GRIGORE T. POPA UNIVERSITY OF
MEDICINE AND PHARMACY IASI

HABILITATION THESIS

The concept of health and the limits between prevention and development of the disease in children

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Contents

Abbreviations

AJG = The American Journal of Gastroenterology
ATP = according-to-protocol
AUC = area under the curve
BCG = bacillus Calmette Guerin
BMI = body mass index
BP = blood pressure
CI = confidence interval
COX = cyclooxygenase
DdLV = diastolic diameters of the left ventricle
DIBU = prophylactically receive delayed ibuprofen
DNA = deoxyribonucleic acid
DPARA = prophylactically receive delayed paracetamol
DTP = diphtheria-tetanus pertussis
ELISA = enzyme-linked immunosorbent assay
ENT = Ear, Nose and Throat
E. coli = Escherichia coli
ESPGHAN = European Society for Pediatric Gastroenterology, Hepatology, and Nutrition
ESBL = extended spectrum beta-lactamases
EURYPA = European Young Pediatricians Association
FHA = filamentous haemagglutinin
GER = gastroesophageal reflux
GERD = gastroesophageal reflux disease
GMCs = geometric mean concentrations
H. pylori = Helicobacter pylori
HAIs = hospital-acquired infections
HBs = hepatitis B surface antigen
HDL-C = high density lipoprotein cholesterol
HGM = heterotopic gastric mucosa
Hib = *Haemophilus influenzae* type B
IBU = ibuprofen
IIBU = prophylactically receive immediate ibuprofen
IPARA = prophylactically receive immediate paracetamol
IPV = inactivated polio vaccine
IVS = interventricular septum
LDL-C = low density lipoprotein cholesterol
LES = lower esophageal sphincter
LL = lower limit
LV = left ventricle
LVH = left ventricular hypertrophy
LVM = left ventricular mass
MDR = multiple drug resistance
MII = multiple intraluminal impedance
MMR = measles, mumps, rubella
MRSA = methicillin-resistant Staphylococcus aureus
NASPGHAN = North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition

NIBU = no ibuprofen
NICE = The National Institute for Health and Care Excellence
NPARA = no paracetamol
PARA = paracetamol
PHiD-CV = *Haemophilus influenzae* protein D conjugate vaccine
PPI = proton-pump inhibitor
PRN = pertactin
PRP = polyribosylribitol phosphate
PT = pertussis toxoid
RWT = relative wall thickness
SAE = serious adverse event
SBIR = internet randomization system
SEM = scanning electron microscopy
Se = sensitivity
Sp = specificity
TC = total cholesterol
TG = triglycerides
TVC = total vaccination cohort
UDE = upper digestive endoscopy
UES = upper esophageal sphincter
UGIE = upper gastrointestinal endoscopy
UL = upper limit
UNESCO = United Nations Educational, Scientific and Cultural Organization
WHO = World Health Organization

Rezumat

Pentru fiecare persoană în parte, procesul de dezvoltare a carierei este original. Anumiți factori, printre care personalitatea, pregătirea și oportunitatea, joacă un rol esențial într-o traiectorie profesională de succes. Asemenea unui perpetuum mobile, acest itinerar continuă de-a lungul vieții, el nu se termină după absolvirea școlii, ci devine o provocare permanentă în a atinge noi și înalte standarde de-a lungul călătoriei maturității profesionale.

Atunci când vocația devine carieră este ideal, iar o astfel de carieră devine cu adevărat remarcabilă atunci când răspunde nevoilor celor din jur și reușește să asocieze practica medicală cu activitatea didactică și cu cercetarea clinică.

Având în vedere că medicina se poate desfășura și evolua doar în preajma pacienților, văd dezvoltarea carierei mele academice în strânsă legătură cu îngrijirile medicale, cercetarea clinică și îndeplinirea sarcinilor administrative. Spitalul Clinic de Urgență pentru Copii "Sf. Maria" și Universitatea de Medicină și Farmacie "Grigore T. Popa", Iași sunt două instituții cu o tradiție cu totul deosebită în acordarea educației medicale și îngrijirii pacienților, în interacțiunea dintre acestea planificându-mi dezvoltarea carierei academice.

Prezenta teză de abilitare descrie evoluția activității mele de cercetare, a ariilor de interes științific în care am fost implicat, în deplină concordanță cu activitatea clinică de la Spitalul Clinic de Urgență pentru Copii "Sf. Maria" din Iași, care implică managementul copiilor cu diferite patologii, în prezența unor posibile complicații atât pe termen scurt cât și pe termen lung cu răsunet în perioada adultă.

Teza de abilitare cu titlul: "Conceptul de sănătate și limitele dintre prevenirea și dezvoltarea bolii la copii" cuprinde cele mai importante rezultate ale activității personale științifice, profesionale și academice care au fost obținute ulterior titlului de Doctor în medicină (din 2011 până în prezent). Aceste studii sunt grupate pe 2 direcții principale de cercetare: cercetări privind medicina preventivă (rolul nutriției și al vaccinării la copil) și cercetări privind boala de reflux gastroesofagian la copii.

Teza de abilitare este alcătuită conform recomandărilor Consiliului Național de Atestare a Titlurilor, Diplomelor și Certificatelor Universitare (CNATDCU), și include trei secțiuni principale axate pe realizările cercetărilor științifice din cele două domenii mai sus enumerate, precum și planurile personale de dezvoltare științifică și profesională, având anexată lista de referințe bibliografice.

În prima parte (SECTION I), intitulată "Overview of personal professional, academic and scientific achievements" prezint rezultatele profesionale, științifice și academice obținute de-a lungul carierei mele în două capitole. În această secțiune sunt prezentate principalele contribuții personale în domeniul pediatriei, cu aplicabilitate directă în viitoarele direcții de cercetare selectate și prezentate.

Aceste două părți componente sunt:

1. Medicina preventivă trebuie să înceapă cu copiii
2. Boala de reflux gastroesofagian la copii

Prima direcție de cercetare pune accent pe medicina preventivă. Este cunoscut faptul că o bună sănătate începe în primii ani de viață. Există studii în literatură care atrag atenția asupra faptului că prevalența obezității și a bolilor cardiovasculare este în continuă creștere, încât pentru prima dată, se preconizează că generația actuală de copii nu va fi la fel de longevivă precum părinții lor. Schimbarea stilului de viață în ceea ce privește activitatea fizică, nutriția și comportamentul de somn au fost puternic legate de dezvoltarea unor serii de afecțiuni cardio-

metabolice, inclusiv obezitate, dislipidemie, hipertensiune și diabet zaharat de tip 2, inclusiv la copii și adolescenți. Educarea părinților ocupă de asemenea un rol central în prevenirea unor boli cu morbiditate crescută precum esofagita postcaustică. Pe de altă parte, una dintre cele mai mari realizări ale sănătății publice este reprezentată de reducerea incidenței bolilor care pot fi prevenite prin vaccinarea copiilor. Beneficiile sociale și economice obținute prin reducerea mortalității și morbidității cauzate de bolile infecțioase au îmbunătățit dramatic viața oamenilor din întreaga lume, dar în același timp trebuie să ținem cont de dezechilibrul pe care îl poate genera creșterea rezistenței la antibiotice. Scopul acestui studiu a fost de a scoate în evidență aceste aspecte și de a contribui la promovarea medicinei prevenitive.

A doua direcție de cercetare subliniază diferite aspecte legate de refluxul gastroesofagian la copil. Refluxul gastroesofagian este un proces fiziologic frecvent întâlnit la copiii de toate vârstele. Cercetările continui și introducerea noilor tehnici de diagnostic a făcut posibilă dezvoltarea unor noi terapii pentru copiii cu tulburări gastroenterologice care necesită o monitorizare atentă, în special pentru efectul lor posibil asupra creșterii și dezvoltării. Scopul acestui studiu este de a contribui la descrierea factorilor de risc intrinseci și extrinseci, de a descrie diferite corelații între refluxul gastroesofagian și alte patologii și în egală măsură la stabilirea unui diagnostic și tratament corect.

Partea a doua tezei (SECTION II) este reprezentată de aspectele cele mai semnificative ale carierei personale, care aduc un plus de valoare abilitării mele în vederea atingerii scopului final, și anume acela de a pune în practica curentă medicală rezultatele cercetării științifice din domeniul pediatriei.

În atingerea acestui scop mă bazez pe o serie de atestate obținute în urma stagiilor de perfecționare medicală continuă pe care le-am urmat și pe creșterea vizibilității naționale și internaționale. Acest lucru a fost și va fi posibil prin publicare de cărți, capitole de carte și articole precum și realizarea de comunicări la congresele de profil. Diseminarea rezultatelor cercetării academice ascunde în spatele acestora o muncă asiduă de echipă și care concură, implicit la creșterea prestigiului Universității pe care o reprezint.

Cercetările din cadrul tezei de doctorat au ridicat o serie de întrebări pe care intenționez să le găsesc o rezolvare. Prin urmare, aprofundarea diferitelor aspecte corelaționale cu boala de reflux gastroesofagian mi-au deschis calea spre alte direcții de studiu.

Ultimului capitol al tezei (SECTION III) prezintă principalele publicații care stau la baza pregătirii mele profesionale și a determinării stadiului actual al cunoașterii în domeniile de interes.

Abstract

The career development process is unique for each individual. A number of factors, including personality, training and opportunity, play a key role in a successful career path. This journey continues throughout life like a perpetuum mobile. It does not end after graduation, instead it becomes a permanent challenge to achieve new and higher standards along the professional maturity journey.

Ideally, vocation becomes a career, and such a career becomes truly remarkable when it meets the needs of the people around it, and manages to combine medical practice with teaching and clinical research.

Given that medicine can only develop and evolve around patients, my view regarding the development of my academic career is that it should be closely linked to medical care, clinical research and administrative duties. "St. Mary" Emergency Hospital for Children and "Grigore T. Popa" University of Medicine and Pharmacy in Iași are two institutions with a very special tradition in providing medical education and patient care. I have planned my career development by interacting with both institutions.

This habilitation thesis describes the evolution of my research activity, the areas of scientific interest in which I have been involved, in full accordance with the clinical activity at the "St. Mary" Emergency Hospital for Children in Iasi, in the field of the management of children with different pathologies, in the presence of possible complications both in the short and long term, with repercussions in adulthood.

The habilitation thesis with the title: "The concept of health and the limits between prevention and development of the disease in children" encompasses the most important results of my scientific, professional and academic work achieved after the degree of Doctor of Medicine (from 2011 to this date). These studies are grouped into 2 main research directions: research on preventive medicine (role of nutrition and vaccination in children) and research on gastroesophageal reflux disease in children.

The habilitation thesis is compiled according to the recommendations of the National Council for the Accreditation of University Degrees, Diplomas and Certificates (CNATDCU), and includes three main sections focusing on scientific research achievements in the two areas listed above, as well as personal plans for scientific and professional development, with a list of bibliographical references attached.

In the first part (SECTION I), entitled "Overview of personal professional, academic and scientific achievements" I speak about the professional, scientific and academic achievements during my career, in two chapters. This section focuses on the main personal contributions in the field of pediatrics, with direct applicability to the future research directions, selected and presented.

These two parts are:

1. Preventive medicine needs to begin with children
2. Gastroesophageal reflux disease in children

The first line of research focuses on preventive medicine. It is well known that good health starts in the first years of life. There are studies in the field pointing to the fact that the prevalence of obesity and cardiovascular diseases is increasing, so much so that for the first time in history the current generation of children is not expected to live as long as their parents. Lifestyle changes with regard to physical activity; nutrition and sleep behavior have been strongly linked to the development of a range of cardio-metabolic diseases, including obesity, dyslipidemia, hypertension and type 2 diabetes, in children and adolescents. Parental education also plays a central role in preventing diseases with increased morbidity such as post-caustic esophagitis. On the other hand, one of the greatest public health achievements is the decrease

in the incidence of vaccine-preventable diseases in children. The social and economic benefits of reducing mortality and morbidity from infectious diseases have dramatically improved the lives of people around the world, but at the same time we must consider the imbalance caused by the increased resistance to antibiotics. The aim of this study was to highlight these issues and help promote preventive medicine.

The second line of research highlights different aspects of the gastroesophageal reflux disease in children. Gastroesophageal reflux is a common physiological process in children of all ages. Ongoing research and the introduction of new diagnostic techniques has made it possible to develop new therapies for children with gastroenterological disorders that require close monitoring, particularly for their possible effect on growth and development. This study aims at contributing to the description of intrinsic and extrinsic risk factors, at describing different correlations between gastroesophageal reflux disease and other pathologies and equally in establishing a correct diagnosis and treatment.

The second part of the thesis (SECTION II) comprises the most significant aspects of my personal career, which add value to my ability to achieve my ultimate goal, namely to translate the results of scientific research in pediatrics into current medical practice.

In achieving this goal, I rely on a series of certificates obtained from the continuous medical training courses I have followed, and on the increased national and international visibility. This has been and will be possible through the publication of books, book chapters and articles as well as the presentation of papers at relevant conventions. The dissemination of the results of academic research is the result of an arduous teamwork that contributes, implicitly, to increase the prestige of the University I represent.

The research in my doctoral thesis has raised several questions that I intend to resolve. Therefore, the deepening of various aspects related to the gastroesophageal reflux disease has opened the way to other lines of research.

The last chapter of the thesis (SECTION III) includes the main publications that form the basis of my professional training and the determination of the current state of knowledge in my areas of interest.

SECTION I. Overview of professional, academic and scientific achievements

Introduction

Career is the upward professional development of a person in their field of work. Professional status is formally recognized through: diplomas, awards, certificates, certifications, but it is a consequence of actions that are continuously carried out and that involve dedication. The success of this evolution depends on internal factors and external influences and conditions. A personal plan for one's own development is necessary. The academic future must be visualized, achievements and failures must be taken into account, priorities, objectives (short, medium and long term) must be identified, and methods and steps must be decided, but without neglecting opportunities and difficulties. Sometimes progress reviews are needed to assess the current state and, where necessary, there must be the capacity to adapt to changing realities.

The stages I have gone through in my teaching career in pediatrics have had periods of continuous training and personal and professional growth. The two components, teaching and research, have been permanently complemented by a medical profession that can be seen as a contribution to the community.

Teamwork and continuous collaboration with all colleagues within the same discipline, as well as interdisciplinary collaboration, have contributed to my professional development.

The teaching profession requires continuous training and development of teachers in order to provide the necessary knowledge to students, as they will contribute to their personal development, training and competence in their future profession.

The habilitation thesis under the title: "The concept of health and the limits between prevention and development of the disease" encompasses the most important results of my scientific, professional and academic work achieved after the degree of Doctor of Medicine (from 2011 to this date). The PhD included several studies on "Gastroesophageal Reflux Disease in Children" that have been published and are still valid and quoted.

There are well-established differences between gastroesophageal reflux (GER) and gastroesophageal reflux disease (GERD) in children. It is known that GERD can be prevented by specific methods, and nutrition plays a central role. Nutrition, as well as vaccinations, contribute significantly to preventing disease and reducing mortality. Of course, the forces shaping the nature and potential of prevention programs can be characterized as falling along a spectrum ranging from the scientific to the social. There are biological, technical, ethnic and economic factors that can alter the positive approach to prevention (McGinnis, 1985).

Professional activity

Trust is a central concept for safe and effective health care. Patients need to trust their doctors, and healthcare providers need to trust each other in a highly interdependent healthcare system. In the teaching environment, supervisors decide when and what tasks they entrust to learners to take on clinical responsibilities. Based on this concept, the entrusted professional activities are units of professional practice, defined as tasks or responsibilities that must be entrusted to the unsupervised execution by a trainee once he has acquired a specific competence. The professional activities entrusted are independently enforceable, observable

and measurable in their process and outcome and therefore appropriate for entrustment decisions. These professional activities can serve as the backbone for graduate medical education (Hicks et al., 2010).

I began my studies in the field of biological sciences in 1990 in the USA by studying Chemistry, Physics and Biology at the California State University Fullerton. In 1998 I completed my undergraduate medical studies at UMF "Grigore T. Popa" in Iasi, and in 2000 I passed the residency in Pediatrics. In 2005 I obtained the title of Specialist Pediatrician and competence in ultrasound, and in 2010 I achieved the title of Consultant and competence in pediatric diagnosis digestive endoscopy.

I obtained the certificate in Health Services Management awarded by the Romanian Ministry of Health in 2008.

In 2015 I became Head of the Department of Pediatrics V, thus becoming part of the first accreditation process of "St. Mary" Emergency Hospital for Children. After six years, in 2021, through the fusion between IVth and Vth Pediatrics Clinics, I become Head of the Ist Pediatric Clinic.

In 2019 I received the title of Specialist in Pediatric Gastroenterology, subsequently the title of Consultant in Pediatric Gastroenterology.

The experience gained from my professional work in the clinical area has helped me in my teaching career, giving me the opportunity to correlate theoretical data with clinical data and to convey them in detail to my students and residents.

Given the intricate pathology, but also the possible complications of diseases, pediatrics must collaborate with other medical specialties (dermatology, endocrinology, pulmonology, gastroenterology, allergo-immunology, nephrology, oncology), but also with pediatric surgery, so I improved my collaboration skills with other colleagues in the discipline, and where appropriate the collaborations extended interdisciplinary.

Academic activity

It is important that academic medicine has not only excellent scientists / researchers, but also excellent clinicians.

From the beginning of my activity in the University, I set out on a continuous and ascending career in higher medical education, being attracted by the reputation of the university of medicine (my father is a pediatrician, himself a doctor of medicine with research activity, literary and medical journalism), but especially by the strong personality of my mentor, Prof. Dr. Marin Burlea, to whom I owe my achievements so far. I also have in mind the beneficial influence on my evolution since the faculty benches of professors Ion Tansanu, Evelina Moraru, Dan Moraru, Stela Goția, Marcel Burdea, AG Dimitriu, through the lectures and especially the pediatric internships that convinced me to dedicate myself to this specialty. The gastroenterological profile of the clinic, thanks to the passion, dedication and tireless work of Prof. Dr. Marin Burlea inspired me, passionate and determined to prepare continuously and to have the evolution presented above. At the same time, I took pedagogy courses with applicability in my activity as an assistant. All this is the basis of my future activity and gives me the conviction of an opportunity for my future development, which I would dare to think with some justified optimism.

I belong to a traditional university, „Grigore T. Popa" University of Medicine and Pharmacy, which has something to transmit to those around and which represents a first-rate brand of Romanian medicine and pharmacy. The accumulated capital must be capitalized and multiplied, especially in its areas of performance.

In 2000 I obtained the position of teaching assistant, and in 2004 I obtained the title of assistant professor at Pediatrics in the Department of Mother and Child Medicine of the "Grigore T. Popa" University of Medicine and Pharmacy in Iasi.

In 2011 I received the title of Doctor of Medicine with the topic "Gastroesophageal reflux disease - diagnosis and therapy issues in pediatric age".

In 2014 I became lecturer, and since 2019 I am an associate professor in the same discipline, where I am still working.

One of my ongoing concerns has been with the accumulation of quality and up-to-date information in the field of pedagogy, communication, and the most efficient transmission of medical information. In order to improve the teaching method for students, I attended the postgraduate course of pedagogy held at the "Alexandru Ioan Cuza" University, Department of Teaching Staff Training in Iasi. I put the accumulated information into practice and constantly improved my teaching skills by constantly interacting with students in many contexts. I have guided students and residents to develop, present and publish scientific papers on pediatrics, I have coordinated annually undergraduate papers of students of the Faculty of Medicine and General Nursing at the university.

Competency-based education aims at standardized levels of competence. In this way, it can be ensured that all students have a sufficient level of competence at the end of the training (ten Cate, 2005; ten Cate, 2007, Mulder et al., 2010).

Malcolm Knowles introduced the term "andragogy", defining it as "the art and science of helping adults learn." Andragogy is based on five presumptions – they are about how young adults learn and their attitude towards and motivation for learning. Knowles later derived seven principles of andragogy. Most theorists agree that andragogy is not really a theory of adult learning, but they regard Knowles' principles as guidelines on how to teach learners who tend to be at least somewhat independent and self-directed. His principles are as follows: establish an effective learning climate, where learners feel safe and comfortable expressing themselves, involve learners in mutual planning of relevant methods and curricular content, involve learners in diagnosing their own needs – this will help to trigger internal motivation, encourage learners to formulate their own learning objectives – this gives them more control of their learning, encourage learners to identify resources and devise strategies for using the resources to achieve their objectives, support learners in carrying out their learning plans, involve learners in evaluating their own learning – this can develop their skills of critical reflection (Kaufman, 2003).

Also, all the time I considered a teacher should have some roles in self efficacy: modelling or demonstration, setting a clear goal or image of the desired outcome, providing basic knowledge and skills needed as the foundation for the task, providing guided practice with corrective feedback, giving students the opportunity to reflect on their learning (Kaufman, 2003).

In the teaching activity itself, where I have 22 years of experience in internships with students and 7 years of teaching, as well as in mentoring through clinical internships, lectures and practical demonstrations of resident doctors, I intend to apply new ways of polarizing the interest of students and younger doctors for this specialty through interactive activities. Thus, I participated as a member-expert in the project "Professional counselling for medical students and integrated practice program in the field of general and dental medicine" (Project Manager Prof. PhD. Norina Consuela Forna) where I introduced students to the profession of Pediatrician, with its pluses and minuses. I shared my experience with them, and I was impressed by the number of students participating in my lectures who wanted to get in direct contact with this profession and who were seeking for guidance in their future choices.

I was and will continue to be greatly involved in working with students studying in English, both in internships and courses. The courses I had taken in 1990-1991 at California

State University Fullerton were very useful to me for this purpose. In addition to continued personal improvement in medical language and fluency, I will also develop tools by producing a new edition of the 2013 book "Lecture Notes in Pediatrics" for the students in the English department.

As part of a competent and concerned team, I participated in the writing of a new Pediatrics course for the students in Dentistry as well as one for the students of General Medicine. I am considering in the near future, the elaboration of practical workbooks for students and not only, in Romanian and English, of course in a larger group.

Equally important is the involvement and responsibility of the residents, the harmonious social integration in the atmosphere and the collegial spirit of the workplace. In case of the resident permanent feedback will be practiced in order to optimize the development of this form of education.

