides in all groups.

Time	**Group**	**Control Group (n=8)**	**C (n=8)**	**C+V (n=8)**
R0	C (n=8)	p = 0.737	-	
	C+V (n=8)	p = 0.884	p = 0.849	-
	C+L (n=8)	p =0.814	p = 0.919	p = 0.928
R1	C (n=8)	p < 0.001*	-	
	C+V (n=8)	p < 0.001*	p < 0.001*	-
	C+L (n=8)	p < 0.002*	p = 0.001*	p =0.0422*
R2	C (n=8)	p < 0.001*	-	
	C+V (n=8)	p < 0.001*	p < 0.001*	-
	C+L (n=8)	p < 0.001*	p < 0.001*	p < 0.001*

Post-hoc analysis: Newman-Keuls test; (*) Marked differences are significant at p < 0.05

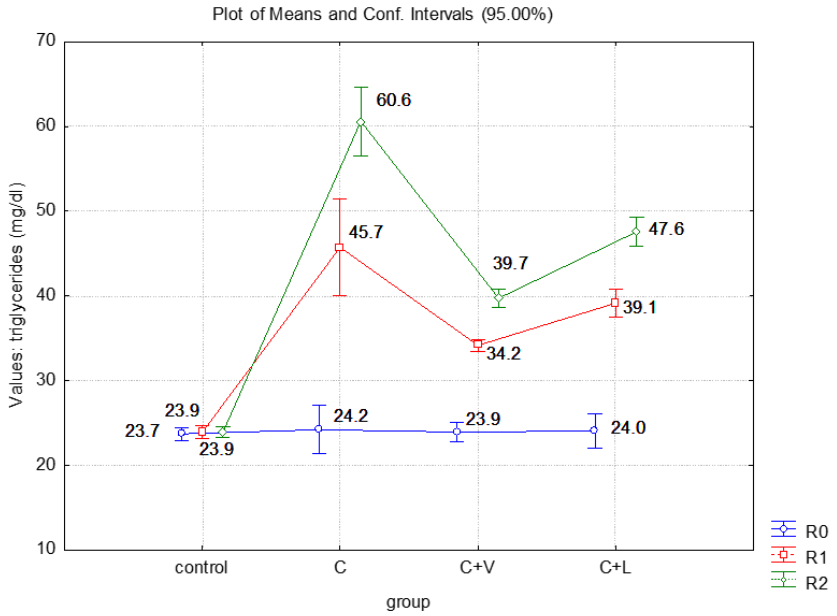


Figure 19. *Average levels of triglycerides in all groups.*

As described before, in previous studies, we evaluated the anti-atherogenic potential of valine and leucine in the context of hypercholesterolemia induced by a cholesterol rich diet, following their effects on lipid metabolism (total cholesterol, HDL-cholesterol, LDL-cholesterol) and oxidative stress parameters. The mean values can be seen in Table XXVIII.

Table XXVIII. Mean values of HDL, LDL and glucose at R0, R1 and R2

	Group	**R0**	**R1**	**R2**	**p–value**
Cholesterol I mean ± SD	Control group	37.14±2.56	37.50±2.09	37.61±1.45	0.0783
	C (n=8)	36.41±4.15	49.89±3.99	76.61±3.46	<0.001*
	C+V (n=8)	36.67±1.28	41.12±1.27	44.87±1.22	0.001*
	C+L (n=8)	36.50±2.70	46.04±2.71	49.53±2.12	<0.001*
	p–value	**0.577**	**0.006***	**<0.001***	
HDL mean ± SD	Control group	23±1.48	22.88±1.22	22.89±1.68	0.911
	C (n=8)	22.43±3.29	19.44±1.45	15.93±1.20	0.004
	C+V (n=8)	22.98±1.48	24.64±2.79	26.85±2.95	0.114
	C+L (n=8)	22.51±2.15	22.97±1.90	23.17±1.81	0.523
	p–value	**0.637**	**0.001***	**<0.001***	
LDL mean ± SD	Control group	9.39±3.39	9.83±2.52	9.93±2.71	0.749
	C (n=8)	7.73±4.54	21.3±3.64	47.94±5.47	<0.001*
	C+V (n=8)	8.9±2.01	9.64±2.79	10.07±2.75	0.486
	C+L (n=8)	9.17±3.43	15.23±2.73	16.84±2.28	0.0004*
	p–value	**0.319**	**0.001***	**<0.001***	
Glucose mean ± SD	Control group	122.75±5.87	122.61±5.84	122.77±6.08	0.962
	C (n=8)	122.78±8.77	149.26±7.73	162.82±5.83	<0.001*
	C+V (n=8)	121.98±4.71	140.93±4.84	142.37±4.70	<0.001*
	C+L (n=8)	121.93±5.13	143.79±6.32	148.08±4.78	<0.001*
	p-value	**0.994**	**<0.001***	**<0.001***	