In addition to the aspects related to the didactic act, the didactic activity also requires operational or organizational skills. Over time, I have been a member of various examination boards, for example, for obtaining the title of specialist and primary doctor. Also, I have participated, as a member, in the commissions for the positions of university assistants, lecturers, as well as in the commissions for the doctoral candidates. I was also part of the commission for supporting the bachelor's theses in the discipline of Pediatrics, in the commission for drawing up the grids for the bachelor's exam for the students of the Faculty of Medicine. For the last years I have been involved in simulating the residency exam and the residency competition.

Teaching position obtained through contest:

- Associate Professor - from 2019 until now, at the Mother and Child Department, Pediatrics, "Grigore T. Popa" University of Medicine and Pharmacy in Iași
- Lecturer - from 2014 until 2019, at the Mother and Child Department, Pediatrics, "Grigore T. Popa" University of Medicine and Pharmacy in Iași
- Assistant Professor - from 2004 until 2014, Pediatrics, "Grigore T. Popa" University of Medicine and Pharmacy in Iași
- Teaching Assistant - from 2000 until 2004, Pediatrics, "Grigore T. Popa" University of Medicine and Pharmacy in Iași

Scientific activity

The main objectives of the research activity are represented by: attracting research grants, capitalizing on research results by publishing scientific papers, increasing the scientific reputation of the faculty.

My scientific activity began when I was admitted to PhD (in 2006). My PhD thesis was "Gastroesophageal reflux disease - diagnosis and therapy issues in pediatric age", and the results of the research lead to the birth of the book "Gastroesophageal reflux in children" published by Amaltea Publishing House.

I intend to open towards multiple fields from the point of view of my scientific activity. I will continue my scientific research by participating in research teams, especially multidisciplinary teams with a permanent focus on a broad spectrum of interests. It is known that healthy eating in children and teenagers is important for proper growth and development and to prevent various health conditions. Healthy eating can help individuals achieve and maintain a healthy body weight, consume important nutrients, and reduce the risk of developing health conditions such as high blood pressure. heart disease. type 2 diabetes, cancer, osteoporosis, iron deficiency, dental caries (cavities). Over the years, vaccines have generated some controversy over safety, but no convincing evidence of harm has been found. Although children can have a reaction to any vaccine, the important thing to know is that the benefits of

vaccinations far outweigh the possible side effects. Knowing these facts, I will extend my scientific concerns to child nutrition and development, vaccination and their impact on the development of systemic infections and especially those of the respiratory system. The research in my doctoral thesis has raised a number of questions that I intend to resolve.

Participating with original studies in national and international conventions has helped me to maintain good communication with my colleagues and a constant update both at home and abroad. I was invited as a lecturer at 34 international congresses and conferences, including: eight editions of MoldMedizin Chisinau 2012-2019, two editions - First and Second Congress of the Pediatric Association of the Balkan, Istanbul in 2013 and Ljubljana in 2015, two editions - 50th and 51st Congress of Turkish Pediatric Association, Antalya in 2014 and Izmir in 2015. Also, for five years I was a lecturer at the national training course "Current Issues in Pediatrics" 2011 - 2015 and I participated in the organization of 13 editions of the Summer School for Residents in Moeciu and Sibiu (2011-2017).

My scientific activity is reflected by:

- Web of Science H-index: 10
- total number of quotations: over 200
- articles in ISI Journals with impact factor: 35 out of which 27 as principal author
- articles indexed in international databases: 73

11 articles published in ISI Journals with impact factor were awarded by the The Executive Agency for Higher Education, Research, Development and Innovation Funding in the Research Results Awards (PRECISI program).

4 papers presented at international congress were awarded, and 8 papers presented at national congresses and conferences were also awarded. For the publishing activity I received the Romanian Medical Association Award and the Family Medicine Society Award.

In 2009 I was appointed Secretary General of the Romanian Society of Pediatrics having as the Chairman Mr. Marin Burlea, Prof. PhD., in which capacity I participated in the organization and support of scientific events in the field of Pediatrics throughout the country until October 2017. With the occasion of the 12th National Congress of Pediatrics in Timisoara and the first Italian-Romanian Conference of Pediatrics, between 2-5 September 2015, I became a founding member and secretary of the Societa Italo-Rumena di Pediatria.

In 2015 I became a founding member of EURYPA (European Young Pediatricians Association), and from 2019 to 2021 I was its Secretary General.

The European Young Pediatricians Association (EURYPA) is a pan-European scientific association created with the vision to support the efforts of young European pediatricians, residents, and trainees to promote children's health, equity, and social justice through mutual collaboration in science, clinical research, and education. The strength of this association lies in the diversity of interests, experience, and training of its members and ongoing collaboration to work toward these common goals. The overall aim of EURYPA is to better the health of European children and young people through networking, education, training, research, and sharing of opportunities across the European pediatric residents, trainees, and young pediatricians. Those eligible to join include all doctors in pediatric or subspecialty pediatric training or those within 5 years of certification/qualification.

The collaboration with EURYPA materialized in 4 articles published in the Journal of Pediatrics (European Young Pediatricians Association: Laying the Foundations for Collaboration, Integration, and Networking among Pediatricians of the Future in 2016, The Evolution of the European Young Pediatricians Association (EURYPA) in 2019, The Diversity of Pediatric Residency Programs across Europe: Admission Procedures, Curricula and Duration of Courses in 2020 and The Diversity of Pediatric Residency Programs across Europe: Quality Assurance of Training, Night Shifts, and Wages in 2020). Also, during this period, we

organized one congress in Istanbul, EURYPA sessions in 3 Europediatrics congresses (2017, 2019, 2021) and sessions in 5 editions of Turkish Young Pediatrics Congress.

Chapter 1. Preventive medicine needs to begin with children

1.1 State of art

As early as the 3rd century BC, leaders in medicine promoted the prevention of disease rather than its management once it occurred, and this concept has been advocated repeatedly over the centuries. Public health prevention initiatives such as sanitation, clean water, immunizations, proper nutrition and food fortification led to huge gains in health and life expectancy (Johnston, 2020).

A growing body links early childhood experiences to the cognitive and social experiences, to health, and to the emotional and physical development of the future adult (Repetti et al., 2002).

Good health starts in the early years of life. In the beginning of childhood, the family is responsible for the care and development of the child. Extreme caution should be applied towards toxics around the house because the small children are in the "researching period" and they tend to touch and tasty everything they can get to. Poor health also starts in the same period of life. Family conflicts, manifested in recurrent episodes of anger and aggression, poor feeding, unsupportive and neglectful families are detrimental to a child's mental and physical health (Johnston, 2020).

There are studies in the literature that point to the fact that the prevalence of obesity and cardiovascular diseases is increasing, so much so that for the first time, the current generation of children in the United States is not expected to live as long as their parents (Olshansky et al., 2005). Although cardiovascular disease is associated with adulthood, the atherosclerotic process often begins in early childhood (Hong, 2010). Lifestyle changes with regard to physical activity; nutrition and sleep behavior have been strongly linked to the development of a range of cardio-metabolic diseases, including obesity, dyslipidemia, hypertension and type 2 diabetes, including in children and adolescents (Reinehr et al., 2006; Giordano et al., 2011).

Also, one of the greatest public health achievements is the decrease in the incidence of vaccine-preventable diseases in children. The social and economic benefits of reducing mortality and morbidity from infectious diseases have dramatically improved the lives of people around the world (Conway and Green, 2011). Antibiotics are medicines used to treat bacterial infections. When bacteria change in response to the use of these medicines, antibiotic resistance occurs. Antibiotic resistance leads to higher medical costs, increased mortality, and prolonged hospital stays. We should use strategies for disseminating knowledge about antibiotic prescription and timing, aiming to limit self-medication, as well as preventing the emergence of resistant strains.

This line of research has been achieved through the following contributions:

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(e20923), **ISI IF 1,889**
2. Duceac LD, Banu EA, Baciu G, **Lupu VV**, Ciomaga IM, Tarca E, Mitrea G, Ichim DL, Damir D, Constantin M, Luca AC. Assessment of Bacteria Resistance

According to antibiotic chemical structure. *Rev chim*, 2019, 70(3): 906-908, **ISI IF 1,775**

3. Chiriac PC, Poroch V, Pascu AM, Hogea MD, Antohe I, **Lupu VV**, Cirlig V. Antibiotic molecules efficacy in pediatric bacterial infections. *Rev chim*, 2018, 69(4): 915-920, **ISI IF 1,412**
4. Lupu A, Paduraru G, Dragan F, Starcea M, Lupu **VV***, Moisa S, Ioniuc I, Pertea LI, Rosu VE, Miron I. Nutrition and oral health in children. *RJOR*, 2019, 11(2): 201-205, ISI indexed journal
5. Drăgan F, **Lupu VV**, Pallag A, Barz C, Fodor K. Rational consumption of nutrients at school-aged children. IOP Conf. Series: *Materials Science and Engineering 200*. 2017, 012063 doi:10.1088/1757-899X/200/1/012063 ISI proceedings
6. Ignat A, **Lupu VV**, Stoleriu G, Ciubară AB, Heller MA, Chatzigianni OE, Burlea M. Preschool children's nutrition affects adulthood health. *Romanian Journal of Medical Practice*, 2017, 12(2):104-107
7. **Lupu VV**, Ignat A, Stoleriu G, Ciubara AB, Ciubara A, Lupu V, Burlea M, Stratciuc S. Vaccination of Children in Romania between Civic Obligation and Personal Choice. *Revista de Cercetare și Intervenție Socială*, 2017, 56: 123-132 **ISI IF 0,838**
8. Falup-Pecurariu O, Man SC, Neamtu ML, Chicin G, Baciu G, Pitic C, Cara AC, Neculau AE, Burlea M, Brinza IL, Schnell CN, Sas V, **Lupu VV**, François N, Swinnen K, Borys D. Effects of prophylactic ibuprofen and paracetamol administration on the immunogenicity and reactogenicity of the 10-valent pneumococcal non-typeable *Haemophilus influenzae* protein D conjugated vaccine (PHiD-CV) co-administered with DTPa-combined vaccines in children: An open-label, randomized, controlled, non-inferiority trial. *Human Vaccines & Immunotherapeutics*, 2017, 13(3): 649-660 **ISI IF 2,229**
9. **Lupu VV**, Ignat A, Paduraru G, Ciubara A, Ioniuc I, Ciubara AB, Gheonea C, Burlea M. The Study of Effects Regarding Ingestion of Corrosive Substances in Children. *Rev chim*, 2016, 67(12): 2501-2503 **ISI IF 1,232**
10. Mărginean CO, Meliţ LE, Moldovan H., **Lupu VV**, Mărginean MO. Lead poisoning in a 16-year-old girl: a case report and a review of the literature (CARE compliant). *Medicine*, 2016, 95(38), e4916, **ISI IF 1,804**

Project

1. Member in project Nutrition and health in school-aged children – project manager Lecturer Drăgan Felicia – 01.03 – 31.05.2016

1.2 The role of nutrition in preventive medicine

1.2.1 The impact of nutrition on oral health

1.2.1.1 Introduction

Mortality associated with dental diseases has a low rate, but they have a considerable impact on self-esteem and nutrition, both in childhood and adulthood. Teeth are important as they enable having a varied diet and they prepare food for digestion. In modern society, teeth are very important for a person's appearance; facial appearance is very important in determining an individual's integration into society. Teeth also play an important role in speech and communication.

Oral health is integrated into general health and it is essential to well-being and quality of life. In 1946, the World Health Organization underlined the correlation between oral health and quality of life. Health is ”the complete physical, mental and social well-being, not just the absence of illness or infirmity” (Scardina and Messina, 2012).

Oral care programs include oral hygiene, fluoride prophylaxis, regular check-ups, professional oral hygiene sessions and secondary prevention programs, and last but not least, healthy eating (Belcastro et al., 2007).

A correct diet means a balanced diet so that the body can get the nutrients needed for proper general health and, thus, oral health (periodontal tissue, saliva quality and quantity, dental elements). Unhealthy eating can affect the integrity of the oral cavity and can cause disease progression at this level (Touger-Decker R, Mobley, 2013).

In 1994, the United States reported 2.9 million acute dental episodes in both adults and children. For the age group of 5-17 years, these dental episodes accounted for 1.2 million days of missed classes in school (Satcher, 2001).

Personal contribution – published paper:

1. Lupu A, Paduraru G, Dragan F, Starcea M, **Lupu VV**, Moisa S, Ioniuc I, Pertea LI, Rosu VE, Miron I. Nutrition and oral health in children. *RJOR*. 2019, 11(2): 201-205

The aim of this review was to highlights the consequences of inadequate nutrition on oral health in children and how certain vitamin or nutrient deficiencies can condition the occurrence of different diseases at this level.

1.2.1.2 Nutrition and development of the oral cavity

A deficiency of vitamins and minerals in the preconception phase influences the development of the future embryo, influencing the dental organogenesis, the development of the skull and jaw.

In infants, the pre-teething phase is influenced by the nutritional condition. Deficiencies of vitamins A, B, C, D and protein are associated with disorders of oral structures (Psoter et al., 2005). Breastfeeding is ideal because of its overall health benefits. The World Health Organization and the American Pediatric Association have shown that breastfeeding influences swallowing, jaw growth, correct teeth alignment and hard palate modeling. Artificial feeding may cause the occurrence of arterial hypertension, obesity, cardiovascular disease and inflammation of the oral mucosa.

1.2.1.3 Nutrition and diseases of the oral cavity

Due to the high prevalence of malnutrition, 5.6 million children under the age of 5 in developing countries contribute to the country's death rate, i.e., 10 children per minute (UNICEF, 2006; Thomaz et al., 2010). Malnutrition not only causes scurvy but also dental development impairments (late tooth eruption), exacerbates infection and periodontal disease (Chicago Dental Society, 2011).

Malnutrition occurs when there are deficiencies in protein and/or energy foods. An insufficient amount of protein can cause lingual papillary atrophy, dentin genesis modification, cimentogenesis alteration, changes in jaw development, malocclusion, or linear enamel hypoplasia (Scardina and Messina, 2008). An insufficient lipid intake can cause inflammatory and degenerative pathologies, hyposalivation, degeneration of the glandular parenchyma, alteration of mucosal tropism (Wilcox et al., 2007). Also, an insufficient amount of

carbohydrates can lead to altered organogenesis, the influence of metabolism on dental plaque, caries, periodontal disease.

Diet influences the health of the oral cavity, it conditions the onset of caries, the development of enamel, the onset of dental erosion, periodontal health and oral mucosa in general.

Thiamine deficiency (vitamin B1) is associated with cracked lips and angular cheilosis, while the deficiency of riboflavin (vitamin B2) and niacin (vitamin B3) causes inflammation of the tongue, angular cheilosis and ulcerative gingivitis. Periodontal disease, anemia, burning sensation in the oral cavity are associated with vitamin B6 deficiency (Psoter et al., 2005). Folate deficiency (vitamin B9) is often associated with neural tube defects, but some recent studies have found a reduction in the occurrence of split lip with or without cleft palate when pregnant women receive supplemental folic acid (Millacura et al., 2017). Also, vitamin B12 deficiency causes angular cheilosis, gingival bleeding, painful mouth ulcers (Thomaz et al., 2010).

Vitamin D plays an important role in the absorption of calcium and phosphorus, allowing proper mineralization of bones and teeth. A deficiency of vitamin D will cause dental hypo mineralization, delay in dental eruption or the absence of lamina dura (Thomaz et al., 2010), also development of dental caries (Wójcik et al., 2018). The American Academy of Pediatrics recommends that all infants (naturally or artificially fed) receive a daily supplement of 400 IU of vitamin D (Wagner and Greer, 2008).

Vitamin A has been shown to prevent cleft palate (Johansen et al., 2008, Finnell et al., 2004). The deficiency of this vitamin causes dental anomalies, tooth fragility, salivary gland degeneration, and caries (Salley et al, 1959).

Vitamin C is essential for collagen synthesis. Deficiency of vitamin C is associated with irregular dentine formation, gingival bleeding, and delayed healing of lesions (Thomaz et al., 2010).

1.2.1.4 Recommendations for the prevention of oral cavity diseases

Poor nutrition, especially consuming sweet foods and drinks is the main cause of tooth decay (Bârlean et al., 2017). Children who do not have a proper diet may develop nutritional deficiencies or obesity and, implicitly, dental pathologies. There are studies that reported that there is an inverse relationship between milk consumption involving adequate calcium intake and the consumption of sweetened beverages (Lande et al., 2004). Families from low social background give sweetened drinks to small children instead of milk.

Cavities represent a demineralization of the inorganic part of the tooth with the dissolution of the organic substance, having multifactorial etiology. Demineralization of enamel and dentin is determined by the presence of organic acids that appear in the dental plaque due to bacterial activity, through the anaerobic metabolism of sugars in the diet. Although cavity development requires the presence of bacteria and sugar, it is also influenced by susceptibility of teeth, bacteria, sugars, and the amount and quality of salivary secretion. For example, lactose produces less acidity than other sugars (Mohan et al., 1998; Bang and Kristoffersen, 1972).

To prevent caries growth, it is advisable to increase the consumption of dietary fiber to reduce sugar absorption; dairy consumption, phosphorus, calcium and casein inhibit caries formation, the proper chewing of food adequately stimulate salivary secretion (Moynihan et al., 1999).

Fluoride is very important in the prevention and control of dental caries. In children, fluoride reduces caries by 20-40% but does not completely eliminate them (Newbrun, 1989).

Malnutrition and poor oral hygiene are two important factors that may lead to necrotizing gingivitis. Programs for oral cavity disease prevention, especially for children,

should include a correct assessment of the immune system and proper nutrition. It should also be considered that some nutrients have a very important role in the inflammatory process, an observation that confirms the relationship between diet and periodontal disease (In-Seok et al., 2016). The amount of vitamin C should be supplemented, which is fundamental for activating repairing mechanisms due to its antioxidant properties (Grosh et al., 2015).

A significant known correlation is between candidiasis and lack of folic acid, vitamins A, B1, B2, C, K, zinc at the expense of a carbohydrate-rich diet (Grosh et al., 2015).

Diets rich in fruits and vegetables, especially tomatoes and their derivates, significantly reduce the risk of leukoplasia. Changes in the tongue, papillary atrophy and ulcerations are possible in the case of deficiencies of iron, folate and vitamin B12. Deficiency of vitamin B12 may cause glossitis, angular cheilitis, recurrent oral ulcers, oral candidiasis and erythematous mucositis (Grosh et al., 2015).

For toddlers, proper oral hygiene is recommended after the first tooth eruption, also encouraging milk consumption, cleaning gums after eating to reduce bacterial transmission. The toddler should not be allowed to fall asleep with the milk bottle. For children 1-3-year-old, teeth should be brushed twice a day for 2 minutes. They should avoid drinking sweetened drinks. For older children, it is recommended to brush their teeth twice a day for 2 minutes. They should consume dairy products, introduce vegetables or fruits when consuming carbohydrates, limiting snacks before bedtime, limiting the consumption of sweet drinks (Clifford et al., 2019; Drăgan et al., 2017; Bobu et al., 2017; Murariu et al., 2017). Proper nutrition along with proper vaccination (Lupu et al., 2017) sets the premises for a good general health.

1.2.1.5 Conclusions

Dietary habits to support oral health and systemic health are similar. Pre-school programs should promote healthy behaviors for proper physical and psychosocial development. Children's oral health is a predictor for the oral health of future adults.

1.2.2 Rational nutrition for a good health

1.2.2.1 Introduction

An essential component of preventive pediatrics is the accurate measurement of growth and development. The growth and development of children are influenced by multiple factors such as genetics, general health, physical activity, sedentary lifestyle, quality of sleep and adequate nutrition (Berentzen et al., 2014; Jackson et al., 2009; Collings et al., 2013). Nutrition is a major event in a child's life. It is necessary to focus the attention of parents and caregivers on it and it can also represent a source of social interaction through verbal and non-verbal communication. Food, in addition to the intake of nutrients, is also a learning opportunity. It affects not only the physical growth and health of children, but also the psycho-social and emotional development. Nutrition is influenced by culture, health, and temperament (Liu and Stein, 2013).

The current generation’s lifestyle differs greatly from previous generations and this can be seen even in the way they eat. Food is at the heart of nutrition. Rational nutrition involves providing the body with energy and nutrients under different physiological and environmental conditions, both in terms of quantity and quality.

The world's population, especially children in developing countries, is going through a phenomenon known as the "nutrition transition". Several changes in food consumption and lifestyle, socioeconomic status and hygiene conditions lead to an accelerated increase in the

percentage of overweight or obese children, as well as a reduction in cases of malnutrition (Hernández-Vásquez et al., 2015; Popkin et al., 2012; Popkin, 2001).

In 2010, overweight and obesity were reported as 6.7% among preschool children worldwide. It is estimated that in 2020, the prevalence of these disorders will increase to 8.6% (de Onis et al., 2010).

Studies in specialized literature (White et al., 2011) point out that poor nutrition is one of the major causes of the appearance and onset of several chronic degenerative disease (Roșu and Popescu, 2008) such as obesity, diabetes or atherosclerosis. Poor nutrition (Graur, 2006) does not refer only to the abundance or, on the contrary, to the lack of some of the nutritive principles, but also to the food industry technology which has brought a number of changes in human nutrition, thereby creating the premises for nutritional imbalances (Negrișanu, 2005).

One may bring into discussion whether today's young people have a healthy lifestyle, whether today's adults had a truly healthier lifestyle in their childhood and to what extent the abundance of food and fast-food products has damaged the quality our children's lives. It is essential to know the principles of a proper nutrition under any life circumstance, depending on age, physiological condition, work, in health and in sickness (Mincu, 2007).

Knowing the types of foods and their nature, the nutritive principles and the nutritional ratio, the physiological changes of foods in the body (Simu et al., 2001), people's health condition and the way foods preserve it as well as setting up a rational and dietetic menu for a healthy person represent essential elements for a healthy lifestyle. All of the above reasons have urged us to set up this exploratory study that we shall present bellow.

Personal contribution – published papers:

1. Drăgan F, **Lupu VV**, Pallag A, Barz C, Fodor K. Rational consumption of nutrients at school-aged children. IOP Conf. Series: *Materials Science and Engineering 200*, 2017, 012063 doi:10.1088/1757-899X/200/1/012063 ISI proceedings
2. Ignat A, **Lupu VV**, Stoleriu G, Ciubară AB, Heller MA, Chatzigianni OE, Burlea M. Preschool children's nutrition affects adulthood health. *Romanian Journal of Medical Practice*, 2017, 12(2):104-107

The aim of this study was to underline that rational nutrition is closely related to the physical and mental development of the young persons, to the adults' work capacity, to the maintaining, as long as possible, of the elderly's health condition, to the prevention of some acute and /or chronic diseases.