Post-hoc analysis: Newman-Keuls test; (*) Marked differences are significant at $p < 0.05$
SD – standard deviation

Our results showed that valine and leucine increased the serum levels of HDL-cholesterol. More specific, the HDL-cholesterol values in animals who received only cholesterol (C) were significantly lower compared with group III who received cholesterol and valine (C + V) or group IV that received cholesterol and leucine (C + L) ($p < 0.001$), after one month and at the end of the experiment. We also showed that valine and leucine decreased the serum levels of LDL-cholesterol proving lipid-lowering properties. In our experiment we also evaluated the glucose value and we found an important increase when cholesterol is added to the diet, but when the amio acids (valine and leucine) are added, the glycemic values decrease compared to group II.

According to our results, the two amino acids proved antioxidant abilities, which could improve the endothelial damage related to atherosclerosis.

II.1.6.4. Discussions

Triglycerides are constituents of lipoproteins called chylomicrons that contain the highest amount of lipids absorbed in the intestines, but also in very low density lipoproteins (VLDL) that contain triglycerides synthesized in the liver. Increased serum triglycerides cause hypertriglyceridemia as a clinical condition that is associated with increased cardiovascular risk (Navar A.M, 2019). In our experimental study we studied the antiatherogenic potential of the valine and leucine amino acids starting from the pathophysiological mechanisms of atherosclerosis. We analyzed many animal models (Leong X.F., Ng C.Y., Jaarin K., 2015) and chose the experimental model of atherosclerosis proposed by Anitschkow in 1913 (Anitschkow N. and Chalatow S., 1913). Male Wistar rats received a high-cholesterol diet that caused cholesterol levels to rise.

Amino acids are essential precursors for the synthesis of numerous molecules playing a major role in homeostasis (Harris R.A., Joshi M., Jeoung N.H, 2004. Wu G., 2009). Leucine, valine and isoleucine are branched-chain essential amino acids involved in protein biosynthesis as well as in regulating the cell-division cycle. Leucine participates in growth and development of cells in a mTOR-dependent manner (Chotechuang N., Azzout-Marniche D., Bos C. et al., 2009).

Also, in 2018 we performed a study in which we evalueted the role of valine, leucine and isoleucine on the occurrence and progression of atherosclerosis in rats receiving hypercholesterolic diet. In our study we observed that of the three essential amino acids valine induced a faster response than leucine and isoleucine on the improvement of biochemical parameters, but no significant differences between the three amino acids in terms of their protective ability, according to the histopathological lesion assessment (Ifrim S., Amalinei C., Cojocaru E. et al., 2018). Still, future studies in order to assess the molecular mechanism by which these amino acids influence the triglyceride levels are necessary.

The exact impact and timing in the modulation of atherosclerotic pathophysiology of these amino acids intervention are not fully elucidated, as research has so far yielded contradictory results (Rom O. and Aviram M., 2017). Moreover, literature data shwo that researchers discussing about both, a possible proatherogenic effect and a potential antiatherogenic role of branched-chain amino acids (Grajeda-Iglesias C. and Aviram M., 2018).

In a study published in 2013, Bhattacharya et al. showed that branched-chain amino acids are responsible for growing cardiovascular-related mortality, being associated with severe forms of ischemic heart disease. They were able to demonstrate that the relationship is there even after the more traditionally accepted risk factors such as diabetes mellitus or insulin resistance have been corrected (Bhattacharya S., Granger C.B., Craig D. et al., 2014).

Ruiz-Canela in 2016 hypothesized that elevated serum levels of branched-chain amino acids as valine, leucine and isoleucine correlate with increased global cardiovascular risk which which was not influenced by diet (Ruiz-Canela M., Toledo E., Clish C.B., 2016). In addition, Sun et al. demonstrated via an experimental study on mice that disturbed catabolism of branched-chain amino acids mediated by Kruppel-like factor 15 (KLF 15) is responsible for cardiac depression manifestations (Sun H., Olson K.C., Gao C. et al., 2016).

On the other hand, numerous published studies support the beneficial effects of branched-chain amino acids in regulating lipid metabolism and functional cardiac parameters. Similar to our results, Noguchi et al. in 2006, highlighted the role that valine and leucine play in counteracting the impact of abnormal lipid concentration, directly involved in the production of atherosclerotic lesions (Noguchi Y., Zhang Q.W., Sugimoto T., 2006).

Terakura et al. demonstrated that branched-chain amino acids supplemention decreases the liver triglycerides accumulation and the chronic inflammatory process in obese mice, most likely by inhibiting IL-6, Tumour Necrosis Factor-alpha and monocyte chemoattractant protein-1 expression. The mice fed with branched-chain amino acids presented lowering of average adiposity, likely mediated by peroxisome proliferator-activated receptor gamma (Terakura D., Shimizu M., Iwasa J., 2012).

Chen et al. in 2012 investigated the influence of leucine on body weight and blood lipid levels and showed that regardless of the mode of administration (oral or intracerebroventricular) determined the regulation of carbohydrate and lipid metabolism (Chen H., Simar D., Ting J.H. et al., 2012).

In another study published by Pedroso et al. in 2014, the authors showed that restricting caloric intake concomitantly with the addition of leucine to the diet in rats with metabolic syndrome improves protein anabolism and causes an increase in leptin and IL-6 levels. The results of the study showed that supplementation of the branched chain amino acid diet alters liver metabolism by influencing the metabolism of fatty acids and cholesterol (Pedroso J.A., Nishimura L.S., de Matos-Neto E.M., 2014).

Also, the role of leucine in lowering the level of triglycerides and LDL and in increasing HDL-cholesterol in diabetic rats that received a diet supplemented with this amino acid was demonstrated in the study published by Sadri in 2017 (Sadri H., Larki N.N., Kolahian S., 2017). Moreover, a possible increase in leptin levels as a result of a leucine-rich diet has been discussed, but the leptin levels obtained have not been shown to be statistically significant (Lynch C.J., Gern B., Lloyd C. and al., 2006).

As we can see, the results of research published by various groups of researchers on the effects of branched chain amino acids on cardiovascular risk are contradictory. Some studies have shown that dietary supplementation with branched-chain amino acids has increased cardiovascular risk, while numerous studies have shown the benefits of dietary supplementation with leucine, valine and isoleucine to reduce lipid metabolism parameters. In our study it was

clearly observed that leucine and valine decreased the level of plasma triglycerides, thus having a positive effect on lipid metabolism and, consequently, on the integrity of the vascular wall.

II.1.6.5. Conclusions

Comparing the measured values of triglycerides between different groups we noticed that essential amino acids such as valine and leucine lower triglyceride levels. Therefore, the vascular endothelium is protected and the risk of endothelial dysfunction has decreased. Also, in our study we found that valine acts more promptly and faster than leucine. The results of this experiment support the idea that valine and leucine play a distinct and specific role in the evolution of induced atherosclerosis. Mode of action of these two amino acids provides multiple ways to further research to therapeutic targets, and our study is an attempt to highlight a new potential therapeutic strategy.

SECTION B. PERSPECTIVES IN THE ACADEMIC, RESEARCH AND MEDICAL FIELD

PERSPECTIVES IN ACADEMIC AND PEDAGOGICAL DEVELOPMENT

Once reaching this specific stage of career, the main objectives will follow several directions of improvement, in terms of teaching activity and scientific research. In other words, the aim is to continue the ongoing professional development and increase the quality of the teaching process. The motivation behind continuous professional development is usually intrinsic in nature and must be doubled by a moral duty that teachers have in terms of training and improvement. It is a form of respect for their profession and students.

To further develop the educational activity, I will continue to improve the teaching methodology in order to properly diversify the interactive teaching methods, so that students develop their intellectual abilities which are specific to pediatric medicine.