1.2.2.2 Material and methods

The study was done in several schools from Bihor County, Romania, using the questionnaire method.

The aim of the research was to present and highlight the dangers of poor nutrition in young people. For this exploratory study, we applied a questionnaire with 26 questions to some school-aged children from Oradea and from the rural area of Bihor County.

The questionnaire, in its form, is divided into two sections. The first section contains questions related to the demographic data used to analyze results as well as more general questions. The second section contains direct questions related to nutrition.

The sample we worked with included 416 children, being an opportunity sampling, a type of nonprobability sampling.

1.2.2.3 Results

From the total of 316 of subjects, 218 of the respondents were female and 198 males, 241 from urban areas and 175 from rural areas.

The distribution of respondents by age is shown in Figure 1.1, the distribution of respondents by age groups is shown in Table 1.1, the distribution of respondents by gender is shown in Figure 1.2 and the distribution of respondents by background is shown in Figure 1.3.

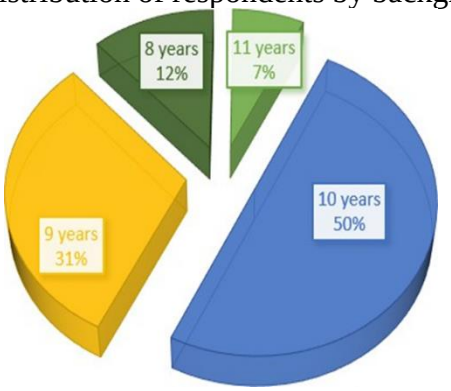

Figure 1.1. *The distribution by age of the respondents from the sample*

Table 1.1. A slightly more complex table with a narrow caption

Age group (Years old)	Male		Female		Total	
	No.	%	No.	%	No.	%
11	14	7.08	15	6.88	**29**	**7**
10	97	48.98	111	50.91	**208**	**50**
9	63	31.82	66	30.27	**129**	**31**
8	24	12.12	26	11.92	**50**	**12**
Total	**198**		**218**		**416**	**100**

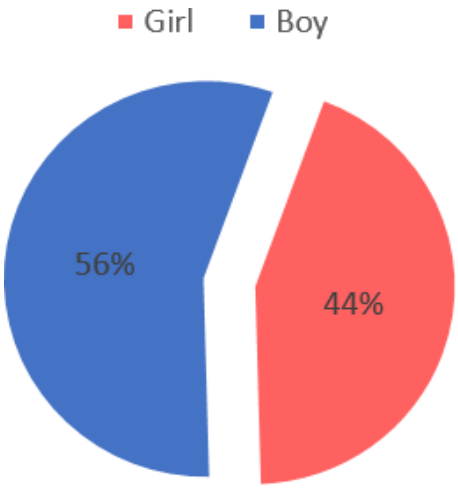

Figure 1.2. *Distribution by gender in the studied sample*

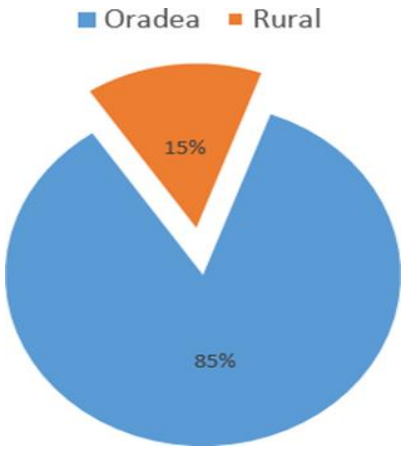

Figure 1.3. *Distribution by background*

It can be observed that respondents to this questionnaire are children aged between 8 and 11 years. Our study aimed to clarify an important aspect related to child nutrition in Bihor and to children adopted, respectively imposed lifestyle.

The child at this age is not aware of the true values of food. This requires proper education on nutrition problems from parents, school and mass media as to ensure a healthy diet. In this questionnaire children were asked whether they eat fruit, vegetables, sweets and of course, fast food products, respectively the frequency of consuming these types of food. Figure 1.4 shows the consumption of fruit for the questioned children.

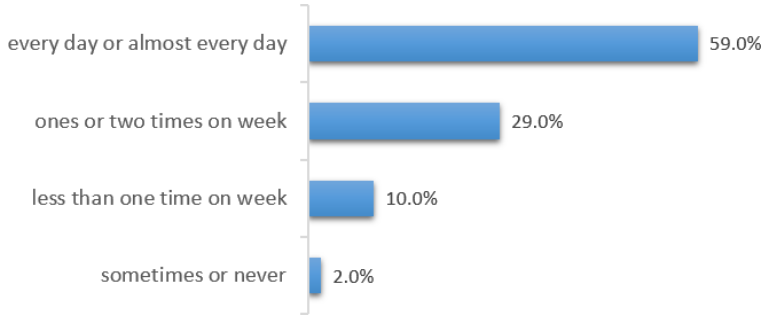

Figure 1.4. *Fruit consumption*

It can be noted that the majority of children (59%) from the studied sample consume fruit every day or almost every day. The percentage of those who do not consume fruit on a daily basis is very small, only 2%. Fruits are rich in antioxidants and fight the oxidative stress that ages the cell, damages the DNA and predisposes the body to disease.

Figure 1.5 shows the vegetable consumption of the questioned children.

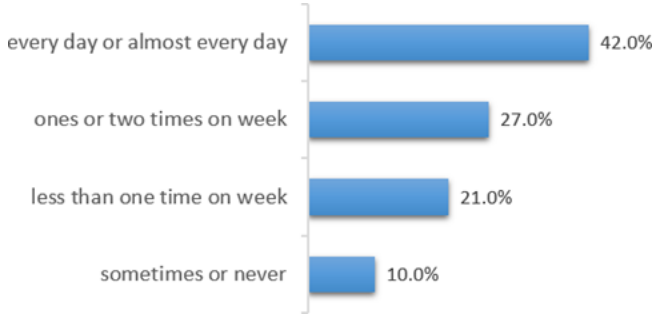

Figure 1.5. *Vegetable consumption*

Responses to the questionnaire show that 42% of the children have vegetables in their meals every day or almost every day, but a quite significant percentage of children (10%) has a diet without these very important nutrients.

Vegetables are rich in water that contains concentrates of nutritional elements in the exact amount required by the human body. Figure 1.6 shows the consumption of sweets.

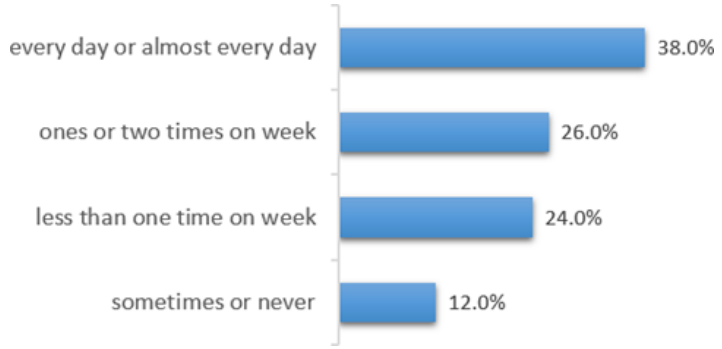

Figure 1.6. *Consumption of sweets*

Analyzing children's answers, one may notice that their nutrition is quite rich in sweets (38%). A small percentage (12%) admits not having introduced sweets in their diet. However, children enjoy sweets; we can say that sweets are their first choice.

Figure 1.7 shows how frequent the questioned children eat fast food products.

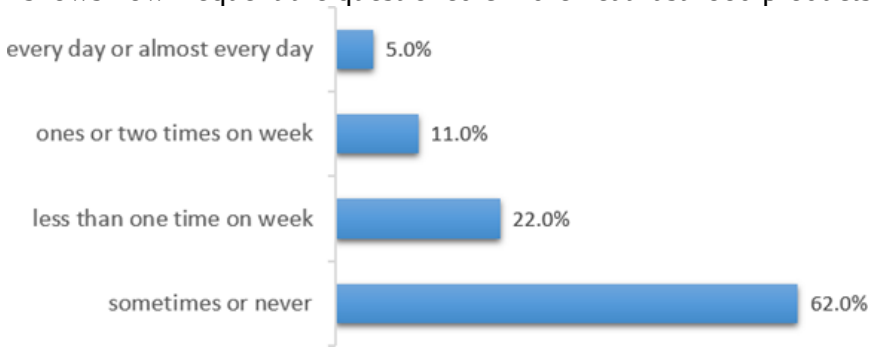

Figure 1.7. *The frequency of eating fast food products*

The results of the study reveal that only a relatively small percentage of children (5%) eat fast food products daily, and a high percentage of the questioned children (62%) is not used to consuming these foods (Figure 1.8).

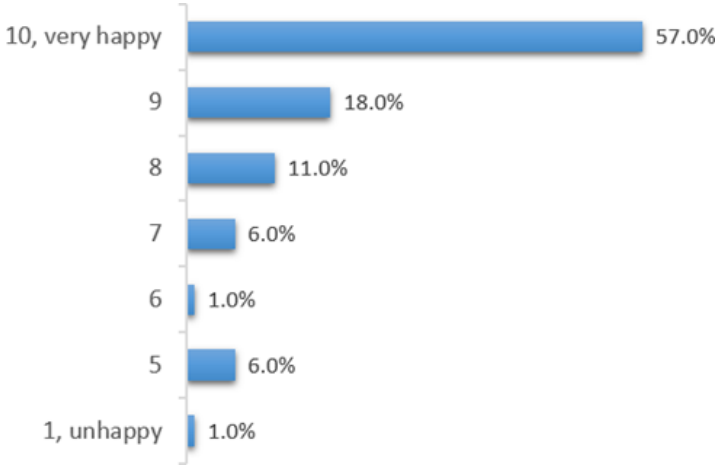

Figure 1.8. *How healthy, thanks to nutrition, and therefore happy are the children from Bihor?*

1.2.2.4 Discussions

Physiological features of the preschool children

The period from 12 months to puberty is a stage in which the increase in weight and length is linear, less spectacular, compared to the important changes in infancy and adolescence (Ministerul Sanatatii, 2006).

Body mass index (BMI) is widely used internationally as a definition of obesity (Inokuchi et al., 2009). A rapid increase in BMI usually occurs in the first year of life. Subsequently, adipose tissue shrinks (objectified by decreased BMI), following an increase in length and a reduction in adipocyte size. The time of maximum reduction is at the age of 6, then increases again throughout childhood, a phenomenon known as the "adiposity rebound" of BMI (Rolland-Cachera et al., 1984). This moment has a predictive role for adult obesity (Whitaker et al., 1988). An early recovery is a risk factor for obesity (Cole, 2004).

Changes in body proportions occur during the preschool period. The limbs lengthen, the head grows less, and the growth of the trunk decreases. During this period, the child's weight increases by 2-3 kg / year, and the height increases by 6-8 cm / year. Increasing weight and height varies depending on changes in appetite and food intake.

Nutritional assessment

Adequate nutritional status reflects the balance between intake and needs. Thus, nutritional assessment represents the foundation of nutritional care provided to the child.

The components of the nutritional assessment consist of nutritional history, clinical examination, anthropometry (weight, length / height, skull circumference, BMI), standard and reference growth curves and paraclinical data. Factors that can affect the nutritional status of the child can be represented by diseases of family members, the evolution of pregnancy, childbirth, birth weight, use of drugs or nutritional supplements.

Nutritional assessment is an important screening tool for assessing the general condition of the child, both malnutrition and obesity. Thus, marked growth and development disorders can be identified. This allows monitoring of children with conditions known to affect growth (kidney, heart, etc.).

The term fast food or junk food usually describes foods rich in calories, salt, fat, but low in nutrients. This combination can produce, in a short time, changes in appearance, overweight, acceleration of the metabolic processes, insulin resistance, dyslipidemia, diabetes.

If we take into consideration the fact that the main predictors of health through nutrition and, in this case, of the happiness of the children from Bihor “a healthy child is a happy child” seem to be: fruit consumption, sweet consumption, fast food consumption, vegetables consumption, we can say, by extrapolation from the studied sample, that the children from Bihor are healthy-happy.

Figure 14 of the study shows the results of the study in terms of children’s health, thanks to nutrition, and their degree of satisfaction regarding food and nutrient consumption in accordance with their age and occupation as pupils in schools from Bihor County.

Caloric requirements

In preschool children, nutritional recommendations are beginning to come close to those for older children and adults. The main difficulties are related to the qualitative food intake and the avoidance of excess caloric intake.

The caloric requirement is the amount of energy needed to compensate for the total energy expenditure corresponding to the desired level of physical activity and to ensure optimal growth and development. The intensity of physical activity varies with age, being lower in children between 2-5 years than in children between 6-10 years (Ministerul Sanatatii, 2006). The energy requirement (ER) is calculated according to the formula: ER (kcal / day) = 1000 + 100 x age (years) (Ministerul Sanatatii, 2006).

When people will understand that through chemical processing and refining, food loses much of its nutritional value and fiber by adding a number of chemical additives and increasing its calorie density, most will be willing to change their diet.

It must also be taken into account that dairy and meat products, although they contain nutrients, are mostly too high in fat, cholesterol and calories and completely lacking in dietary fiber.

Nutritional interventions

There are 10 steps to a healthy diet in children (web page):

1. relaxing meals with the family;
2. parents decide the quality, children decide the quantity;
3. food from all 5 food groups every day (cereals, fruits and vegetables, dairy, meat, fats) is offered;
4. 3 regular meals and 2 snacks a day are recommended;
5. 6-8 glasses (100-150 ml) of liquids per day (milk, water, very diluted fruit juice) are recommended;
6. vitamin D is administered;
7. children's tastes and preferences are respected; they must not be forced;
8. the child is rewarded carefully; food and drink are not used as a reward;
9. restriction of fried foods, chips, cakes, biscuits and prohibition of sugar juices, sour drinks, fruit juice, tea, coffee, whole nuts and small, round foods that can be aspired;
10. physical activity is encouraged for at least 3 hours each day and approximately 12 hours of sleep are recommended.

It is known that preschool children learn eating behavior through observation and participation in various common activities, so the parenteral model and caregivers is an ideal opportunity to promote positive nutritional behaviors. The researchers demonstrated similarities in different aspects between mother and daughter in terms of beverage consumption, fruit and vegetable consumption, consumption of fats, minerals, and vitamins. Family meals are recommended, when children are encouraged to eat the same healthy diet as their parents (Nicklas et al., 2001; Addesi et al., 2005). Parents and caregivers have a vital role to play in developing healthy eating behavior by providing healthy foods. They must be accessible to children. Cutting fruit and vegetables and serving them as 'finger food' is a way to promote their consumption (Davison and Birch, 2001).

Vegetables play an important role in nutrition, being a source of vitamin A and C, folic acid, minerals, protecting the body and defending it against various diseases.

Fruits also contain fibers, necessary for the proper functioning of the bowel, as well as vitamins, necessary for a harmonious development of the children’s body.

Strategies such as "eat everything" or "finish everything from the plate" have led to increased caloric intake in children, increased fat intake, reduced consumption of fruits and vegetables, increased feeding time of the child and increased obesity (Birch, 1999).

Sugar-sweetened sour drinks and fruit juices are frequently consumed by children. They are high in calories and low in nutrients. There are studies that have shown the link between the consumption of sugary drinks and overweight in young children (Hu and Malik, 2010). Although the consumption of fruit juices (100% natural) is not related to overweight, the energy intake is higher in children who consume these juices. It is therefore recommended to limit 100% natural fruit juice to once a day in a cup (Baker et al., 2001). Water is an alternative to sugary drinks and fruit juices to reduce total energy intake (Wang et al., 2009).

The results of a study conducted in Oradea on 316 children showed that the diet of children is adequate, they consume fruits and vegetables frequently, and the consumption of "fast food" is still uncharacteristic (Dragan et al., 2017).

An increase in portion sizes is an important factor contributing to the obesity epidemic. At larger portion sizes, older children will consume larger amounts compared to younger children (Rolls et al., 2006). Educating parents about portion sizes in relation to children's age is considered key to promoting healthy eating. Also, avoid using plates of the same size as adults, thus encouraging larger portions.

By setting your routine and regular meals at set intervals and limiting your diet to three lunches and two snacks a day, your caloric intake can be controlled. Often, in preschool children, the three lunches fail to cover the necessary nutrients, so two snacks from the first four food groups are needed. Lunches and snacks should be spaced at regular intervals throughout the day to prevent starvation and overeating.

Sweets are unhealthy primarily because of the contained E numbers and preservatives, but also because of the high sugar content. Sugar-based products lead to an altered lipid profile, atherosclerotic plaques, increased risk of coronary heart disease and cardiovascular dysfunction, rapid rise of the blood sugar concentration, followed by an increased insulin secretion.

The caffeine and theobromine content of chocolate has stimulating effects on the central nervous system. The beneficial effect of antioxidants, contained in sufficient quantities in dark chocolate, should also be mentioned here.

However, there are sugar-based products such as cakes, ice creams and chocolate that contain milk, butter, dried fruit and oilseeds and that provide some of the nutrients that are necessary for the body. Even here moderation is necessary.

Physical activity and sedentary behavior

Inactivity during the early years contributes to childhood obesity and impaired physical, cognitive, and emotional development. A study on the impact of sedentary behavior in children under 4 years of age showed that they spend up to 84% of their waking hours being inactive: immobilized in a cart, in a car seat or in front of a screen (LeBlanc et al., 2012). The associations were evident between sedentary behavior and adiposity, bone health, motor skills, psychosocial health, cognitive development and cardiometabolic health.

The authors of a study of 1,314 young children reported an association between watching television and increasing waist circumference. Preschool children who sat on television for more than two hours a day had a waist circumference increase of 7.6 mm by the age of 10 (Fitzpatrick et al., 2012). While watching TV, children are not only inactive, but are exposed to advertisements for unhealthy eating. A study of 15144 children in Europe showed that those who receive lunch in front of the TV prefer a diet high in fat and sugar (Lissner et al., 2012).

The guides recommend 3 hours of physical activity a day for preschool children (Department of Health, UK). In UK, most of them have a physical activity of 120-150 minutes a day, which means that they have to add another 30-60 minutes a day (Department of Health, UK). Physical activity can occur through unstructured active play but can also include more structured activities. Activities can be of any intensity (light or more energetic) (Department of Health, UK). Preschool children are not able to sustain long periods of activity, so breaks are recommended.

1.2.2.5 Conclusions

The growth and the development of children are influenced by multiple factors such as genetics, general health, physical activity, sedentary lifestyle, quality of sleep and proper nutrition. The disruption of these factors in the preschool period can negatively influence the health of the older child and later, the adult's one. Prevention represents an essential component of pediatrics. Preschool children's care should provide opportunities for the promotion of healthy behaviors for proper physical and psychosocial development.

In conclusion to the above-mentioned issues, one may notice that a large number of children (57%) can be considered healthy and thus happy as far as nutrition is concerned.

One may notice that questioned children have a proper consumption of healthy foods such as fruit and vegetables.

Questioned children admit the pleasure of consuming sweets and this is a good thing as long as sweets are consumed in moderation.

Consumption of fast-food products is not yet characteristic to the studied sample, even if advertisements for these products are aggressive and present everywhere.

Parenting and caregiver practices associated with child nutrition and physical activity include eating styles, the degree to which parents and caregivers ensure a healthy environment (access to healthy foods and physical activity facilities), the role of modelling healthy behaviors and nutrition knowledge. Preschool children's care should provide opportunities for the promotion of healthy behaviors.

1.2.3 Prevention of heart disease starts in childhood

1.2.3.1 Introduction

Lately, childhood obesity has reached epidemic proportions worldwide. Early identification of infants at high risk of becoming obese in childhood or adolescence can be extremely important in preventing obesity. There are studies that point out that the atherogenic process starts in childhood, which in adulthood can manifest itself in myocardial infarction and stroke, which are the major clinical consequences of atherosclerosis (McGill et al., 2000).

There is an upward trend in the prevalence of obesity globally, from 0.7% in 1975 to 5.6% in 2016 in girls and from 0.9% to 7.8% in boys (NCD Risk Factor Collaboration, 2017). With the increasing prevalence of obesity in pediatric age, the number of complications associated with obesity has also increased: dyslipidemias, type 2 diabetes mellitus, fatty liver disease, sleep apnea, microalbuminuria, elevated triglycerides (TG), total and low-density lipoprotein cholesterol (LDL-C), and insulin levels, as well as a reduced high density lipoprotein cholesterol (HDL-C) levels. All of them represent risk factors for the occurrence of cardiovascular diseases (Cozzolino et al., 2015; Alpert et al., 2016).

In the obese children, the signs of cardiovascular dysfunction are: significantly higher arterial blood pressure, changes in the structure and function of the myocardium (left

ventricular hypertrophy, left ventricular diastolic dysfunction, and myocardial dysfunction), and the occurrence of long-term epicardial fat (Alpert et al., 2016; Elshorbagy et al., 2016).

The waist circumference is an easy-to-determine clinical parameter for assessing the nutritional status of the child, independent of body mass index (BMI) (Bassali et al., 2010). Waist circumference correlates with visceral obesity, this is the reason why obese children with elevated waist circumference need to be carefully monitored to prevent long-term cardiometabolic complications (Savva et al., 2000; Romero-Velarde et al., 2013).

There are evidences that central obesity is an independent risk factor for coronary artery disease, arterial hypertension, dyslipidemia (Larsson et al., 1984; Ducimetiere et al., 1986). Moreover, waist circumference at the ages of 5 to 17 years was shown to be associated with abnormal concentrations of TG, LDL-C, HDL-C, and insulin (Freedman et al., 2007).

However, the association of abdominal obesity in children and adolescents with cardio-metabolic risk factors remains controversial.

The purpose of our paper was to establish if waist circumference could be a predictor of cardiovascular complications in children.

Personal contribution – published papers:

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(e20923), **ISI IF 1,889**

The aim of this study was to establish if waist circumference could be a predictor of cardiovascular complications in children.

1.2.3.2 Material and methods

In this retrospective study was included 160 children with overweight and obesity, without associated pathologies, hospitalized between January 1, 2016 and December 31, 2018 in the "Saint Mary" Emergency Children Hospital, Iasi, Romania. Obese patients with associated medical illness, eating disorders, chronic medications were excluded.