I wish to contribute to the improvement of teaching activities within the Pediatrics Discipline through the intensive use of the latest technological means. I will continue to develop the research and teaching directions both of the Pediatric Discipline and of the University of Medicine and Pharmacy „Grigore T. Popa" Iasi. I believe that all teachers must work together to develop a plan for organizing the teaching activity and the topics that are addressed during a university year to meet the aspirations of students, residents, PhD students in accordance with current European requirements. From the moment, I have tried to acquire a continuous improvement and a development of the professional training in order to offer students, residents and PhD students the highest quality teaching activity. Thus, I want to be an example for my students, residents and future PhD students, trying to be always up to date with the news in medical guidelines and also making time to answer their questions.

I will develop educational activities for training students, residents and PhD students by encouraging acquisition of "hands-on" technique in basic care of the patient and stimulating their training for teamwork. I will encourage the learning of problem-based techniques for differential diagnosis of the main symptoms in pediatrics, a crucial element in approaching the pediatric clinical cases. Also, I aim to achieve a certain independence of the student from laboratory investigations and invasive techniques through a good knowledge of pediatric semiology. I would like to continue the clinical case presentation sessions for students and pediatric residents as well as those in related specialties. By organizing learning sessions of clinical research techniques and basic research with clinical applicability I will try to introduce students into the critical thinking and academic achievement model, with the recruitment of students with potential in research field since the early years of faculty. Using cascading clinical cases, various clinical case scenarios will help students engage in discussions, apply knowledge and skills in problem solving and address issues from different perspectives. To this end I wish to introduce the critical analysis of scientific papers courses, statistical data interpretation necessary for a future medical research. Also, I want to encourage students, residents and PhD students to attend to national and international congresses for dissemination of scientific knowledge.

As a coordinator for the discipline of Pediatrics at the Simulation Center of UMF "Grigore T. Popa" Iasi, I will be involved in the modern learning process based on laboratory simulation on mannequins of clinical cases that students, residents, PhDs are likely to encounter in the clinic. This concept is used to describe the interactive simulations used in medical training, performed on the mannequin, which are focused on replicating clinical stages. It is an efficient approach meant to develop students' and residents' clinical skills while making use of modern technologies, starting from the innovative concept of learning based on games. These virtual patient applications offer to the future doctors the chance to experience what exactly it is like to be in the medical staff's position, further developing clinical and decision-making skills by working with the high-performance mannequins. These mannequins are an important part of the equipment available at the Simulation Center of our university. These programs stimulate teamwork both of the students involved in solving the clinical case, but also of the colleagues in the pediatric discipline who will be involved in this form of controlled education. This type of educational activity can significantly increase confidence in students' clinical skills.

I am also a member of the Erasmus + project "Case-based learning and virtual cases to foster critical thinking skills of students (CLEVER)". Education for clinical practice is a complex process, involving the development of a body of knowledge, skills and multiple aspects of professionalism. Traditional approaches, with modules in single discipline biosciences, bear little relationship to eventual learner needs for clinical practice. Clinicians act by synthesizing a range of relevant information, identifying and testing solutions. Developing competence in this crucial process requires an approach that differs from traditional teaching, where the students are recipients of information. Case-Based Learning (CBL) is one example of widespread form of learning which uses patient simulation to create a learning style close to the needs of practice. Virtual patient cases (VPCs) are encouraging students to use their knowledge base to explore simple management decisions as they work through patient scenarios. We believe that implementation of the CLEVER project's outcomes through VPC/CBL approach is in accordance with the horizontal priority: "Achievement of relevant and high-quality skills and competences" and with the sectoral priority: "Enhancing the quality and relevance of students' knowledge and skills". The CLEVER project promotes innovating pedagogy methods through technology-enhanced learning, such as case-based learning CBL and VPCs to enable future physicians and healthcare professionals simulating important steps in the diagnostic and therapeutic process in a safe environment.

I will continue to support activities aimed at medical education by coordinating postgraduate courses. I will also be a lecturer in postgraduate courses in different areas of Pediatrics. Thus, I want to further promote postgraduate courses with mixed lecturers' teams from other Universities from Romania. This will allow a better relationship of our teachers with colleagues from other pediatric disciplines in Romania and a welcome exchange of experience for both students and doctors. At the same time, residents, PhD students and physicians from different areas of Moldova will have access to the clinical experience of other university centers from our country.

I also intend to increase visibility nationally and internationally, by participating in conferences, symposiums, congresses, at the national, European, and global level, as before. Developing and nurturing connections with leaders in the field creates the perfect space for continuous development and a perpetual refreshment of information which further translates

into the high standards and quality of the didactic and research activities. Workshop events, training, postgraduate courses, international exchanges with prestigious partners will provide me with the basis for constant individual training. At the same time, all these activities will give me access to information of immediate interest which has a high chance to later materialize in new projects and research topics.

PERSPECTIVES IN THE RESEARCH ACTIVITY

Scientific research is a basic component of the educational-formative development that any university fosters, a duty and, simultaneously, a privilege of any professor. Previous scientific experience, training in the field, the collaborative relationships we have achieved so far, and scientific accomplishment represent an important starting point. This will ensure both the continuation of research and the development of new research projects in pediatrics, gastroenterology and pediatric nutrition. The research directions will include the continuity of the previously initiated studies, but also new research directions. Based on my expertise to date in the field of clinical nutrition and pediatric gastroenterology, future scientific activity will focus on nutritional pathology ranging from malnutrition to obesity associated with a variety of comorbidities. The high level of academic interaction that I have developed in recent years will allow the extension of the interdisciplinarity of the research team that I am part of, stimulating the active involvement of PhD students from other clinical or laboratory specialties. In order to carry out the research topics I propose, I will need to identify funding sources for these projects, such as internal grants of the "Grigore T. Popa" University of Medicine and Pharmacy Iași, national grants from UEFISCDI, international grants from several international scientific societies, but also private funding from the pharmaceutical industry.

The chance to specialize in two important fields of pediatrics, nutrition and pediatric gastroenterology opens new research horizons for me. On the one hand, it will allow me to continue to study gastrointestinal pathology in patients with obesity and malnutrition and, on the other hand, it will allow me to deepen the role of current nutritional strategies in patients with malabsorption syndrome, such as cystic fibrosis and celiac disease.

Thus, in direct connection with my own experience and areas of interest, I aim to develop the following research directions:

RESEARCH STUDIES IN THE FIELD OF PEDIATRIC OBESITY

As the founder of the "Center for Diagnosis, Counseling, and Monitoring of Overweight and Obese Children" within our university, I want to continue the projects started but also develop new ones.

1. Studies on the prevention of obesity in childhood

The first nutritional screening project I coordinated was entitled "Proper nutrition in pediatrics - a chance for a healthy future adult" and took place between June 2016 and June 2017. The aim of our project was to identify overweight and obese children in kindergartens, schools and high schools from Iasi and the northeaster region of Romania. Identification of children with nutritional disorders was made possible by measuring children by students and pediatric residents. Thus, in our project, 7138 children and adolescents from 19 kindergartens, 16 schools and 7 high schools weighed and measured. Some of these children who were

identified as overweight and obese received nutritional, psychological, and physical therapy counseling at the Center for Diagnosis, Counseling, and Monitoring of Overweight and Obese Children. I also offered nutritional education in kindergartens and schools from Iasi. I would like to continue nutritional screening projects in children in order to identify risk factors for obesity, both locally and nationally, early identification of overweight and obese children and continuing the nutritional counseling program for children and their families. These projects will involve multidisciplinary teams including physicians (family physicians, pediatricians, epidemiologists, nutritionists, psychologists, physiotherapists), as well as students from medical school and nutrition college.

2. The study of complications and comorbidities of obesity in pediatric age

Nowadays, childhood obesity risks are not to be underestimated. Obese children are at least twice as likely to become obese as adults. Short-term complications include increased risk of non-alcoholic fatty liver disease, hypertension, CVD, IR and T2DM, and psychological issues (Kinlen D., Cody D., O'Shea D., 2018). So, it is very important to identify hormonal and inflammatory markers from childhood. An adequate understanding of the inflammatory processes characteristic of obesity constitutes a crucial factor for the prevention of the disease and its complications in obese pediatric patients. In this respect, I want to focus especially on the study of metabolic, cardiovascular and liver complications in obese children. In obese children, insulin resistance and chronic inflammation have an essential role in the pathogenesis of obesity-associated comorbidities (Lopez-Sandoval J., Sanchez-Enriquez S., Rivera-Leon E.A. et al, 2018). My concern for this topic has already materialized through the publication of the results of our study on predictive markers of early cardiovascular impairment and insulin resistance in obese pediatric patients. Our study highlighted the importance of early markers of cardiovascular risk in obese pediatric patients represented by IL-6, Intercellular Adhesion Molecules (ICAM), endotoxemia and their correlation with metabolic markers of IR represented by insulinemia, HOMA index and plasma cortisol. Thus, inflammatory markers, IL-6, ICAM 1 and endotoxemia, show significantly higher values in our pediatric obese patients, leading to chronic and systemic inflammation. The results of our study indicated that these markers can be considered significant predictors of cardiometabolic diseases in obese pediatric patients. Of particular interest is the link between low-grade inflammation, IR and endothelial dysfunction and obesity. The significant correlation between the HOMA index and the BMI percentile confirmed in our study that obesity is a major risk factor for the development of IR. It can clearly be considered that the BMI percentile has significant predictive power for metabolic markers of insulin resistance. However, our study highlights the need for detailed research in the dynamics of obese pediatric patients by age group.