The patients were included in 2 groups: group A: children between 6 and 11 years old; group B: adolescents between 12 and 18 years old. The data were collected from the patient's observation sheets. Patients were evaluated completely anthropometrically, biologically, and imagistic. The anthropometric data tracked were height, weight, waist circumference, and the BMI. Interpretation of BMI values was based on BMI Z score and BMI percentile, applicable for age and sex, according to WHO standards, using WHO AnthroPlus software. Depending on the BMI Z score, the patients were classified into overweight (BMI Z score >+1SD or BMI percentiles between 85 and 97th), obese (BMI Z score >+2SD or BMI percentiles between 97 and 99.9th), and severe obesity (BMI Z score >+3 SD or BMI percentiles>99.9th) (de Onis and Lobstein, 2010; Rolland-Cachera, 2011). Visceral obesity was defined by values over the 90th percentile of waist circumference (Bassali et al., 2010).

The lab blood tests included: total cholesterol (TC), low-density lipoprotein cholesterol (LDLc) and high-density lipoprotein cholesterol (HDLc), triglycerides (TG), glucose levels, alanine aminotransferase values, urea and creatinine values. The reference standards were used to interpret the values of biological parameters.

The blood pressure (BP) value was interpreted according to the National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents. Children hypertension was defined as systolic BP and/or diastolic BP >95th percentile, adjusted for height, age, and sex, at least 3 separate determination. Normally, high

blood pressure was considered for values between the 90 and 95 percentile values (National Institute for Health, 2004). In our study, BP values ≥90th percentile were defined as ”elevated BP” or vascular impairment.

All children performed echocardiography, the following parameters being thickness of the interventricular septum (IVS), diastolic diameters of the left ventricle (DdLV), left ventricular mass (LVM), the relative thickness of the ventricular wall (RWT), the presence of epicardial fat. Diastolic dysfunction was evaluated through the E/A ratio and the pulmonary venous flow through the S/D ratio.

The interpretation of the LVM was made according to LVM index or LVM-for-height Z score (LVM divided by height raised to a power of 2.7) (Foster et al., 2008). Left ventricular hypertrophy (LVH) was defined as LVM index >95th percentile for normal children and adolescents (De Simone et al., 1995). Relative wall thickness (RWT) was measured to assess the LV geometric pattern (De Simone et al., 1992). The RWT value above >0.41 is considered pathological. Patients with increased LVM index and elevated RWT (>0.41) had concentric LVH; those with increased LVM index and normal RWT (<0.41) had eccentric LVH. Concentric remodeling was defined as elevated RWT, but with normal LVM index. Pathological epicardial fat quantified by echocardiography was considered over 4.1 mm (Fang et al., 2019).

Patients who had concentric or eccentric LVM hypertrophy, concentric remodeling, and/or epicardial fat with pathological values were considered to have cardiac impairment.

Statistical analyzes of the variables were performed using SPSS software v.20 (IBM Corporation, North Castle Drive, Armonk, NY 10504-1785, U.S.A.) type variables were reported as mean with standard deviation. Comparisons between the analyzed groups were performed using Student t test or Mann–Whitney U test for continuous variables. The qualitative variables were presented as absolute (n) and relative (%) frequencies, and the comparisons between groups were made based on the results of McNemar, Yates Chi-square, or Fisher exact test. Univariate and multivariate analysis of prognostic factors regarding cardiovascular complications was performed using the Logistic regression model. The significance level calculated in the used tests (P-value) was considered significant for P values <.05.

1.2.3.3 Results

The authors analyzed the clinical, biological, and imaging characteristics of the all patients included in the study group. Group A (children) included 97 patients aged 6 to 11 years old (mean age 9.82±2.2 years) and group B (adolescents) included 63 patients aged 12 to 18 years old (mean age 14.7±1.6).

Between the 2 groups of patients, the sex distribution showed predominance of the male sex in both groups (59.8% in group A compared with 60.3% in group B).

In both groups, obesity was more prevalent than overweight (53.61% in children vs 49.21% in adolescents). In the study group A, 32.99% of children had severe obesity while in the group B, 44.44% of adolescents had overweight.

These results are concordant with the classification of the study groups according to the nutritional status, with significant differences regarding the classification of children and adolescents in overweight, respectively morbid obesity.

Thus, overweight is far more common in adolescents, while obesity and severe obesity are significantly more common in children. Obesity and severe obesity are significantly associated (P<.001) with the age of <12 years old.

Therefore, the BMI percentile indicated higher mean values for children compared with adolescents (P<.0001).

Comparing the average values of the systolic BP and diastolic BP does not objectively reflect the presence of vascular impairment due to the different criteria for age group specific classification.

Considering also the age criterion and the classification of hypertension properly, it is clearly shown that in obese adolescents (8.3% vs 30.2%; P=.0003) pre-hypertension and hypertension are significantly more frequent (20.6% vs 14.4%; P=.0003).

Also, we compared several biological parameters between children and adolescents. The assessment of total cholesterol values showed that there are no statistically significant differences between the 2 age groups.

Regarding triglycerides, it is observed that there are statistically significant differences between children and adolescents (P=.0142).

We evaluated the correlation between epicardial fat and visceral obesity in children and adolescents (Table 1.2).

Table 1.2. *Evaluation of the association of epicardial fat versus visceral obesity*

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

[a]Chi-square test (McNemar Chi-square/Yates).
*Marked effects are significant at P<.05.

The presence of visceral obesity was unquestionably associated (x2=11.72, P=.0006) with the presence of pathological epicardial fat: from the 46 cases without visceral obesity only 2.17% had pathological epicardial fat, whereas of the 92 cases with visceral obesity, 21.74% presented pathological epicardial fat. Area under the curve (AUC) showed an increased predictive power of the presence of pathological epicardial fat (AUC=0.668, 95% confidence interval [CI]: 0.562–0.775, P=.014) in the presence of visceral obesity (Figure 1.9).

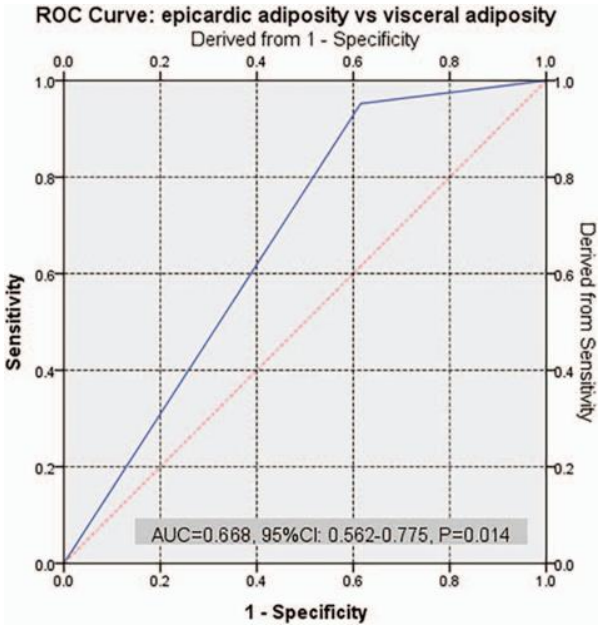

Figure 1.9. *AUC: Epicardial fat versus visceral obesity. AUC = area under the curve*

Our analysis revealed that BMI is not a significant predictor for vascular impairment for either children or adolescents (AUC=0.57, P=.327 vs AUC=0.54, P=.53). However, BMI is an important predictive factor for the occurrence of cardiac impairment in children (AUC=0.62, P=.041) and adolescent (AUC=0.66, P=.036). In the age group 6 to 11 years, among the factors analyzed (BMI, visceral obesity, TC, TG, LDLc, HDLc), none of them are important predictive factors for vascular impairment (P>.05), but BMI is a significant predictive factor for cardiac impairment.

In the age 6 to 11 years, visceral obesity is not a risk factor for vascular or cardiac impairment, compared in adolescents, where the results showed that visceral obesity is an important predictive factor for the occurrence of vascular (AUC=0.669, P=.021) and cardiac (AUC=0.697, P=.037) impairment.

Also, increased levels of TG and LDLc are predictable for the occurrence of cardiac impairment in adolescents (AUC=0.67, P=.044; AUC=0.66, P=.038), but total cholesterol is not a predictive factor for cardiac manifestations.

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

1.2.3.4 Discussions

Follow-up studies from childhood to adulthood have shown that early obesity or overweight may be associated with increased atherosclerosis, as well as increased cardiovascular morbidity and mortality (Fang et al., 2019).

Literature studies show that in overweight and obese adults, heart failure will develop 10 years faster compared with subjects with normal BMI (Abaci et al., 2009). This is why it is extremely important to identify the clinical and biological parameters for predicting cardiovascular risk from childhood.

Among the clinical parameters, BMI and waist circumference were the first cardiovascular risk factors discussed in childhood and adolescence.

Waist circumference provides an indicator of visceral adipose tissue. Imaging techniques such as abdominal computed tomography, magnetic resonance imaging, and dual-energy x-ray absorptiometry provide accurate measurements of visceral adipose tissue, but are expensive and often unfeasible to use in the clinical setting. Studies showed that waist circumference provides almost an identical measurement of visceral adipose tissue among adults and can be used to identify those at risk of developing cardiovascular disease and type 2 diabetes mellitus (Harrington et al., 2013; Schneider et al., 2007).

The evaluation of fat distribution is a compulsory procedure in obese children because it is known very well the relationship between visceral adiposity and cardiovascular risk (Roever et al., 2016). In evaluating overweight children, waist circumference plays an important role in the early identification of metabolic syndrome and cardiometabolic risk (Maffeis et al., 2008). In the study published by Bin and Li in 2011, the authors stated that increased waist circumference was an indicator of high BP in preschool children, especially in boys. Moreover, waist circumference was independently associated with high BP in this category of children. Thus, high BP in children has been associated with increased waist circumference, and childhood obesity is associated with high risk of adult hypertension (Mehta,

2015). Regarding the predictive power of BMI and waist circumference on elevated BP, literature studies have shown that increased waist circumference is associated with elevated BP even when BMI is normal (Finucane et al., 2008).

In this study, we observed that waist circumference is an important predictive factor for the occurrence of vascular impairment (pre-hypertension and hypertension) only in adolescents, not in children under 12 years. Regarding the predictive value of BMI, this is not a significant predictive factor for vascular impairment neither in children or adolescent in our study.

In our study, waist circumference above the 90th percentile is a predictive factor for increased LVM index and concentric hypertrophy in both children and adolescents. Moreover, concentric remodeling and the presence of eccentric hypertrophy were not significantly influenced by the presence of visceral obesity.

This study has a number of limitations. Primarily, the definition of visceral obesity based on the waist circumference, in the absence of abdominal magnetic resonance imaging or dual energy x-ray absorptiometry. In addition, the impossibility of performing the vascular ultrasound and evaluation of intima media thickness for the evaluation of subclinical atherosclerosis, a marker of morphological vascular damage.

1.2.3.5 Conclusions

Overweight and obesity in childhood are associated with early cardiovascular dysfunction. Visceral obesity is an important predictive factor for the occurrence of vascular impairment (prehypertension and hypertension) especially in adolescents compared with children. Waist circumference above the 90th percentile is a predictive factor for increased LVM index and concentric hypertrophy in both children and adolescents. Obese pediatric patients with elevated waist circumference need to be carefully monitored to prevent long-term cardiovascular complications.

1.3 The role of vaccination in preventive medicine

1.3.1 Why childhood immunizations are important?

1.3.1.1 Introduction

Immunity is the body's way of preventing disease. The immune system recognizes the antigens that enter the body, and it produces proteins called antibodies to fight them off. The first time a child is infected with a specific antigen, the immune system produces antibodies designed to fight it. Initially, the immune system cannot work fast enough to prevent the antigen from causing the disease, so the child becomes ill. Later, the immune system “remembers” that specific antigen. If it ever enters the body, even after many years, the immune system can produce antibodies quickly enough to prevent it from causing the disease a second time. This protection is called immunity. Vaccines contain the same antigens or parts of antigens that cause the disease, but the antigens in vaccines are either destroyed or weakened to the point where they do not cause that specific disease. However, it is strong enough to make the immune system produce antibodies that lead to immunity. Thus, a vaccine is a safer substitute for a child's first exposure to a disease. The child receives protection without having to become ill. Through vaccination, children can develop immunity without suffering from the diseases themselves.

One of the greatest accomplishments of medical sciences was the discovery and implementation of vaccination against the main infectious-contagious diseases, often spread in pandemic proportions, which shook humanity throughout history through the epidemics that decimated entire populations (Centers for Disease Control and Prevention, 1999). The biological aggression has always been the most powerful in the relation of men to their life environment, therefore, in the fight against infectious agents there were three main moments that marked the odyssey of the combat against infectious and contagious diseases produced by them.

The first great accomplishment was the implementation of active immunizations by the agents of vaccination, once the smallpox (black pox) vaccine was discovered and applied in 1786 by the English physician Edward Jenner (1749-1823) and applied starting 1831 (Riedel, 2005; Lakhani, 1992). This method was continued and developed for the main epidemic diseases until today, when after almost two hundred years from the beginning of the vaccination practice as a prophylactic method, there are vaccines for 26 diseases with massive spread (Baxby, 1999).

The second great accomplishment was the discovery in 1867 of asepsis and antisepsis by British surgeon Joseph Lister (1827-1912), and this method proved to be salutary in the management of different surgical diseases, in terms of prevention and combat against contamination and inter-contamination during procedures, medical and surgical interventions on sick people, antiseptic measures in locations, public institutions, public interest networks (water supply, sewage) etc., because they considerably reduced contamination and disease spread (Sherwin, 1988; Pitt and Aubin, 2012). His work was published in 2 papers in the Lancet (in March and in July, 1867) (Ackerknecht, 1982). As a result, postoperative mortality decreased from the 45% generally reported to 15%. "If I could, I would even singe my hands", Lister rightfully said in his time, and this dictum remained as valid today (Lister, 1870).

The third moment, as important as the first two, was the introduction of antibiotics in the therapy of infectious-contagious diseases, and this era begins with the discovery of penicillin in 1928 by Alexander Fleming (1881-1955), which was turned to advantage as late as 1945 (Maurois, 1965; Tan and Tatsumura, 2015), and this practice continues today through a true arsenal of antibiotic classes available to medical practice. These three moments with historic impact in eradicating or epidemiologically controlling these contagions led to the fading of one of the Malthusian methods (besides hunger and wars) involved in the demographic control of population (Lupu and Lupu, 2014).

By far, though, vaccination has proved to be the most powerful weapon that science made available to humanity with the purpose of preventing and even eradicating the main diseases of massive spread (Plotkin and Plotkin, 2004). The essential condition in accomplishing and maintaining this desideratum is the achievement of a reasonable immunity in population, which can only be obtained by vaccinating the entire receptive population, according to a well-established schedule (Chiotan, 1998).

Personal contribution – published papers:

1. **Lupu VV**, Ignat A, Stoleriu G, Ciubara AB, Ciubara A, Lupu V, Burlea M, Stratciuc S. Vaccination of Children in Romania between Civic Obligation and Personal Choice. *Revista de Cercetare și Intervenție Socială*, 2017, 56: 123-132 **ISI IF 0,838**

The aim of this review was to discuss actual theories concerning immunization, to underline idea that vaccination is the only individual and collective defense against a personal and collective disaster generated by any of the infectious diseases with epidemic potential.

1.3.1.2 Romanian actualities in the vaccinology field

Romania was one of the countries that introduced and actually contributed to the development of vaccinology as a science and practice from the second half of the 19th century, through reference names in the pantheon of autochthonous and international medical sciences (Pistol, 2011; Lambert et al., 2015). It is worth reminding here, that the first bacteriology textbook was elaborated by Victor Babes (1854-1926) in collaboration with Victor Andre Cornil, and published in 1885 (Petri Dish). The bacteriology schools in Bucharest and Cluj, founded and led by Victor Babes, contributed decisively to the progress of bacteriology as a science. We mention that, among other things, Victor Babes is the discoverer of over 50 bacteria and viruses and a species of parasites that bear his name (babesiidae) producing the disease known as babesiosis (Iftimovici, 2010).

His name is related to the introduction of the rabies vaccination in our country and of serotherapy in the treatment of diphtheria, achieved together with his disciple Mihail Manicatide, the founder of the pediatrics school in Romania (Nastase, 1972). Victor Babes also discovered the antibiosis process that would be the basis of antibiotherapy (Chiotan, 1998).

Another great name of Romanian medicine is Ion Cantacuzino, who discovered and put into practice the cholera vaccination in the second Balkan war, which is a disease that decimated Napoleon Bonaparte's troops in his campaign in Russia and influenced decisively the denouement of the first Balkan war. He laid the foundations of the autochthonous production of vaccines in the institute bearing his name, which today is unfortunately taken over by malefic structures and persons. On this basis, vaccinations were introduced rapidly in our country, and what is more, Romania became the largest producer of vaccines from the South-East of Europe through Ion Cantacuzino Institute, which lasted until the end of the 20th century (Mesrobeanu, 1965).

Vaccination in Romania is organized on three important lines (Ivan and Azoicai, 1995):

- ***Compulsory vaccination*** – for a number of nine diseases, performed until this year within the national vaccination program for children, as follows: (1) BCG vaccination in new-born, between the second and the seventh day from birth, (2) hepatitis B vaccination administrated immediately after birth (after two hours), then at the ages of two months, six months and nine years; (3) diphtheria-tetanus pertussis vaccination (DTP); at the ages of two months, four months, six months, 1 year and three years (4) polio vaccination; at the ages of 2, 4, 6 months, 1 year and 9 years; (5) MMR (measles, mumps, rubella) triple vaccination at the ages of 15 months and five years. These vaccinations are compulsory; they are administrated by family physicians at small ages and in school vaccination campaigns for the school population. The degree of coverage of the vaccinations reached 95 – 98% of the population, the difference being related to incompatibilities, temporary or permanent contraindications, refusal of vaccination (very rare in the past) and other reasons. In appreciating the efficacy of the vaccination, we must not omit the fact that vaccination itself has variable immunization percentages, as follows: the BCG vaccination provides immunization against tuberculosis to up to 80% of the vaccinated individuals, the DTP vaccination up to 96-97%, the polio vaccination 95-100%, MMR – 95%, and the hepatitis B vaccination to 65-92% of the vaccinated individuals. Corroborating these data with the situations in which immunization is not achieved for other reasons, we can say that the actual coverage of the population by means of vaccination used to be of 95%. This year, a new calendar of compulsory vaccinations has been elaborated, and according to it by the 18 months of age, each child should receive at least three doses of hepatitis B vaccine, three doses of the DTP, IPV and Hib vaccines, the first dose of MMR by 1 year and the second dose by the age of 5 years (Ministerul Sanatatii, 2015).

- ***Vaccination of selective compulsoriness*** – it is a method of immunization of a given population, with the purpose of solving certain epidemiologic situations emerged in an area or

community, involving a major risk of sickening. This category includes a few special situations: (1) particular epidemiologic conditions (floods, earthquakes, other acts of God), in which the typhus, dysentery, hepatitis A vaccines used in areas and communities with high epidemiologic risk are compulsory; (2) in emergencies: tetanus, rabies, anti-venom vaccination in case of animal bites, rubella vaccination in rubella epidemics; (3) regional interest vaccinations: cholera, malaria, plague vaccines etc. in areas where there is an epidemiologic potential for these diseases; (4) vaccinations of interest for professional groups, such as: anthrax, rabies vaccination for those working in animal husbandry and influenza or hepatitis B vaccination for those working abroad; (5) population groups with high epidemiologic risk, such as: elderly, suffering from chronic diseases or consumptive diseases, where certain types of vaccine are necessary, such as the influenza vaccines. Both vaccine categories are free of charge and covered from the budget of the health ministry, being at the same time compulsory.

- ***Optional vaccinations*** – generally performed based on a medical recommendation, but paid for by the patient, including: influenza, hepatitis A, pneumococcal, meningococcal, chickenpox vaccines etc. (World Health Organization, 2012).

1.3.1.3 The accomplishments and actualities of the past 10 years in the field of Romanian vaccinology

We may say that the Romanian accomplishments generally keep up with the world accomplishments and meet the WHO recommendations in the field. We would like to point out the fact that the national coverage of compulsory vaccinations shifted massively to polyvalent vaccines in the shape of tetra-, penta- or hexa-vaccine, which include the classic triad diphtheria-tetanus-pertussis, polio vaccination with antigenic fractions, antigenic fractions for hepatitis B and *Haemophilus influenza type B* (Tavakol, 2014).

Live attenuated strains, such as in the polio vaccine, are no longer used, consequently oral administration is no longer a choice. A legal framework has been created regarding the vaccination acceptance or unacceptance by participants, which is a phenomenon that, for the past few years, has become a public debate subject generated mainly, by the fact that the market of vaccines is diversified enough and there is even commercial pressure that predisposes to an exaggeration in the prescription and use of vaccines, which led to the unwanted consequence of a public perception, that is not quite favorable and even to the refusal of vaccination, even for compulsory vaccines (Lupu, 2012).

The great accomplishments of vaccinology seem to be eclipsed by the actualities of the past few years, when in certain areas, even in the Romanian capital, there has been a decrease in the vaccination percentage by 20% (Bucharest), and the population immunization percentage has decreased under 75%, which means that the critical mass necessary for epidemic outbursts has been exceeded (Ministerul Sanatatii, 2015). The main causes for this situation gravitate – in our opinion – around defective communication associated to a strong anti-vaccine offensive in the media exercised by unprofessional structures, starting from certain prejudices that are not confirmed in reality. The emergence of these situations was also favored by the fact, that there were discontinuities and dysfunctionalities in the provision of imported vaccines that were not precisely appropriate for our area, because our national source of vaccines was willingly and tendentiously impaired, in complicity with the authorities in the field.

Besides the mentioned dysfunctionalities, the liquidation of our national production of vaccines led to their purchase from abroad, for prices 10 times higher. Apart from the economic impact, which is not at all negligible, this situation will unfortunately end a tradition of research and production built across one century at Ion Cantacuzino Institute.

1.3.1.4 Ethical aspects regarding the vaccination of children

For the past few decades, as vaccinology got consecrated as a science, a strong trend developed against the vaccination of children supported by different structures, some of which, unfortunately, even supported by professionals in the field (Dubé et al., 2013; Burton-Jeangros et al., 2005; Benin et al., 2006; Brown et al., 2011). To this situation a few elements contributed, the first is the lack of communication between physician and patient due, today, to a large extent to the bureaucratic approach of this relationship by the authorities that – we have to say – fail to encourage in any way this type of relationship. This becomes visible through the fact that, in the physician's activity, the necessary time is not allocated to certain discussions on medical topics, which would represent that sanitary education confirmed and validated by time. What is more, unfounded and even contradictory information polluting the media and especially the virtual space get to question the physician's authority in outlining the correct attitudes of the patient and population, as regards the usefulness of vaccines (Smith et al., 2007; Mason and Donnelly, 2000). There are studies indicated that parents' choice was often based on following what is recommended, rather than based on specific knowledge about vaccine or vaccine-preventable disease (Streefland et al., 1999; Tickner et al., 2006; Benin et al., 2006).

We would also add here, an unconvincing positioning especially online by experts, whose presence in the public space is rather poor. This is where we could answer scientifically the ethical question: Why should we get vaccinated? This is all the more, so as the 200-year-old practice highlighted the benefits that vaccination brought to humanity (Lakhani, 1992). We dare to mention: (1) due to vaccination, extremely serious infectious-contagious diseases such as: smallpox, plague, poliomyelitis, diphtheria, cholera etc. were eradicated (whose mortality in epidemic outbursts exceeded 50% of the affected population) and also, by means of vaccination, diseases such as rabies, tetanus, rubella etc. are kept under control; (2) beyond the extremely high mortality rate generated by these diseases, vaccination spares the individual, the family and community from the risks and discomfort produced by the diseases, that can encumber the life of communities by means of the emergency measures imposed by an epidemic outburst (isolation, treatments, quarantines, restrictions, interdictions and drastic anti-epidemic measures) with serious perturbations of the daily existence of the individual and of the community; (3) vaccination responds in an extremely convincing way to two major desiderata. The first is medical and corresponds to the Hippocratic dictum "prevention is better than cure", because the ongoing disease, apart from the discomfort it generates, implies vital risks and permanent sequelae, the most convincing example here being poliomyelitis. The second desideratum is economic, it is subsumed to the first, and according to it "it is much cheaper to prevent than to treat", because an epidemic, besides the necessary reorientation of resources, can, economically and socially, disorganize a community; (4) nowadays, there are sufficient specialized studies and other studies that unequivocally attest the unquestionable value of vaccination for the individual and public health.

It is enough to remind that many of the great scourges of human kind are now history (the plague, smallpox, cholera etc.) precisely due to massive vaccination.

The insufficient knowledge of these epochal accomplishments due to vaccinations created the corridor necessary for the manifestation of a true offensive against vaccinations, fed on the one side, by the distrust of a considerable part of the population towards vaccination, and on the other side, by a commercial overstrain on vaccinations other than those epidemiologically important, due to the current technical possibilities (Kane, 1998).

1.3.1.5 The causes for the distrust regarding vaccines and possible remedies

This situation is based on a few aspects, of which the first is precisely the lack of knowledge regarding the actualities presented above; therefore, we need to publish them in any way available. Then, there is the deep-rooted idea that the best immunization is provided by

the disease, therefore the human body should be allowed to face the disease. It is true that, from the biological point of view, the confrontation with the disease provides the most durable immunization, but provided people survive the disease. What happens to the ones that do not survive it? (Mortality by diphtheria, for instance, was 50%) or are left with permanent sequelae for the rest of their lives? (The paralyses caused by poliomyelitis, subacute sclerosing panencephalitis following measles etc.), let alone the risks of the extremely fast spread of the diseases to the population, which are medically and epidemiologically dramatic aspects that can only be prevented through vaccination.

It is not less true that the industry of vaccines overbids immunization in population, even for common conditions, which is biologically and medically justified only in part and only for certain population categories (see optional vaccinations). Their extension to the entire population could be assimilated to the category of unnecessary interventions, that can lead to additional risks through the fact that the most sensitive system of the body structure – the immunity system – is overstrained and its response is often unpredictable (Lupu and Lupu, 2014). These complaints are also accompanied by the unjustified fear of the contamination of vaccines with other germs or inorganic and organic components harmful for the body, or the fear that the rush of releasing them on the market makes the testing period uncertain and doubtful (Tavakol, 2014). We would also like to mention that it is not rarely, that the fierce and disloyal competition between companies feeds and supports this suspicion and distrust.

The maintenance of such a climate is favored by the intense circulation in the media – especially online media – of certain empiric and unfounded information from many people untrained in the field, who exaggerate with regard to certain particular cases, many of which are isolated and unchecked.

The speculations on the contamination must be regarded suspiciously, because today the technique of vaccine manufacture is safer than ever (Tavakol, 2014).

The wide propagation through the media of inappropriate charges must be seen rather as diversions in the commercial war. We should remember here, that the manufacture of vaccines uses strains that circulate in our area and not exotic strains with a small impact in our area but a high risk.

Refusal of vaccines has also been linked with religious convictions. Orthodox Protestants in The Netherlands and the Amish in the United States are religious communities well-known for rejecting to vaccination based on religious motives (Streefland, 2001; Ruijs et al., 2012).

Another cause for the refusal to get vaccinated is a defective perception of informed consent, seen rather as a possibility to refuse, than an expression of autonomy by virtue of the fundamental human rights.

Therefore, this leads to the unconscious assumption of risks that regard equally the individual and the community.

In approaching this last aspect, we must consider that, in a given epidemiologic context, in which the risk of sickening and equally of contamination of healthy persons is imminent, the refusal of vaccination raises fundamental ethical issues that overcome the strictly personal interest and must be seen as a collective right that must be respected as being of general interest.

Before the major risks implied by an epidemic outburst, general interest prevails, because both the freedom and security of a person ends where they meet the freedom and security of another person.

In addition, the refusal of vaccination, beyond its condemnable character (drastically sanctioned in some states), entails a series of inconveniences that encumber the child's and individual's social integrity, such as: integration in school communities, filling-in a position, access to certain professions and the right to travel worldwide.

1.3.1.6 Conclusions

Furthering the idea that vaccination is the only individual and collective defense against a personal and collective disaster generated by any of the infectious diseases with epidemic potential must become a permanent concern of the medical system and the civil society.

The correct understanding of the role of vaccination in the community life, of the fact that it is the only manner that ensures personal and collective security against epidemic diseases and equally corresponds to the ethical principles of cohabitation in society must prevail before any considerations. Here, the physician, and equally the medical system must take full responsibility in accomplishing this ethical desideratum implied by the profession.

1.3.2 The effect of antipyretic analgesics on immune response to vaccination

1.3.2.1 Introduction

Antipyretic analgesics are widely used during vaccination to relieve fever and pain. Pneumococcal conjugate vaccines (PCVs) provide protection against invasive pneumococcal disease (IPD) and other diseases such as acute otitis media or pneumonia (Deceuninck et al., 2015; Domingues et al., 2014; Palmu et al., 2013; Tregnaghi et al., 2014). PCVs have been included in many national childhood immunization programs. Co-administration of PCVs with standard infant vaccines was shown to induce a higher incidence of fever in children compared to single-vaccine administration (Knuf et al, 2006; Olivier et al., 2004; Stockwell et al., 2013). Antipyretics, most commonly paracetamol and ibuprofen, are sometimes administered prophylactically to prevent fever during pediatric immunization (Taddio et al., 2007). The authors of a study showed that while acetaminophen prophylaxis significantly reduced fever after routine childhood immunization, it simultaneously affected the immune response to several vaccine antigens (Prymula et al., 2009).

Prophylactic paracetamol administration (immediate and 6–8 hours post vaccination) with 10-valent pneumococcal non-typeable *Haemophilus influenzae* protein D conjugate vaccine (PHiD-CV) transiently lowered immune response after primary and booster vaccination. Induction of immunological memory and persistent impact of PHiD-CV on carriage rates were observed until at least 28 months post-booster vaccination (Prymula et al., 2013). The observed trend toward lower antibody geometric mean concentrations (GMCs) prior to boosting may have significance for those children who might miss their booster dose, as their antibodies may decline faster than if they had not received paracetamol. Prophylactic administration of paracetamol also seemed to interfere with immune responses to the PCV13 in infants, while ibuprofen appeared to reduce responses to pertussis filamentous haemagglutinin (FHA) and tetanus antigens without impacting pneumococcal responses (Wysocki et al., 2014).

Another study demonstrated that prophylactic administration of paracetamol in children after concomitant vaccination with a multicomponent meningococcal serogroup B vaccine (4CMenB), DTPa-HBV-IPV/Hib and PCV7 decreased fever and reactogenicity, with no apparent clinically relevant effect on immune responses (Prymula et al, 2014).

There are no published data concerning the impact of prophylactic ibuprofen administration on the immune response to PHiD-CV (Das et al., 2014). This study aimed to demonstrate non-inferiority of the immune response to PHiD-CV administered as a 3-dose primary course with immediate (IIBU) or delayed (DIBU) versus no prophylactic ibuprofen (NIBU) administration, in terms of percentage of infants with anti-pneumococcal antibody concentrations $\geq$0.2 mg/mL. Non-inferiority was to be demonstrated if, for $\geq$7/10 serotypes,

the upper limit (UL) of the 98.25% confidence interval (CI) of the difference between groups (NIBU vs IIBU and NIBU vs DIBU) was <10%, in compliance with the European Medicines Agency Guideline on the Choice of the Non-inferiority Margin (CHMP, 2005). Also, the study aimed to demonstrate a lower incidence of febrile reactions with immediate or delayed ibuprofen administration vs no ibuprofen administration.

Personal contribution – published papers:

1. Falup-Pecurariu O, Man SC, Neamtu ML, Chicin G, Baciu G, Pitic C, Cara AC, Neculau AE, Burlea M, Brinza IL, Schnell CN, Sas V, **Lupu VV**, François N, Swinnen K, Borys D. Effects of prophylactic ibuprofen and paracetamol administration on the immunogenicity and reactogenicity of the 10-valent pneumococcal non-typeable *Haemophilus influenzae* protein D conjugated vaccine (PHiD-CV) co-administered with DTPa-combined vaccines in children: An open-label, randomized, controlled, non-inferiority trial. *Human Vaccines & Immunotherapeutics*, 2017, 13(3): 649-660 **ISI IF 2,229**

The aim of this study was to evaluate the effect of paracetamol administration (immediate or delayed, the latter not yet studied) on the immunogenicity and reactogenicity of PHiD-CV and the co-administered routine infant vaccines after primary and booster vaccinations.

1.3.2.2 Material and methods

The study was conducted between 12 November 2010 and 08 December 2012. Of 850 participants randomized, 812 were included in the total vaccination cohort (TVC) for primary vaccination and 768 in the TVC for booster vaccination; 647 (79.7%) children from the primary and 575 (74.9%) children from the booster epoch were included in the according-to-protocol (ATP) cohort for immunogenicity.

Demographic characteristics were similar between groups. The mean age at primary vaccination was 13.1 (standard deviation: 1.18) weeks at first dose, 18.0 (1.48) weeks at second dose, and 23.1 (1.78) weeks at third dose; the mean age at booster vaccination was 12.3 (0.62) months.

There were no major differences between groups in the total daily dose of administered antipyretics. Two children in the TVC were withdrawn due to a serious adverse event (SAE) during the study period; these SAEs were not considered to be causally related to vaccination.

Study design and participants

In this phase IV, multicenter, open-label, randomized, controlled study performed in Romania, infants aged 12–16 weeks at the time of first vaccination (Figure 1.10), born after a gestation period of 36–42 weeks and without any obvious health problems, were enrolled. Written informed consent was obtained from each participant's parents or legally authorized representatives.

The study was conducted according to Good Clinical Practice, the Declaration of Helsinki, and the local rules and regulations of the country; when deviations from these guidelines and regulations were detected, corrective actions were implemented where needed, including exclusion of participants from analyses.

This was the case for one study site, at which all study related activities were terminated during the study due to lack of confidence in the integrity of the data. The infants enrolled at this site were withdrawn from the study, offered continuation of vaccination outside the study and excluded from analyses.

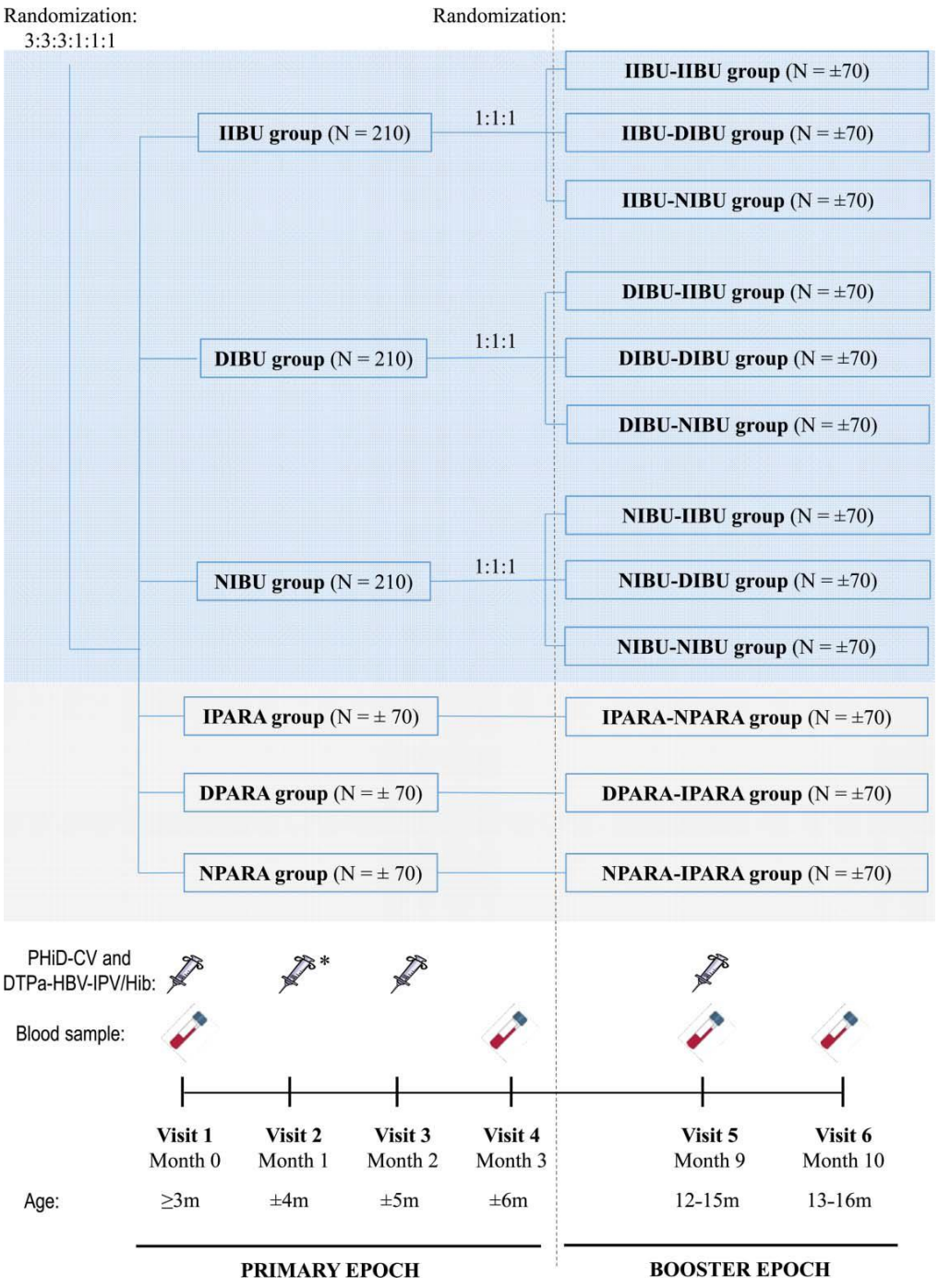

***Figure 1.10**. Study design. Footnote: Primary vaccination: PHiD-CV and DTPa-(HBV)-IPV/Hib at 3, 4, and 5 months of age, with the following prophylactic antipyretic regimen: IIBU, immediate ibuprofen; DIBU, delayed ibuprofen; NIBU, no ibuprofen; IPARA, immediate paracetamol; DPARA, delayed paracetamol; NPARA, no paracetamol. Booster vaccination: PHiD-CV and DTPa-HBV-IPV/Hib at 12–15 months of age, with the following prophylactic antipyretic regimen: at primary vaccination: immediate ibuprofen, and at booster: immediate (IIBU-IIBU), delayed (IIBU-DIBU) or no ibuprofen (IIBU-NIBU); at primary vaccination: delayed ibuprofen, and at booster: immediate (DIBU-IIBU), delayed (DIBU-DIBU) or no ibuprofen (DIBU-NIBU); at primary vaccination: no ibuprofen, and at booster: immediate (NIBU-IIBU), delayed (NIBU-DIBU) or no ibuprofen (NIBU-NIBU); immediate paracetamol at primary vaccination and no paracetamol at booster (IPARA-NPARA); delayed paracetamol at primary vaccination and immediate paracetamol at booster (DPARA-IPARA); no paracetamol at primary vaccination, and immediate paracetamol at booster (NPARA-IPARA). N, number of children per group; m, months; DTPa-IPV/Hib instead of DTPa-HBV-IPV/Hib.*

Randomization and masking

Enrolled infants were randomized using a blocking scheme (3:3:3:1:1:1) into 3 ibuprofen (IBU) groups and 3 paracetamol (PARA) groups, to receive after each dose of primary vaccinations immediate, delayed, or no ibuprofen or paracetamol prophylactic administration. At booster vaccination, each IBU group (immediate, delayed, or no ibuprofen at priming) was further randomized (1:1:1) into 3 groups (immediate, delayed, or no ibuprofen at booster), while for the 3 PARA groups, treatment (immediate, delayed, or no paracetamol at priming) was re-allocated as defined in the protocol (Figure 22). The randomization lists were generated at GSK using MATEX for SAS to number the study vaccines and the antipyretic doses given at primary and booster vaccination. Treatment allocation at the site was performed using GSK's internet randomization system (SBIR): the site investigator accessed the randomization system on the internet and provided the identification number for eligible infants. The randomization system then used a minimization algorithm to determine the treatment number for the study vaccines and antipyretic doses to be used for the infant.

The study was conducted in an open manner; the participants' parent(s) or legally acceptable representative, the investigator, and all study staff involved in the clinical evaluation of participants were aware of treatment allocation.

Procedures

Participants received 3-dose primary vaccination with PHiDCV (SynflorixTM, GSK, Belgium) at 3, 4, and 5 months of age and booster dose at 12–15 months of age (intramuscular, in the right thigh, or deltoid for children >12 months); 2 doses of DTPa-HBV-IPV/Hib (Infanrix hexaTM, GSK, Belgium) at 3 and 5 months of age and booster dose at 12–15 months of age (intramuscular, left thigh or deltoid); and one dose of DTPa-IPV/Hib (Infanrix-IPV/HibTM, GSK, Belgium) at 4 months of age (intramuscular, left thigh). The first dose of antipyretic (ibuprofen (NurofenTM, Reckitt Benckiser, UK) – 10 mg/kg/dose, with a maximum daily dose of 30 mg/kg, or paracetamol (Panadol BabyTM, GSK, UK) – 15 mg/kg/dose with a maximum daily dose of 60 mg/kg) was administered orally either immediately after vaccination at the study site (immediate administration) or by the parents at home 4–6 hours after vaccination (delayed administration). The second and third dose of antipyretic were administered by the parents at home, 6–8 hours after the previous dose; if a child slept overnight, the dose was deferred to the following morning.

Outcomes

The primary study outcome was to assess the percentage of infants with anti-pneumococcal antibody concentrations $\geq$0.2 mg/mL for each of the 10 PHiD-CV serotypes, in order to demonstrate non-inferiority of immune response to PHiD-CV administered as a 3-dose primary vaccination course with immediate or delayed prophylactic ibuprofen compared to PHiD-CV without prophylactic ibuprofen administration.

Secondary outcomes included determination of the percentage reduction in fever episodes with immediate or delayed prophylactic ibuprofen administration after primary PHiD-CV vaccination (confirmatory objective). The percentage of participants with local and general adverse events within 4 days, with unsolicited AEs within 31 d after each vaccine dose, and the occurrence of SAEs during the entire study were also assessed. Another secondary outcome was the evaluation of the immune responses to the components of PHiD-CV and the co-administered DTPa-HBV-IPV/Hib and DTPa-IPV/Hib vaccines, in terms of antibody concentrations one-month post-primary immunization, prior to and one month after booster immunization.

Statistical analysis

Immunogenicity analysis

Immunogenicity analyses were performed for the primary and booster ATP immunogenicity cohort, comprising all evaluable participants (meeting all eligibility criteria and no elimination criteria), who complied with protocol-defined procedures/intervals with results available for primary or booster immunogenicity endpoint measures.

Confirmatory inferential analysis for the primary objective

The global type I error for each pair-wise comparison was adjusted to 1.25% using a Bonferroni adjustment to ensure that the overall type I error was below 2.5%, considering that the 2 IBU groups were compared to the control group (without ibuprofen).

The non-inferiority to the control group was further adjusted to account for endpoint multiplicity using the method by Lehman et al. (Lehman et al., 2005), leading to a nominal type I error D 1.25% - (7/10) D 0.875%. The statistical decrease in GMC was also adjusted to account for the 11 endpoints (10 serotypes and anti-protein D) using a Bonferroni adjustment, leading to a nominal type I error D 1.25%/11 D 0.11364%.

The study had no less than 92.1% power to detect a statistical difference for a true GMC decrease equal to 2-fold. To obtain a power of 92.7% using an adjusted one-sided a of 0.875%, a sample size of 180 participants for each primary IBU group was necessary. Anticipating that »14% of vaccinated participants would not be evaluable for the ATP cohort for immunogenicity, we planned to enroll 210 participants in each ibuprofen group.

Standardized asymptotic 98.25% CIs were computed using StatXact for the difference between groups in the percentage of participants with anti-pneumococcal antibody concentrations $\geq$0.2 mg/mL one-month post-dose 3 (NIBU minus IIBU, or NIBU minus DIBU). Non-inferiority was demonstrated for one of the 2 pair-wise group comparisons if the UL of the 2-sided 98.25% CI was below 10% for at least 7 of the 10 vaccine pneumococcal serotypes.