However, I know that performing clinical and translational research is not possible without access to the latest technology. Thus, the inclusion of the Emergency Clinical Hospital for Children "Sfanta Maria" as a partner in the cross-border project "Cross-border network for research and management of medical services in the field of Obesity and Diabetes - OBDIANET" opened new research opportunities. The equipment purchased through this project will allow us to conduct clinical studies on the complications and comorbidities of obesity in pediatric age.

The development of research projects on cardiometabolic complications will allow the stimulation of teamwork, these projects involving entire research team consisting of pediatric

specialists, residents, students and PhD students at the University of Medicine and Pharmacy "Grigore T. Popa". As before, I will be involved in continuing to equip the obesity center with the necessary equipment and kits to conduct quality research through the application of future grants and projects. The studies will respect all ethical and confidentiality standards. Because interdisciplinarity in essential to research, the Emergency Clinical Hospital for Children "Sfânta Maria" Iași will be involved in partnerships with several institutions from Iași: Departments of the University of Medicine and Pharmacy "Grigore T. Popa", The Advanced Center for Research and Development in Medicine Experimental (CEMEX), The Emergency Clinical Hospital "Sfantul Spiridon", The Rehabilitation Hospital. I will also get involved in international collaboration activities.

Considering the collaboration with our colleagues from 6th Medical Clinic of Rehabilitation Hospital Iasi, on a long term, we would like to continue the collaboration started within the "Obdianet" project **on development of cardiovascular disease prevention in adolescents and young adults.** Thus, we will be able to optimally achieve the transition of the young adult into the adult network. In this manner, we will be able to continue the complex and multidisciplinary long-termnd monitoring of the obese patient. I would like to see if early establishment of nutritional therapy and regular physical activity program will have long lasting effects. Thus, I propose that these children be monitored during time of 20 years by our colleagues from the Medical Rehabilitation Clinic to see the evolution of possible cardiometabolic complications.

3. Another research direction that I want to develop in the future is **the study of the intestinal microbiota in obese children.**

The study for intestinal flora has become the new frontier in understanding the development of obesity and represents a new strategy to prevent and treat obesity through intervening the intestinal flora (Gao X., Jia R., Xie L. et al., 2018). Obesity was associated with an altered gut microbiota characterized by elevated levels of Firmicutes and depleted levels of Bacteroidetes. Members of the Bacteroidetes were generally better predictors of BMI z-score and obesity than Firmicutes. The main metabolites produced by gut bacteria, short chain fatty acids (SCFAs), were higher in obese children, suggesting elevated substrate utilisation. Multiple taxa were correlated with SCFA levels, reinforcing the tight link between the microbiota, SCFAs and obesity. The actual studies suggest that gut microbiota dysbiosis and elevated fermentation activity may be involved in the etiology of childhood obesity (Riva A., Borgo F., Lassandro C., 2017).

RESEARCH STUDIES IN THE FIELD OF CYSTIC FIBROSIS IN CHILDREN

I wish to continue my research activity in the field of cystic fibrosis, a disease that I started to study 20 years ago in my doctoral thesis. Cystic fibrosis is an important cause of intestinal malabsorption in children, with serious consequences on the growth and physical and neuro-intellectual development of children. Cystic fibrosis is the most common autosomal recessive genetic disease found in the Caucasian population. Globally, we are witnessing an increase in the survival rate due to early diagnosis through neonatal screening, rapid establishment of symptomatic treatment and adequate nutritional support. Unfortunately, in Romania there is no neonatal screening at birth for the early diagnosis of cystic fibrosis. Therefore, the late diagnosis of children sometimes caused by the multitude of clinical

manifestations and the absence of curative treatment make CF a chronic disease that requires complex, multidisciplinary care in which quality of life must occupy an important place.