99.8% CIs for antibody GMC ratios (IIBU/NIBU and DIBU/NIBU), one-month post-dose 3, were computed for each of the 10 vaccine pneumococcal serotypes and protein D using a one-sided ANOVA test on the logarithm 10 transformation of the concentrations. A statistically significant difference in post-dose 3 antibody GMCs was established if the UL of the 2-sided 99.8% CI was <1 for at least one of the 10 vaccine pneumococcal serotypes or protein D.

Factorial analysis

The nine randomized booster IBU groups were designed to enable a factorial analysis in which 2 factors (factor A – ibuprofen administration at primary vaccination, factor B – ibuprofen administration at booster vaccination), and 3 levels for each factor (immediate, delayed or no ibuprofen administration), could be evaluated. Further details are provided in supplementary methods.

Exploratory analyses

The exploratory analyses for the IBU and PARA groups are detailed in the supplementary methods. Briefly, the exclusion of 0 from the 95% CI of difference between groups in percentage of participants with antibody concentrations above the threshold, and the exclusion of 1 from the 95% CI of antibody GMC ratios were used to highlight potential group differences.

For other comparisons, non-overlapping 95% CIs were used as indicator of potential differences.

Within-group assessments

Antibody GMCs with 95% CIs were tabulated for each group, at each timepoint with a blood sample result available, and seropositivity/seroprotection rates with exact 95% CIs were calculated for each appropriate serotype/antigen. Antibody GMC calculations were performed by taking the anti-log of the mean of the log concentration transformations. Antibody concentrations below the cut-off of the assay were given an arbitrary value of half the cut-off for the purpose of GMC calculation. The 95% CI for the mean of log-transformed concentration was first obtained, assuming that log-transformed values were normally distributed with unknown variance. The 95% CI for the GMCs was then obtained by exponential-transformation of the 95% CI for the mean of the log-transformed concentration.

Safety analysis

Safety analyses were performed for the TVC, comprising all children who received at least one primary vaccine dose (primary TVC) or the booster dose (booster TVC). Because more than 5% of vaccinated participants were excluded from the ATP safety cohort, a complementary analysis was performed based on this ATP cohort, which included participants who met all eligibility criteria and with no elimination criteria, who had received at least one vaccine dose and antipyretic (if applicable) according to their random assignment (primary ATP cohort for safety analysis) or who had received all primary vaccine doses with antipyretic (if applicable) plus the booster dose and antipyretic, if applicable (booster ATP cohort for safety analysis), in compliance with the protocol defined vaccine administration route and antipyretic dose.

Confirmatory inferential analysis

Standardized asymptotic 97.5% CIs for the difference between groups in percentage of participants with rectal temperature $\geq 38^{o}C$ within 4 d after at least one primary dose (NIBU minus IIBU, or NIBU minus DIBU) were computed using StatXact. This secondary confirmatory objective was assessable if the primary objective was reached and was demonstrated if the lower limit (LL) of the 97.5% CI around the difference was higher than 0%.

1.3.2.3 Results

Effect of ibuprofen on PHiD-CV immunogenicity

One-month post-primary vaccination, for each of the 10 vaccine serotypes, the percentage of children in the IBU groups with antibody concentrations $\geq$0.2 mg/mL was at least 98.7%, except for serotypes 6B and 23F (6B at least 84.0%; 23F at least 89.2% in each group). Non-inferiority in terms of the percentage of infants with antibody concentrations $\geq$0.2 mg/mL was demonstrated since the UL of the difference was <10% for 9 out of 10 serotypes for each comparison (IIBU vs NIBU and DIBU vs NIBU). The UL was >10% for serotypes 6B (IIBU vs NIBU: percentage difference 0.69; UL D 10.99%) and 23F (DIBU vs NIBU: percentage difference 2.73; UL D 11.04%). No statistically significant differences in antibody GMCs for vaccine pneumococcal serotypes or protein D were observed (Table 1.3).

Table 1.3. *Serotype-specific pneumococcal and protein D antibody responses with pairwise group comparisons for the ibuprofen groups, at one-month post-dose three (ATP cohort for immunogenicity)*

Proportion of children with antibody concentrations ≥ 0.2 μg/mL					
	% ≥ 0.2 μg/mL (95% CI)			Difference in % ≥ 0.2 μg/mL	
Serotype	IIBU N = 154	DIBU N = 158	NIBU N = 164	NIBU minus IIBU 98.25% CI (LL; UL)	NIBU minus DIBU 98.25% CI (LL; UL)
Vaccine serotypes					
1	100 (97.5; 100)	100 (97.6; 100)	99.4 (96.6; 100)	−0.62 (−4.52; 3.17)	−0.62 (−4.52; 2.91)
4	99.3 (96.2; 100)	100 (97.6; 100)	99.4 (96.5; 100)	0.06 (−3.94; 4.38)	−0.63 (−4.57; 2.91)
5	100 (97.5; 100)	100 (97.6; 100)	99.4 (96.5; 100)	−0.64 (−4.63; 3.19)	−0.64 (−4.63; 2.92)
6B	84.0 (77.0; 89.6)	87.1 (80.8; 91.9)	84.7 (78.1; 90.0)	0.69 (−9.40; **10.99**)	−2.38 (−12.02; 7.22)
7F	99.4 (96.4; 100)	100 (97.7; 100)	100 (97.8; 100)	0.65 (−2.70; 4.71)	0.00 (−3.34; 3.48)
9V	99.3 (96.2; 100)	100 (97.6; 100)	98.7 (95.5; 99.8)	−0.58 (−5.05; 3.82)	−1.27 (−5.66; 2.32)
14	100 (97.5; 100)	99.4 (96.4; 100)	99.4 (96.5; 100)	−0.65 (−4.68; 3.15)	0.00 (−4.08; 4.12)
18C	99.3 (96.2; 100)	99.4 (96.4; 100)	98.7 (95.5; 99.8)	−0.58 (−5.04; 3.85)	−0.62 (−5.08; 3.54)
19F	100 (97.5; 100)	98.7 (95.4; 99.8)	99.4 (96.5; 100)	−0.63 (−4.60; 3.14)	0.67 (−3.40; 5.20)
23F	91.9 (86.3; 95.7)	89.2 (83.3; 93.6)	92.0 (86.7; 95.7)	0.08 (−7.66; 8.10)	2.73 (−5.30; **11.04**)
Vaccine-related serotypes					
6A	44.2 (36.0; 52.6)	47.4 (39.2; 55.6)	43.3 (35.4; 51.4)	NA	NA
19A	53.1 (44.6; 61.4)	52.0 (43.7; 60.1)	40.1 (32.4; 48.2)	NA	NA

Antibody GMCs					
	Antibody GMC (95% CI)			Antibody GMC ratio	
Serotype	IIBU N = 154	DIBU N = 158	NIBU N = 164	IIBU / NIBU 99.8% CI (LL; UL)	DIBU / NIBU 99.8% CI (LL; UL)
Vaccine serotypes (μg/mL)					
1	1.82 (1.59; 2.09)	1.71 (1.49; 1.95)	1.90 (1.67; 2.17)	0.96 (0.71; 1.29)	0.90 (0.67; 1.21)
4	2.25 (1.97; 2.57)	2.21 (1.95; 2.51)	2.21 (1.96; 2.50)	1.02 (0.77; 1.35)	1.00 (0.76; 1.32)
5	2.93 (2.58; 3.33)	2.39 (2.13; 2.69)	2.77 (2.44; 3.15)	1.06 (0.80; 1.41)	0.86 (0.66; 1.14)
6B	0.67 (0.55; 0.81)	0.76 (0.63; 0.92)	0.60 (0.49; 0.72)	1.12 (0.72; 1.74)	1.28 (0.83; 1.97)
7F	2.87 (2.52; 3.27)	2.83 (2.52; 3.17)	2.77 (2.49; 3.09)	1.04 (0.79; 1.35)	1.02 (0.80; 1.31)
9V	2.10 (1.81; 2.42)	2.01 (1.79; 2.27)	2.18 (1.91; 2.50)	0.96 (0.70; 1.31)	0.92 (0.69; 1.22)
14	4.76 (4.10; 5.51)	4.52 (3.91; 5.21)	4.77 (4.08; 5.58)	1.00 (0.71; 1.40)	0.95 (0.68; 1.32)
18C	3.85 (3.23; 4.60)	3.80 (3.24; 4.46)	4.34 (3.65; 5.15)	0.89 (0.60; 1.31)	0.88 (0.60; 1.27)
19F	6.11 (5.26; 7.10)	5.04 (4.35; 5.86)	4.96 (4.22; 5.83)	1.23 (0.87; 1.75)	1.02 (0.72; 1.44)
23F	1.04 (0.86; 1.26)	0.92 (0.76; 1.11)	1.07 (0.91; 1.26)	0.97 (0.66; 1.44)	0.86 (0.58; 1.27)
Vaccine-related serotypes (μg/mL)					
6A	0.17 (0.14; 0.21)	0.18 (0.15; 0.23)	0.15 (0.12; 0.19)	NA	NA
19A	0.23 (0.18; 0.28)	0.20 (0.16; 0.25)	0.16 (0.13; 0.19)	NA	NA
Protein D (EL.U/mL)					
	1461.28 (1267.4; 1684.8)	1353.13 (1191.3; 1537.0)	1557.75 (1355.4; 1790.3)	0.94 (0.69; 1.28)	0.87 (0.64; 1.17)

Post-booster immune responses were in similar ranges in all groups; for each of the vaccine serotypes, percentages of children with antibody concentrations ≥0.2 mg/mL were 91.5–100%. An increase in antibody GMCs post-booster compared to post-primary vaccination was observed in all groups, for each vaccine serotype except serotype 14 in the DIBU-NIBU group and serotype 19F in the NPARA-IPARA group. Because very few participants per group had available results from the opsonophagocytic activity and poliomyelitis neutralization assays, these results could not be presented and interpreted.

For vaccine-related serotypes 6A and 19A, antibody concentrations ≥0.2 mg/mL were observed for ≥43.3% and ≥40.1% of children in each IBU group at one-month post-primary vaccination (Table 23), and for ≥80.4% and ≥78.0% children post booster, respectively.

Effect of paracetamol on PHiD-CV immunogenicity

Post-primary vaccination, the percentage of children with antibody concentrations ≥0.2 mg/mL generally tended to be lower in the immediate (IPARA) and delayed paracetamol (DPARA) groups than in the no-paracetamol (NPARA) group (however, the 95% CI of the differences included 0) and the highest difference in point estimates vs control NPARA was observed for serotype 6B (~8% for IPARA and ~14% for DPARA). Compared to the NPARA

group, antibody GMCs were lower for 6 of the PHiD-CV serotypes and protein D in the IPARA group, and for serotypes 1 and 6B in the DPARA group (Table 1.4).

Table 1.4. *Exploratory analysis: serotype-specific pneumococcal and protein D antibody responses with pairwise group comparisons for the paracetamol groups, one-month post-dose three (ATP cohort for immunogenicity)*

Proportion of children with antibody concentrations ≥ 0.2 μg/mL					
	% ≥ 0.2 μg/mL (95% CI)			Difference in % ≥ 0.2 μg/mL	
Serotype	IPARA N = 55	DPARA N = 55	NPARA N = 56	NPARA minus IPARA 95% CI (LL; UL)	NPARA minus DPARA 95% CI (LL; UL)
Vaccine serotypes					
1	96.3 (87.3; 99.5)	98.0 (89.6; 100)	100 (93.5; 100)	3.70 (−3.01; 12.59)	1.96 (−4.69; 10.37)
4	96.4 (87.5; 99.6)	100 (93.0; 100)	100 (93.6; 100)	3.64 (−2.96; 12.38)	0.00 (−6.48; 7.07)
5	100 (93.3; 100)	100 (92.9; 100)	100 (93.4; 100)	0.00 (−6.70; 6.82)	0.00 (−6.70; 7.20)
6B	79.2 (65.9; 89.2)	72.5 (58.3; 84.1)	87.3 (75.5; 94.7)	8.03 (−6.35; 22.68)	14.72 (−0.55; 30.18)
7F	100 (93.5; 100)	100 (93.5; 100)	100 (93.6; 100)	0.00 (−6.47; 6.58)	0.00 (−6.47; 6.58)
9V	100 (93.3; 100)	100 (92.9; 100)	98.1 (90.1; 100)	−1.85 (−9.83; 5.03)	−1.85 (−9.83; 5.41)
14	100 (93.3; 100)	100 (92.9; 100)	100 (93.3; 100)	0.00 (−6.82; 6.82)	0.00 (−6.82; 7.20)
18C	98.1 (89.9; 100)	100 (92.9; 100)	100 (93.4; 100)	1.89 (−4.88; 10.00)	0.00 (−6.70; 7.20)
19F	100 (93.3; 100)	100 (92.9; 100)	100 (93.4; 100)	0.00 (−6.70; 6.82)	0.00 (−6.70; 7.20)
23F	87.0 (75.1; 94.6)	81.1 (68.0; 90.6)	90.9 (80.0; 97.0)	3.87 (−8.61; 16.76)	9.78 (−3.55; 23.71)
Vaccine-related serotypes					
6A	35.8 (23.1; 50.2)	30.0 (17.9; 44.6)	49.1 (35.1; 63.2)	NA	NA
19A	41.5 (28.1; 55.9)	50.0 (35.5; 64.5)	56.6 (42.3; 70.2)	NA	NA

Antibody GMCs					
	Antibody GMC (95% CI)			Antibody GMC ratio	
Serotype	IPARA N = 55	DPARA N = 55	NPARA N = 56	IPARA / NPARA 95% CI (LL ; UL)	DPARA / NPARA 95% CI (LL ; UL)
Vaccine serotypes (μg/mL)					
1	1.32 (1.04; 1.67)	1.38 (1.09; 1.74)	1.95 (1.64; 2.32)	0.68 (0.51; **0.90**)	0.71 (0.53; **0.94**)
4	1.57 (1.21; 2.04)	1.95 (1.63; 2.32)	2.59 (2.07; 3.24)	0.61 (0.43; **0.85**)	0.75 (0.56; 1.00)
5	1.95 (1.53; 2.48)	2.36 (1.89; 2.94)	3.05 (2.53; 3.68)	0.64 (0.47; **0.86**)	0.77 (0.58; 1.03)
6B	0.49 (0.34; 0.69)	0.42 (0.28; 0.62)	0.72 (0.51; 1.02)	0.67 (0.41; 1.09)	0.58 (0.35; **0.97**)
7F	2.18 (1.75; 2.70)	2.45 (2.01; 2.99)	2.95 (2.37; 3.69)	0.74 (0.54; 1.00)	0.83 (0.62; 1.11)
9V	1.67 (1.30; 2.13)	1.82 (1.48; 2.23)	2.40 (1.87; 3.10)	0.69 (0.49; **0.98**)	0.76 (0.55; 1.05)
14	3.44 (2.55; 4.62)	4.12 (3.21; 5.29)	5.17 (4.20; 6.36)	0.66 (0.46; **0.95**)	0.80 (0.58; 1.10)
18C	3.08 (2.29; 4.15)	4.08 (3.15; 5.29)	4.96 (3.75; 6.55)	0.62 (0.42; **0.93**)	0.82 (0.57; 1.20)
19F	4.95 (3.74; 6.54)	5.20 (3.94; 6.85)	6.98 (5.48; 8.88)	0.71 (0.49; 1.02)	0.75 (0.52; 1.07)
23F	0.77 (0.54; 1.09)	0.74 (0.51; 1.08)	1.00 (0.73; 1.36)	0.77 (0.48; 1.22)	0.74 (0.46; 1.20)
Vaccine-related serotypes (μg/mL)					
6A	0.11 (0.08; 0.16)	0.12 (0.08; 0.17)	0.19 (0.13; 0.27)	NA	NA
19A	0.15 (0.11; 0.22)	0.17 (0.12; 0.25)	0.25 (0.17; 0.36)	NA	NA
Protein D (ELU/mL)					
	1109.64 (876.9; 1404.1)	1348.55 (1048.2; 1734.9)	1667.91 (1401.9; 1984.4)	0.67 (0.50; **0.89**)	0.81 (0.60; 1.09)

One-month post-booster vaccination, for each of the 10 vaccine serotypes, at least 91.7%, 93.2% and 97.9% of children in the IPARA-NPARA, DPARA-IPARA and NPARA-IPARA groups, respectively, had antibody concentrations ≥0.2 mg/mL. Post-booster antibody GMCs tended to be lower than in the control NIBU-NIBU group for all vaccine serotypes in the IPARA-NPARA group and the majority of serotypes in the DPARA-IPARA group, as well as for protein D in both groups. No major differences in antibody GMCs were observed when paracetamol was administered only during booster vaccination (NPARA-IPARA group).

For vaccine-related serotypes 6A and 19A, antibody concentrations ≥0.2 mg/mL were observed for ≥30.0% and ≥41.5% of children in each PARA group at one-month post-primary vaccination (Table 24), and for ≥83.0% and ≥77.3% children post booster.

Effect of ibuprofen on co-administered antigens

Effect of ibuprofen on co-administered antigens post-primary vaccination, a borderline significant difference in antibody GMCs was observed in the IIBU vs NIBU comparison for FHA (UL D 0.99), in the DIBU vs NIBU comparison for tetanus (UL D 1.00) and in the IIBU vs NIBU comparison for hepatitis B surface antigen (HBs) (UL D 1.01). Post booster, a difference in pertussis antibody GMCs was observed in the IIBU-DIBU (anti-pertussis toxoid [PT], anti-pertactin [PRN] and anti-FHA antibody GMCs) and IIBU-NIBU (anti-PT antibody GMCs) groups. Seroprotection and seropositivity rates were not affected.

Effect of paracetamol on co-administered antigens

Concerning the co-administered vaccine antigens for which the results were interpretable (diphtheria, tetanus, pertussis [PT, FHA, and PRN], HBs and *Haemophilus influenzae* type b polyribosylribitol phosphate [PRP]), antibody GMCs seemed to be reduced in the IPARA and DPARA groups and in the NPARA-IPARA group for some antigens; nevertheless, seroprotection/seropositivity rates remained high ($\geq$95.5%). In detail, the antibody GMCs tended to be lower for post-primary anti-PRP (ratio of 0.66) and anti-tetanus (ratio of 0.78) in the IPARA group and for post-primary anti-tetanus (0.81) in the DPARA group, as well as for post-booster anti-PT in the NPARA-IPARA and IPARANPARA groups compared to the control group.

Factorial design analysis

Comparison of antibody GMCs in the 9 booster groups that received ibuprofen did not indicate a combined effect (interaction) of prophylactic ibuprofen administration at primary and booster vaccination, and no individual effect of ibuprofen at primary vaccination or at booster on the PHiD-CV post booster immune response.

Safety results

The confirmatory analysis of the difference in fever incidences in the IIBU or DIBU groups compared to NIBU did not demonstrate any statistically significant reduction. Fever during primary vaccination was reported for 122 children (61.3%) in the NIBU group, compared to 121 (61.4%) in the IIBU group (difference: -0.11% [97.5% CI: -11.04; 10.82]) and 101 (51.3%) in the DIBU group (difference: 10.04% [97.5% CI: -1.15; 20.98]). Grade 3 fever was reported only in the NPARA and DIBUDIBU group (Figure 23).

Similar results were obtained in the complementary descriptive analysis on the ATP cohort: fever incidence in the NIBU group was 61.4%, vs 62.6% in the IIBU group (group difference: -1.16% [97.5% CI: -12.55; 10.39]) and 49.7% in the DIBU group (group difference: 11.72% [97.5% CI: 0.04; 23.13]).

Exploratory analyses indicated a trend for decrease in the rates of reported fever post-primary vaccination in the groups receiving immediate or delayed paracetamol (32.9% and 38.0% of participants, respectively) versus the control group (54.1% of participants) (Figure 1.11).

Other solicited local and general symptoms seemed to be reported in similar ranges across groups during primary and booster vaccinations.

Twenty-seven SAEs were reported for a total of 15 children from the TVC. All SAEs resolved and none were considered by the investigator to be causally related to vaccination. In addition, of the 35 children at the study site eliminated from the TVC, 4 children reported SAEs including one fatal SAE (DPARA group; craniocerebral injury 132 d post-dose 3); none of these SAEs were considered by the investigator to be causally related to vaccination.