The priority areas in the field of pediatric cystic fibrosis that I identified, included:

1. Exploring the role of genetics/genomics (e.g.modifying genes, gene-environmental interactions, and epigenetics) in early CF pathogenesis. In this sense, I wish to continue the study of genetic mutations in patients with CF in order to establish the correct diagnosis and the genotype-phenotype correlations which are pivotal for determining the long-term prognosis. Also, the correct identification of the genetic mutation will offer access to the latest, innovative therapies. The partnership with the Medical Genetics Center from the Emergency Clinical Hospital "Sfânta Maria" gives patients the opportunity to access genetic testing and genetic counseling for children`s families. This collaboration will make it possible to conduct research in this specific direction so we can further identify the genetic profile of CF in the Northeast Romania.

2. Developing a spectrum of biomarkers of early gastrointestinal and nutritional disease that reflects cystic fibrosis pathophysiology, clinical outcome, and response to treatment. Intestinal inflammation of CF has been extensively studied and plays an important role in intestinal obstruction by various mechanisms represented by inappropriate secretion of digestive enzymes (maldigestion), intestinal transit disorders, intestinal resections (e.g., intestinal ileus). The inflammatory markers of CF enteropathy are IgG, IgM, interleukin- (IL-) 1β, IL-8, neutrophils, elastase and eosinophilic cationic protein. In patients with pancreatic insufficiency caused by chronic pancreatitis, a mononuclear cell infiltrate expressing the intercellular adhesion molecule (ICAM)-1, IL-2 alpha (CD25) receptor, IL- 2 and interferon-γ has been observed at the level of the lamina of the duodenal mucosa obtained through intestinal biopsy. Also, fecal calprotectin and nitric oxide are non-invasive markers of intestinal inflammation in CF.

3. Study of intestinal microbiota in children with cystic fibrosis

We know that the GI microbiota play important roles in maintaining gastrointestinal tract health and in processing and absorbing nutrients. Microbial diversity is strongly correlated with healthy status and diversity depends on both the number of microbial species and the abundance of each represented species (Albenberg L. and Kelsen J., 2016). Reduced gut microbial diversity is observed in several inflammatory, metabolic, immune-related, and systemic diseases (Rinninella E., Raoul P., Cintoni M. et al., 2019) Dysbiosis represents the imbalance in the composition, diversity, or function of these intestinal microorganisms. (Thavamani A., Salem I., Sferra T.J. et al., 2021).

Children with CF have difficulty absorbing nutrients and, as a result, with growth and with their health. Therefore, the GI microbiota in children with CF could contribute to early growth disorder and disease. Intestinal dysbiosis due to both disturbance of intestinal secretions and prolonged antibiotic therapy for respiratory diseases represents an important mechanism of early gastrointestinal and nutritional disease. All these different microbiotas correlated with measures of both nutrient absorption (stool fat content) and inflammation (calprotectin). These results support the concept that the GI microbiota are either impacted by CF GI dysfunction, contribute to that dysfunction, or both. Thus, I want to research the GI microbiota in the first year of life and their relationship with the growth and health in infants and children with CF. I

will try to understand how these changes of intestinal microbiota contribute to health and disease in these children using clinical studies and in vitro and animal models.

4. Further research on the nutritional status of patients with CF. A particular interest assigned to CF and its multiple aspects is that this disease can now be perceived as a condition leading to obesity. The relation between malnutrition and pulmonary death in patients with CF has resulted in intensive nutritional intervention over the last few decades, ultimately leading to a significant decline in underweight and the emergence of overweight/obesity as a potential new problem.

To summarize, in terms of research, I wish to ensure the quality of scientific activities that further align with European standards while strictly respecting ethical requirements.

PERSPECTIVES IN MEDICAL ACTIVITY

Medical activity in a Pediatric Clinics is extremely dynamic. There is a permanent need for training in order to be up to date with the latest developments in the field. There is a couple of reasons for which it is necessary to provide such permanent training. Firstly, to be ready to approach all the cases as competently as possible, regardless of their complexity. Secondly, to ensure the continuous improvement of the medical act through constant access to information and participation in training and specialization courses.

The objectives proposed in this plan are focused both on a continuous process of professional and personal development as well as on the contribution offered in preparing future generations of specialists of high human and professional quality.

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