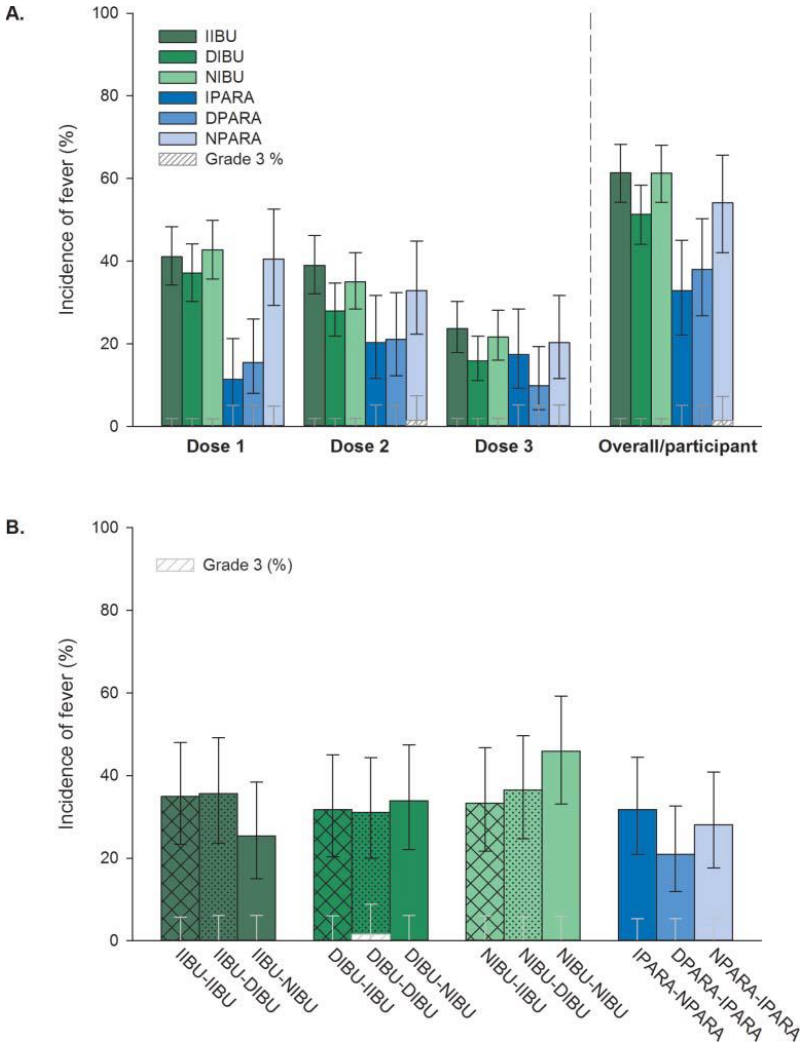

Figure 1.11. *Incidence of fever post-primary (A) and post-booster (B) vaccination (TVC). Footnote: Primary vaccination: PHiD-CV and DTPa-(HBV)-IPV/Hib at 3, 4, and 5 months of age, with the following prophylactic antipyretic regimen: IIBU, immediate ibuprofen; DIBU, delayed ibuprofen; NIBU, no ibuprofen; IPARA, immediate paracetamol; DPARA, delayed paracetamol; NPARA, no paracetamol. Booster vaccination: PHiD-CV and DTPa-HBV-IPV/Hib at 12–15 months of age, with the following prophylactic antipyretic regimen: at primary vaccination: immediate ibuprofen, and at booster: immediate (IIBU-IIBU), delayed (IIBU-DIBU) or no ibuprofen (IIBU-NIBU); at primary vaccination: delayed ibuprofen, and at booster: immediate (DIBU-IIBU), delayed (DIBU-DIBU) or no ibuprofen (DIBU-NIBU); at primary vaccination: no ibuprofen, and at booster: immediate (NIBU-IIBU), delayed (NIBU-DIBU) or no ibuprofen (NIBU-NIBU); immediate paracetamol at primary vaccination and no paracetamol at booster (IPARANPARA); delayed paracetamol at primary vaccination and immediate paracetamol at booster (DPARA-IPARA); no paracetamol at primary vaccination, and immediate paracetamol at booster (NPARA-IPARA). Fever: rectal temperature >38.0°C; Grade 3 fever: rectal temperature >40°C or axillary/oral/tympanic temperature >39.5°C; TVC, total vaccinated cohort. Error bars indicate 95% confidence intervals*

1.3.2.4 Discussion

This study found no clinically relevant impact of immediate or delayed prophylactic administration of ibuprofen during primary or booster vaccination on the immune response to PHiDCV.

A factorial design analysis indicated neither a combined effect (interaction), nor separate effects of prophylactic ibuprofen administration at primary and booster vaccination on the post-booster immune response to PHiD-CV.

For the primary objective, a threshold of 0.2 mg/mL antipneumococcal antibody concentrations were used (equivalent to 0.35 mg/mL measured by the non-22F ELISA of the WHO reference laboratory). For most vaccine serotypes, almost all children in each study group (≥96.3%) reached this antibody concentration at 1-month post-primary vaccination, except for serotypes 6B (≥72.5%) and 23F (≥81.1%).

Prophylactic administration of paracetamol during primary series showed a trend for reduced post-primary antipneumococcal antibody GMCs when given immediately after vaccine administration (for the majority of vaccine serotypes) or when given in a delayed manner (for some serotypes). The proportion of children with post-primary antibody concentrations ≥0.2 mg/mL for PHiD-CV serotypes was not impacted except for serotypes 6B and 23F, thus the clinical relevance remains unknown. Observations related to immediate administration of paracetamol are in line with previous findings (Prymula et al., 2009).

When paracetamol was given immediately only at the booster dose, corresponding to the age with highest risk of febrile seizures (Leung et al., 2007), we observed no effect on immune response to PHiD-CV while fever was reduced. This suggests that paracetamol can be used for prophylaxis of febrile reactions at booster dose. In contrast, the post-booster immune response to PHiD-CV appeared to be impacted when paracetamol was administered either immediately at primary vaccination but not post-booster, or in a delayed manner at primary vaccination and immediately at booster dose.

Descriptive comparisons of the response to co-administered antigens showed a trend for lower anti-FHA post-primary antibody GMCs in the group with immediate prophylactic ibuprofen administration. No major differences were observed in post-booster antibody GMCs in the ibuprofen groups except for pertussis antigens (PT, FHA, and PRN in the IIBU-DIBU group, and PT in the IIBU-NIBU group vs the NIBU-NIBU group). However, seroprotection and seropositivity rates for the co-administered antigens one month after primary vaccination and one month after booster dose were not affected by ibuprofen prophylactic administration, suggesting no clinically relevant impact.

Our findings differ from in vitro assessments, in which ibuprofen was found to have a dose-dependent effect on antibody production in human peripheral blood mononuclear cells and in purified B cells, with the major influence on antibody production observed when ibuprofen was administered early (day 2 and 3) to the culture (Bancos et al., 2009).

Immediate or delayed prophylactic administration of paracetamol during primary vaccination did not reveal major differences in seroprotection and seropositivity rates or in antibody GMCs of co-administered antigens. When no antipyretics were given at booster dose, a trend for decreased post-booster antibody GMCs was observed for the majority of co-administered vaccine antigens, with no impact on seroprotection and seropositivity rates, indicating no or limited clinical relevance.

A previous study assessing the effect of prophylactic immediate administration of paracetamol at the time of vaccination with PHiD-CV and DTPa-HBV-IPV/Hib found generally lower antibody GMCs for antibodies against diphtheria, tetanus, PRN and PRP antigens after primary vaccination. After booster vaccination, this tendency was only observed for antibodies against tetanus. Moreover, this study showed that the seropositivity or seroprotection rates were not impacted and remained in line with previous experiences with DTPa based or pneumococcal vaccines with the exception of serotype 6B after primary vaccination (Prymula et al., 2009).

Our results correspond with findings recently reported for PCV13, in which immediate prophylactic paracetamol administration seemed to interfere with infant series immune

response to PCV13, while immediate prophylactic administration of ibuprofen did not interfere with pneumococcal responses but may reduce responses to pertussis FHA and tetanus antigens. These effects were especially apparent when antipyretic prophylaxis was administered at the time of primary vaccination, while no differences were observed after the booster dose (Wysocki et al., 2014).

In contrast, another recent study did not show any apparent clinically relevant impact on immune responses to 4CmenB and to the concomitantly administered routine vaccines (DTPa-HBV-IPV/Hib and PCV7) when paracetamol was administered prophylactically to prevent post-immunization fever in children (CHMP, 2005). The different outcomes might be related to differences in the study design, including vaccination schedule, age of children at the time of vaccination, route of administration of the antipyretic, and different vaccines used for immunization.

The diverging effects of prophylactic paracetamol and ibuprofen administration on vaccine immunogenicity could be explained by differences in the antipyretic's mode of action and pharmacokinetics in infants and children (Tucci et al., 2009). Ibuprofen nonselective inhibits both cyclooxygenase (COX)-1 and COX-2, while paracetamol is thought to selectively block COX-3 in brain and spinal cord, (Jozwiak-Bebenista and Nowak, 2014) although this latter mechanism of action has been disputed (Kis et al., 2005). While ibuprofen and other non-steroidal anti-inflammatory drugs inhibit cyclooxygenase through competing with arachidonic acid for the active site of the enzyme, paracetamol acts by reducing ferryl protoporphyrin IX at the peroxidase site of the cyclooxygenase enzyme (Jozwiak-Bebenista and Nowak, 2014). Furthermore, it was hypothesized that non-steroidal anti-inflammatory drugs lead to lower levels of produced antibodies due to a decreased expression of B lymphocyte-induced maturation protein 1, which in turn leads to less terminal differentiation of proliferating B-cells into plasma cells (Purssell, 2014). Unlike ibuprofen, paracetamol inhibits myeloperoxidase-catalyzed oxidant production and, by decreasing hypochlorite production at the inflammation site, could impair immunogenicity by decreasing antigen processing and cross priming (Prokopowicz et al., 2010). Both ibuprofen and paracetamol can rapidly cross the blood-brain barrier, and the latter may act in a synergistic manner on the opioidergic and serotonergic systems (Parepally et al., 2006; Smith, 2009).

The specific impact of paracetamol on vaccine response could also be explained by the generation of an active metabolite which inhibits the uptake of anandamine and increases its concentration in the brain and blood (Ottani et al., 2006; Sinning et al., 2008). Anandamine is a powerful modulator of immune cell functions, especially of primary T cells (Cencioni et al., 2010). Paracetamol also decreases protein kinase C epsilon translocation in cultured sensory neurons, leading to a decrease of monocyte and macrophage function, as well as Th1 responses (Aksoy et al., 2004; Vellani et al., 2013). Although no clinical confirmation was found, it has been previously proposed that the impact of paracetamol administration on immune responses to primary vaccination with PHiD-CV may be due to interference with early interactions between dendritic, B and T-cells. Because this impact is not as evident post-booster, it has been suggested that paracetamol has a higher effect on plasma-cell differentiation than memory-cell differentiation of B-cells. Regardless of the mechanism of action, the overall effect of paracetamol on immunogenicity probably depends on multiple target sites.

For incidence of fever after primary vaccination, results from the confirmatory analysis showed no impact of prophylactic immediate or delayed use of ibuprofen after vaccination.

Point estimates of differences in febrile reaction reporting rates between ibuprofen and no-ibuprofen groups during the primary series were close to 0 (immediate manner, reporting rate around 60%) or 10% (delayed manner, reporting rates 61.3 and 51.3%, respectively). Post-booster, no differences in reporting rates of fever between prophylactic ibuprofen groups and the no-ibuprofen group were observed. Yet, immediate or delayed paracetamol administration

tended to decrease fever incidence (32.9% and 38.0% of participants, respectively versus 54.1% of participants from the NPARA group). In another study, prophylactic paracetamol administration effectively prevented fever and other reactions in children vaccinated with PCV7 co-administered with hexavalent vaccine, mainly during the infant series; however, less impact in fever prevention was observed after the booster dose (Rose et al., 2013). This might be explained by an over-estimate of the fever rate after primary vaccination or by a weak anti-inflammatory effect of paracetamol on the more frequent local reactions after booster dose (Rose et al., 2013).

Another article reported that the administration of ibuprofen did not induce differences in fever incidence after DTwP or DTaP vaccination, when compared to placebo administration (Manley and Taddio, 2007).

Generally, ibuprofen is known to be an antipyretic at least as efficacious as paracetamol (Autret et al., 1997; Perrott et al., 2004; Sullivan and Farrar, 2010). However, limited data are available regarding its prophylactic administration (Wysocki et al., 2014).

Of note, ibuprofen and paracetamol use and labels in the assessed age group differ across countries, which complicated the study set-up and the choice of country in which to perform it. Moreover, ibuprofen is only licensed for use in children from the age of 3 months in Romania or even from 6 months in other European countries, while the first dose of PHiD-CV can be given as early as from the age of 6 weeks. The choice of antipyretic use during pediatric immunization is therefore expected to vary from one country to another, regardless of how the nature of the prophylactic drug impacts the immune response elicited by vaccination.

The study had several strengths: the factorial design addressed all possible combinations of ibuprofen use, a parallel assessment of paracetamol in the same study was performed, and good compliance with the complex study procedures was observed.

A limitation of the current study is that very few results were available from the opsonophagocytic activity and poliomyelitis neutralization assay due to insufficient sera volumes; thus, these results could not be interpreted. In addition, no adjustment for multiplicity was performed for the exploratory group comparisons so the results based on these analyses should be interpreted with caution.

1.3.2.5 Conclusion

Because prophylactic administration of paracetamol at primary vaccination tends to impact post-primary and post booster antibody GMCs while ibuprofen was shown not to affect immunogenicity, ibuprofen could be considered as the antipyretic of choice for prophylaxis during primary vaccination courses. However, ibuprofen prophylaxis appeared to have no or only limited effect on fever rates. Thus, prophylactic use of ibuprofen and its benefit/risk ratio should be cautiously considered when deciding in choice of prophylactic antipyretic.

Paracetamol may be more suitable for prevention of febrile reactions after booster vaccination in the second year of life, as it appeared to have no detrimental effect on immunogenicity when administered at booster dose only. However, its use around primary vaccination and benefit/risk ratio should be assessed individually.

Finally, a more conservative approach would be to not provide prophylaxis at all, except when the individual patient would require it. Results of our study may help in guiding general practitioners, pediatricians, and policy makers in their recommendation and choice of antipyretics for prophylaxis of post-vaccination febrile reactions in children.

1.4 Parents' education in preventing accidental toxic ingestion

1.4.1 Introduction

Corrosive esophagitis is one of the most frequent forms of esophagitis in children and is found to a larger extent in the rural environment, where the supervision of small children is faulty. The ingestion of corrosive substances in children is usually accidental (Baskin et al., 2004). Voluntary ingestion is found mostly in suicidal adolescents. In the United States, studies reported approximately 5000 – 15000 new cases a year (Anderson et al., 1999; Arévalo-Silva et al., 2006).

The ingestion of corrosive substances can cause lesions in the lips, oral cavity, pharynx and upper airways. The respiratory system is mostly affected especially due to its exposure to corrosive vapors. In children, 18 to 46% of all ingestions of corrosive substances are associated to esophageal lesions (Lupa et al., 2009).

The gravity of lesions is determined by certain factors: the nature of the substance (acid, base or oxidant), pH, ingested amount, form of presentation (liquid corrosives cause more extended lesions than solid ones), duration of the contact with the corrosive substance, stomach repletion or depletion, the time of reaching the hospital, the time of the original endoscopic examination, the time of the decision on surgical or instrumental correction, and the time of the initiation of pharmaceutical treatment (Atabek et al., 2007).

Corrosive substances cause tissue lesions by means of chemical reactions. These substances can be acid or alkali. The most important lesions are produced by acids with a pH less than 3 or by bases with a pH greater than 11 (Salzman and O'Malley, 2007).

Acids, by denaturing superficial tissue proteins, cause coagulation necrosis generating scar tissue. Strong acids have immediate, almost instantaneous effect. Scar tissues have a protective role, which limits the penetration of deep layers. The stomach and lower esophagus are affected most frequently. The denudation of the scar tissue exposes to a major risk of perforation. Pyloric stenosis often occurs. The acids in commercial products are represented by the hydrochloric acid, sulfuric acid and silver nitrate (Espinola and Amedee, 1993). They are found in the bathroom, pool and rust cleaning products (Cummings et al., 2005).

Base ingestion causes liquefactive necrosis with quick and severe tissue penetration. Lesions are generated by fat saponification, denaturation of proteins and vascular thrombosis (Espinola and Amedee, 1993; Cummings et al., 2005). Even low concentrations can produce extended prejudice. A study conducted in Brazil reports the fact that a solution with 1.83% concentration can cause esophageal necrosis within an hour (Mattos et al., 2006). The oropharynx and the esophagus are affected most often. Severe gastric damage is not rare. The complications of base ingestion are represented by esophageal strictures and fistulas, and posterior cricoarytenoid muscle fibrosis. There is a risk of malignant transformation of fibrous lesion. Basic substances are represented by sodium hydroxide (caustic soda), potassium hydroxide, sodium carbonate (washing soda) and potassium carbonate.

From the anatomical-pathological point of view, the lesions caused by corrosive substances in the digestive tract evolve to 3 stages: acute – immediately after the ingestion (day 1 – 4) generating erythema, edema and intense cellular necrosis; latent (day 5 – 15) generating cellular edema, ulcerations and granulation tissue; chronic (after day 15) generating fibrosis and scars with stenosis.

The maximum risk of esophageal perforation is present during the first 3 days from ingestion and between days 6 to 10.

Because the morbidity of caustic esophageal burn is still a problem, the education of parents is necessary. Attention to the storage conditions and secured bottle cap seems to be the easiest and simplest way to prevent corrosive substance ingestion.

Lead is a soft, pliable, bluish-grey metal resistant to corrosion, that exists in both organic and inorganic forms (Dapul and Laraque, 2014). This metal does not conduct electricity and it owns antiradiation properties (Dapul and Laraque, 2014). Lead poisoning in children is an important health problem, accounting for 0.6% of the global burden of the disease according to the World Health Organization (leadguidance, 2016). According to the National Health and Nutrition Examination Survey data, from 2007 to 2010, approximately 535,000 children aged 1 to 5 years, meaning 2.6%, presented blood lead level above 5mg/dL (Blood lead levels in children aged 1–5 Years—United States, 1999–2010). Even though lead is everywhere, the industrialized areas carry a higher risk for lead exposure (Dapul and Laraque, 2014). The ways of contamination include ingestion, inhalation, prenatal exposure, and dermal exposure, but the most important and frequent ones are ingestion and inhalation (Dapul and Laraque, 2014). The half-life of lead is between 30 and 40 days in men, while in children and pregnant women it can be longer (Dapul and Laraque, 2014). It binds to the sulfhydryl group of proteins leading to toxicity for multiple enzyme systems (Dapul and Laraque, 2014). The clinical presentation of lead poisoning involves nervous, hematologic, and renal systems impairment, but it can also lead to gastrointestinal disorders (anorexia, vomiting, constipation, abdominal pain), hypertension, and fertility impairment (Dapul and Laraque, 2014). Neurological symptoms include ataxia, stupor, coma, convulsions, hyperirritability, reduced IQ, shortened attention span, increased antisocial behavior, reduced educational attainment, and even death (Dapul and Laraque, 2014). Impairment of the hematological system may involve either disruption of heme synthesis or hemolysis, leading to anemia with its specific clinical signs, like weakness and fatigue (Dapul and Laraque, 2014). The effects of lead on the renal system consist in proximal tubular function impairment leading to aminoaciduria, glycosuria, and hyperphosphaturia (Loghman-Adham, 1998), interstitial nephritis in chronic exposure, and also impairment of calcium metabolism by interfering with activation of vitamin D 1,2- dihydroxy cholecalciferol (Dapul and Laraque, 2014). The diagnosis is established on the blood lead level, higher than 40mg/dL for occupational and 30mg/dL for nonoccupational exposure. Primary and secondary preventions should be the first steps in the management of lead poisoning as public health problem (Dapul and Laraque, 2014). If a patient is found with high blood lead level, the test must be repeated before considering any therapy. Chelating agents are recommended only if the level is above 45mg/dL, and the type will be chosen according to the blood level and symptoms (Dapul and Laraque, 2014). The available agents nowadays include: 2,3 dimercaptosuccinic acid (DMSA), dimercaprol, ethylene diamine tetra-acetic acid (CaNa2EDTA), D-penicillamine. In certain cases, the management can also include supportive therapy, like airway protection in acute encephalopathy or antiepileptic drugs in case of seizures (Dapul and Laraque, 2014).

We present a case report of lead poisoning in a 16-year-old girl with the aim of highlighting the difficulty in diagnosing this condition, and the fact that even though occupational exposure is the main cause of lead poisoning in adults by inhalation, it can also be present in children in certain circumstances, as in our case. The informed consent was given by the patient's father (legal guardian) for publication of this case report.

Personal contribution – published paper:

1. **Lupu VV**, Ignat A, Paduraru G, Ciubara A, Ioniuc I, Ciubara AB, Gheonea C, Burlea M. The Study of Effects Regarding Ingestion of Corrosive Substances in Children. *Rev chim*, 2016, 67(12): 2501-2503. **ISI IF 1,232**
2. Marginean CO, Melit LE, Moldovan H, **Lupu VV**, Marginean MO. Lead poisoning in a 16-year-old girl: a case report and a review of the literature (CARE compliant). *Medicine*, 2016,95(38):1-4 **ISI IF 1,804**

The aim of this study was to underline the importance to prevent the ingestion of corrosive substances in children because the morbidity of caustic esophageal burn is still a problem.

Also, we present a case report of lead poisoning in a 16-year-old girl with the aim of highlighting the difficulty in diagnosing this condition, and the fact that even though occupational exposure is the main cause of lead poisoning in adults by inhalation, it can also be present in children in certain circumstances, as in our case.

1.4.2 Material and methods

- ***Effects regarding ingestion of corrosive substances in children***

We conducted a retrospective study on a batch of 115 children of both genders, coming both from the rural and from the urban environment, aged 1 to 18 years, admitted in the Vth Pediatrics – Gastroenterology Clinic of "St. Mary" Children Emergency Hospital, Iaşi, Romania, for ingestion of corrosive substances.

In case of ingestion of corrosive substances, the upper gastrointestinal endoscopy (UGIE) is performed as early as possible (the first 48 hours, preferably during the first 12 hours), immediately after the patient is stabilized. This allows the exact mapping of the lesions and the exact determination of the esophagitis degree (Table 1.4).

Table 1.4. *Endoscopic classification of corrosive esophageal lesions modified according to Zargar et al. (Zargar et al., 1991)*

Degree 0	Normal endoscopic image
Degree I	Erythema and edema limited to the mucous membrane [+/- small non-bleeding erosions]
Degree II a	Friability, erosions, superficial ulcerations, whitish membranous exudate
Degree II b	Deep or circumferential ulcerating lesions
Degree III a	Deep hemorrhagic ulcerations, small necrosis areas
Degree III b	Extensive necrosis
Degree IV	Perforation

UGIE determined the reduction of mortality by approximately 50% during the past 25 years. It is the most efficient method of monitoring the evolution. It plays an important role in prognostic orientation and in the dilative or protein therapy of stenosis. It plays an essential role in establishing the type of medical treatment and in the indication of surgical treatment.

The treatment of esophageal stenosis – complication of corrosive esophageal lesions – consists in successive esophageal dilations using Savary bougies (Figure 1.12) or balloons (Siersema and de Wijkerslooth, 2009).

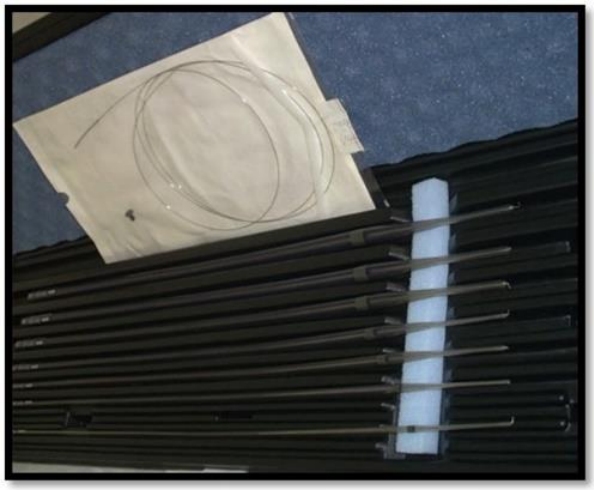

Figure 1.12. *Savary bougies*

The equipment necessary for esophageal dilations is made of dilators, guiding wire and an endoscope. The ideal dilator must easily pass through the oropharynx and the stenotic area, allow the physician to feel the stenosis, require minimum passages through the pharynx, have a radio-opaque marker, be usable in case of hiatal hernia or an operated stomach, pass on a guiding wire with imperfections, and be cost-efficient. The dilation technique is similar for all types of dilators. The basic principle consists in introducing through the stenosis dilators of increasing sizes. The dilation with Savary bougies is performed in left lateral decubitus. The guiding wire is passed through the biopsy channel under direct vision into the gastric antrum. If the stricture area cannot be surpassed with the endoscope above it, the guiding wire is introduced through the biopsy channel and through the stenosis and is placed under radiologic control into the stomach (the diaphragm is used as a marker). After placing the guiding wire, the endoscope is gradually retracted and simultaneously the guiding wire is introduced a few centimeters further through the endoscope. The proper dilation procedure begins by introducing the smallest dilator and continuing until resistance is felt. The Savary probes have a radio-opaque marker and ease radiologic monitoring of the correct introduction in special cases.

In moderate stenosis, progressive endoscopic dilations were applied by means of Savary bougies (Figure 1.13A, 1.13B, 1.13C, 1.13D).

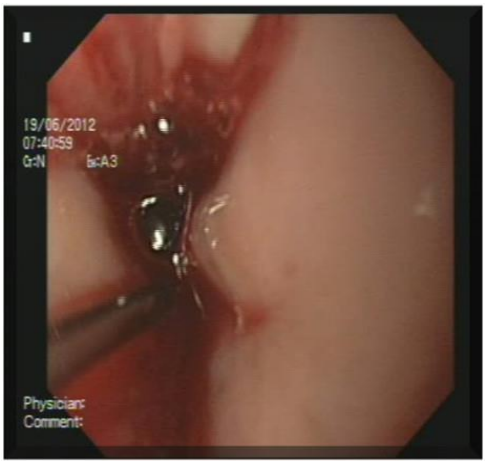

Figure 1.13A

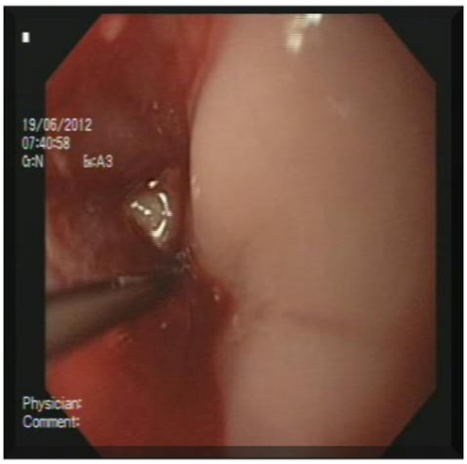

Figure 1.13B

Figure 1.13C

Figure 1.13D

Figure 1.13 A, B, C, D. *Dilations with Savary bougies*

- ***Lead poisoning in a 16-year-old girl***

We present the case of a 16-year-old girl, admitted to our clinic with severe abdominal pain, loss of appetite, nausea, and vomiting. The anamnesis revealed that the girl comes from a family of potters, and that she also participated in the process of pottery, her father being diagnosed with lead poisoning 2 years before. The patient's personal history underlined that approximately 1 year ago she presented with severe abdominal pain, being diagnosed with acute appendicitis and she underwent appendectomy, but the pain persisted, thus due to family history of lead poisoning, the suspicion of saturnine colic rose, and she was diagnosed with lead poisoning (urinary lead: 219mg/L), but she received only symptomatic treatment. Approximately 3 weeks before admission to our clinic, she was admitted to the regional hospital with another episode of saturnine colic (blood lead: 113.2mg/dL), and chelation therapy with EDTA (4 days before the admission in our clinic) was initiated, with a dose of 2 tablets daily, one in the morning and one in the evening associated with calcium supplements.

1.4.3 Results and discussions

- ***Effects regarding ingestion of corrosive substances in children***

All children with post ingestion esophagitis followed corticoid therapy for one month and antibiotherapy. They were evaluated by UGIE at 1 month and at 6 months to notice the esophagitis' evolution and eventually the esophageal stenosis.

The ingestion of corrosive substances was more frequent in small ages (1-4 years old) 94 cases (81.74%).

Regarding the esophagitis degree, according to Zargar classification, most frequent was: type I esophagitis 57 children (49.56%), type II esophagitis – 33 children (28.69%), type III esophagitis – 25 children (21.73%).

Out of the 115 cases of corrosive esophagitis, 46 cases complicated with esophageal stricture.

Out of the 46 cases of esophageal stenosis, 4 cases underwent surgery (in 3 cases a digestive tract graft was performed, and in 1 other case a left colon graft was performed).

The distribution of children with esophageal strictures was: small children (1 – 3 years) – 33 children (71.74%), in big children (4 – 13 years) – 9 children (19.57%), and in adolescents (14 – 18 years) – 4 children (8.69%). Their distribution according to gender showed a slight preponderance of females: 25 (54.35%) girls and 21 (45.65%) boys. As regards their environment of origin, the rural environment prevailed: 71.74% cases and 28.26% cases from the urban environment.

The polymorphic clinical picture depends on the lesions caused by the ingestion of corrosive substances, as well as on the individual intrinsic activity and is represented by pain in the digestive segment in question (oropharyngeal, retrosternal, epigastric), of variable intensity, depending on the depth of the lesions, heartburn, dysphagia, sialorrhea, dysphonia, nausea, vomiting, hematemesis and melena. Dyspnea appears due to laryngeal edema or pulmonary aspiration, and stridor appears due to glottal edema. Sometimes, these require tracheotomy and are usually associated to extended esophageal lesions (Haller et al., 1971). Anxiety or psychomotor agitation can also appear.

General status varies depending on the gravity of lesions. An examination of the oral cavity can reveal hyperemia, edema and ulcerations. Fever can appear in medium and severe forms and is a gravity indicator. Abdominal muscle contraction can be present in gastric perforations (Espinola and Amedee, 1993).

The relation between symptoms and the gravity of lesions is uncertain (Zargar et al., 1992). In specialized literature, the incidence of coexisting gastric lesions is reported as being between 20% and 62% and varies from simple hyperemia to diffuse transmural necrosis

erosions (Chaudhary et al., 1996; Ceylan et al., 2011). Immediate or delayed gastric perforation is associated to high mortality and is much more rarely reported in children than in adults (Turner and Robinson, 2005).

Biochemical changes following the ingestion of corrosive substances have not been studied in detail in the specialized literature. Leukocytosis (>20,000/ mm3), the presence of the inflammatory syndrome and esophageal ulcer have been associated to a high mortality rate in adults (Rigo et al., 2008). Arterial pH less than 7.22 or an excess of bases lower than -12 were considered an indicator for severe esophageal lesions associated to emergency surgery (Cheng and Kao, 2003).

Simple abdominal X-ray can show the presence of radiopaque pills, gastric dilation, ileus or pneumoperitoneum.

A computed tomography scan is the most sensitive method for the early detection of perforation.

The treatment immediately following the ingestion of corrosive substances implies respiratory, cardiovascular, renal, electrolyte, and acid-basic monitoring. No food should be administrated for 12 hours. Painkillers and tranquilizers can be used. There are contradictory studies regarding the administration of corticoid therapy. Antibiotic therapy is used as prophylaxis. Anti-secretory drugs can also be administrated: proton-pump inhibitors.

Progressive endoscopic dilations by means of Savary bougies were performed in 42 cases of moderate esophageal strictures and 4 cases underwent surgery.

Complications of esophageal stenosis dilations are rare. No post-dilation discomfort should be neglected. Perforation is the most severe complication. Generally, it occurs at a level close to the stenosis, in the case of the balloon method. Post-dilation hemorrhage, aspiration pneumonia or bacteremia can also emerge. The rate of perforations after the dilation of corrosive stenosis varies between 0.4% and 32%, decreasing to 17.6% - 4.5% as the experience of the personnel is higher (Contini et al., 2011). We had no case with perforation.

When esophageal dilations are not possible or an appropriate long-term esophageal caliber cannot be ensured, surgical treatment is recommended. It is also recommended in digestive tract perforations. Mortality and morbidity are low when the surgeon is experienced (Javed et al., 2011).

Anyway, the main aim of the therapy is to manage the early complications and to prevent stricture formation. It has been observed that along with supportive therapy, leaving esophagus to rest provided success for preventing the stricture development and the other complications (Gün et al., 2007).

- ***Lead poisoning in a 16-year-old girl***

Clinical findings

The clinical examination performed at the time of admission revealed the following pathological elements: influenced general status, ailing face, jaundice of the sclera, blue pigmentation of the nails, painful abdomen at palpation, and weight: 45kg.

Diagnostic focus and assessment

The laboratory test performed upon admission revealed hypochromic anemia (hemoglobin (Hb): 10.9 g/dl, hematocrit (Htc): 31.6%, medium cellular volume (MCV): 77.6 fL), increased level of liver transaminases (alanine-aminotransferase (ALAT): 158.9 U/L, aspartate-aminotransferase (ASAT): 63U/L, gamma-glutamyl-transferase (GGT): 128U/L), conjugated hyperbilirubinemia (direct bilirubin (DBi): 1.432 mg/dL), hyponatremia (Na: 132mmol/L), and hypopotassemia (K: 2.85mmol/L). The systolic arterial pressure was 156 mm Hg, and the diastolic was 96 mm Hg. The blood lead level was 66.28mg/dL, the urinary one was 419.7mg/L (normal <50mg/L) and the value of delta-aminolevulinic acid was 7.66mg/L (normal <4.5 mg/L).

We also performed abdominal ultrasound which revealed a disappearance of the delimitation between the cortical and medullar parts in both kidneys. We requested consultation from an occupational healthcare specialist, who recommended the continuation of chelation therapy with EDTA, increasing the dose at 4tablets/day. We also required a neurological consultation, and the specialist established the diagnosis of behavioral disorders with depressive elements, and recommended psychotherapy.

Based on all these clinical and laboratory findings, we established the diagnosis of lead poisoning.

Therapeutic focus and assessment

We initiated an intense i.v. hydration in order to favor lead elimination, approximately 3 liters per 24hours initially, and we decreased progressively the quantity once she ceased to vomit, and she was able to consume liquids. We associated diuretics, initially furosemide by vein, but the values of the arterial pressure persisted above the upper limit, therefore we were forced to introduce also an angiotensin-converting enzyme inhibitor, with the remission of arterial hypertension. Regarding the liver function, we administered amino acids intravenously, associated with liver protectors by mouth. We also administered vitamins of the B complex in order to improve the neurological impairment.

The evolution was slightly favorable, in the first 3 days after the admission, the patient continued to present severe abdominal pain, vomiting, and she also complained of pain in the lumbar area. All the laboratory parameters presented normalization of the values after approximately 10 days of treatment. On the 6^{th} day of admission, we ceased the chelation therapy with EDTA.

We also repeated the blood and urinary lead levels. The blood level was 45.57mg/dL, and the urinary one was 836.4mg/L before discharging the patient. The abdominal ultrasound reevaluationrevealed no pathological modifications.

Follow-up and outcome

After 14 days of admission, the patient was discharged without any complaints, and we recommended no further exposure to lead, avoiding the contact and the working in the pottery process.

The long-term outcome of this case depends on further exposure to this heavy metal. Nevertheless, we intend to repeat the blood lead levels after 12 and 24 months, assessing also the renal (urea, creatinine, urinary exam) and hepatic functions (ASAT, ALAT, GGT, bilirubin).

Lead poisoning is a severe condition with potential multiorgan damage and even death if lead is present in large amounts in the blood, representing a major health problem (Dapul and Laraque, 2014). In children, there is no safe lead level, although according to the World Health Organization, this problem represents approximately 0.6% of the global burden of disease (leadguidance, 2016). The 2 main ways of lead poisoning are through ingestion and inhalation. The former is more common in children due to their tendency of chewing everything, while the latter is more frequent in occupationally exposed adults (Dapul and Laraque, 2014). In the case presented here, lead entered the blood by inhalation as occupational risk because our teenager patient had helped her parents in the pottery process.

Other occupations with an increased risk for lead toxicity can be battery manufacturing and recycling plants, demolition, remodeling, and renovation projects, rubber and plastic industries, ammunition and manufacturing, automotive/ radiator repair, lead soldering and welding, painting, plumbing, and so on (Dapul and Laraque, 2014).

There are certain storages of lead in the organism, such as bones, teeth, hair, and nails, where it is bound tightly and it does not seem to be harmful since it is not available to other tissues (Rubin et al., 2008).

However, in children, only 70% of the absorbed lead will end up in the bones, in comparison with adults in whom 94% will deposit there, fact that can be responsible for the more expressed clinical effects of this condition in small ages (Barbosa et al., 2005). According to Lamas and collaborators, it seems that the deposit of active metals, like cadmium and lead, represents a major risk factor for cardiovascular disease (Lamas et al., 2016). Therefore, chelation therapy with edetate disodium can provide important benefits for those with atherosclerotic cardiovascular disorders (Lamas et al., 2016). Lead is well known as a risk factor for arterial hypertension (Lamas et al., 2016). The case we presented above also had increased values of arterial pressure. It seems that this metal is also incriminated for reducing the bioavailability of nitric oxide, and therefore promoting oxidative stress and inflammation (Lamas et al., 2016; Vaziri and Khan, 2007). A recent study assessed the relationship between blood lead levels and childhood asthma, 2 conditions frequently encountered in small children, both related to environmental factors. The study concluded that even though blood lead levels are not significantly associated with asthma diagnosis, the elevated blood levels of this metal lead to a more severe form of asthma in children, being related to eosinophilia and elevated immunoglobulin E levels (Mohammed et al., 2015). The toxicity of blood lead levels on the nervous system is a major concern for children's normal development. According to the data of the Agency for Toxic Substances and Disease Registry (ATSDR), blood lead level in children is associated with encephalopathy (ATSDR-lead, 2016). Even though the results vary from study to study, it seems that a blood lead level of 70 to 80mg/dL or greater represents a serious risk (ATSDR-lead, 2016). The neurological sequelae of lead poisoning include reduced IQ, shortened attention span, increased antisocial behavior, and reduced educational attainment (Dapul and Laraque, 2014). It was proved that an average increase of blood lead level of 10mg/dL in children will lead to an IQ decline between 1.9 to 3.2 points (Grosse et al., 2002). Other sequelae of chronic lead exposure consist in interstitial nephritis, reduction of sperm concentration, total sperm counts, and total sperm motility (Dapul and Laraque, 2014). On a study performed on 769 American adolescents, Fadrowski found that decreased kidney function is positively correlated to a blood lead level less than 10mg/dL, therefore the US government recommends repeated blood lead level testing at 12 and 24 months (Fadrowski et al., 2010). We also intend to repeat the blood lead level in our patient at 12 and 24 months. A study performed in developing countries showed that in many Latin American countries, lead-glazed ceramics represent a major source of exposure for those who live there (Kianoush et al., 2015). Even though the most common cause of lead poisoning in adults is represented by occupational exposure (Staudinger and Roth, 1998; Hettmansnerger and Mycyk, 2002), in our case we detected elevated blood lead levels in a teenage girl after occupational exposure because the patient supported the family pottery business.

The pottery-making process involves ceramic glazes and decorative paints, which both contain lead. Therefore, these hand-made traditional products can represent a real danger not only for potters, but also for those using these products. The diagnosis establishment of this condition can represent a challenge for every physician, especially for a pediatrician since the initial symptoms are nonspecific, such as abdominal pain, anorexia, or irritability (Dapul and Laraque, 2014), suggesting a gastrointestinal pathology. The case presented above was also initially misdiagnosed as an acute appendicitis. Prevention of lead exposure is the most important step in lead poisoning management because the neurocognitive damages induced by lead toxicity are irreversible (Kianoush et al., 2015), and also because in children, this metal is much more easily stored than in adults and it impairs the function of multiple organs, such as kidneys, bones, blood, and the brain (Woolf et al., 2007). For example, in the United States, in 1988, control disease center (CDC) Childhood Lead Poisoning Prevention Program was created which provided local governments lead poisoning programs (Blood lead levels in children aged 1–5 Years—United States, 1999–2010). These programs include public health

education, policy development, screening protocols, and case management guidelines (Dapul and Laraque, 2014). We think that it would be of great importance for the potters to benefit at least from public health education about lead toxicity and screening programs for lead poisoning. The screening for lead poisoning would be an important diagnostic tool for the family members involved in the pottery process, diminishing the rate of unreported cases, and also due to the cluster occurrence of this condition. Due to the multiple system involvement, the management of lead poisoning presents multiple critical points. Therefore, the patient submitted to chelation therapy must be closely monitored, because even the elimination of this heavy metal itself can impair the renal function. Even though lead is a toxic metal that is easily stored in the body, its removal being almost impossible, chelation therapy remains the standard treatment of lead poisoning (Kianoush et al., 2015).

1.4.4 Conclusions

- ***Effects regarding ingestion of corrosive substances in children***

Esophageal strictures occupy an important place in pediatric pathology, with multiple implications. Caustic soda ingestion is one of the most invalidating intoxications, as in over 90% of the cases esophageal stricture sets in and seriously affects life quality. Dilations with adjustable Savary bougies with metallic wire endoscopically guided and calibrated – a modern method of solving esophageal stenosis, especially corrosive ones – diminish as much as possible the perforation risk and allow the avoidance of surgery in many cases. Using this kind of treatment, the evolution towards healing of corrosive esophageal stricture is possible.

- ***Lead poisoning in a 16-year-old girl***

Lead toxicity is a life-threatening condition because of its severe acute and chronic complications. In children, there is no safe blood lead level, prevention methods are, therefore, very important to avoid toxic multiorgan effects of this metal. Even though the diagnosis represents a challenge in case of children mostly due to its rare incidence in teenagers, a physician must always include this possibility in the differential diagnosis for cases with suggestive symptoms.

1.5 Prevention of antibiotic resistance

1.5.1 Introduction

One of the greatest achievements in medicine is the discovery of antibiotics. These drugs improved clinical outcomes from infections by reduction of morbidity and mortality in transplant, surgical and critical care patients. Long-term administration of broad-spectrum antibiotics determined antibiotic resistance to be a worldwide threat. Many hospitals acquired infections are caused by multidrug resistant pathogens, making antibiotic therapy progressively difficult.

Bacteria resistance refers to opposition of microorganisms to an antimicrobial agent to which they were firstly sensitive. The evolution of this phenomenon was increased by the misuse of antimicrobial medicines and the global spread of antimicrobial resistance affecting unhealthy patients thus giving rise to superbugs. Different antibiotics faced with the resistance in last few years, a resistance that may be generated and transmitted in various ways.

Antibiotics represent the most prescribed drugs in pediatric hospitals, children receiving antibiotics more frequently than any other type of pharmacological treatment.

Excessive or inappropriate antibiotic prescription has increased the risk of bacterial strains that are resistant even to the third generation cephalosporins.

The main cause of antimicrobial resistance is the lack of public knowledge about antibiotics and their overuse. Therefore, self-medication affects the effective therapy and the correct diagnosis would avoid the administration of last-line antimicrobials (Martinez and Baquero, 2000; Fair and Tor, 2014; Bhullar et al., 2012).

Some Italian authors suggested the idea of using online educational tools for the general population as useful strategies for disseminating knowledge about antibiotic prescription and timing, aiming to limit self-medication, as well as preventing the emergence of resistant strains (Logan et al., 2014; Shah et al., 2004).

Personal contribution – published paper:

1. Duceac LD, Banu EA, Baciu G, **Lupu VV**, Ciomaga IM, Tarca E, Mitrea G, Ichim DL, Damir D, Constantin M, Luca AC. Assessment of Bacteria Resistance According to antibiotic chemical structure. *Rev chim*, 2019, 70(3): 906-908. **ISI IF 1,755**
2. Chiriac PC, Poroch V, Pascu AM, Hogea MD, Antohe I, **Lupu VV**, Cirlig V. Antibiotic molecules efficacy in pediatric bacterial infections. *Rev chim*, 2018, 69(4): 915-920. **ISI IF 1,412**

The aim of this studies was to evaluate descriptively the antibiotic resistance of pathogens isolated from patients admitted in a pediatric hospital. The connection between antibiotic chemical structure and the bacteria resistance to those types of drugs was investigated. Scanning electron microscopy micrographs focused on investigation the effect of ampicillin on E. coli biofilm formed on silicone and glass surface materials.

Knowing these facts, we could improve the prevention of antibiotic resistance.

1.5.2 Material and methods

- ***Antibiotic molecules efficacy in pediatric bacterial infections***

The descriptive study was carried out on a group of 411 patients admitted to the Sf. Maria Clinical Emergency Hospital for Children Iasi, between January 1st and March 31st, 2016. The age groups were established according to the new internationally published regulations (Williams et al., 2012). The exclusion criteria were the repetition of the same microbial agent in the same patient collected from various sites (in order not to overload the data), as well as the age over 18, because in the microbiology laboratory of the hospital there were also performed analyzes for adults as patients' attendants and employees of the hospital. The data collection was performed using the hospital's statistic reports, hospital recordings and microbiological and epidemiological reports.

- ***Assessment of bacteria resistance according to antibiotic chemical structure***

Purchased *E. coli* bacterial suspension was prepared inoculating 500 µL of a glycerol stock in a total volume of 200 µL of inoculation medium. Culture medium consisted of 5.5 g/L glucose, 2.5 g/L peptone and 1.25 g/L yeast extract in phosphate buffer was grown on a 1 L shake flask incubated at 37°C under agitation. Cell were then harvested by centrifugation and suspended in broth for removing of all traces of medium and again harvested by centrifugation and suspended in broth for obtaining an inoculum containing about 1x107 cell/ml.

Glass and silicone surfaces were prepared by washing and drying them for further determination.

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

1.5.3 Results and discussions

- ***Antibiotic molecules efficacy in pediatric bacterial infections***

The distribution of the cases by age groups showed that newborns and infants (0–1-year age group) prevailed (n =182 cases, representing 44.29% of total isolates) (Figure 1.14). In the 0–1-year age group bacterial infections were most prevalent - more than half of the total number of samples (n = 226 isolates, representing 54.98%) (Table 1.5).

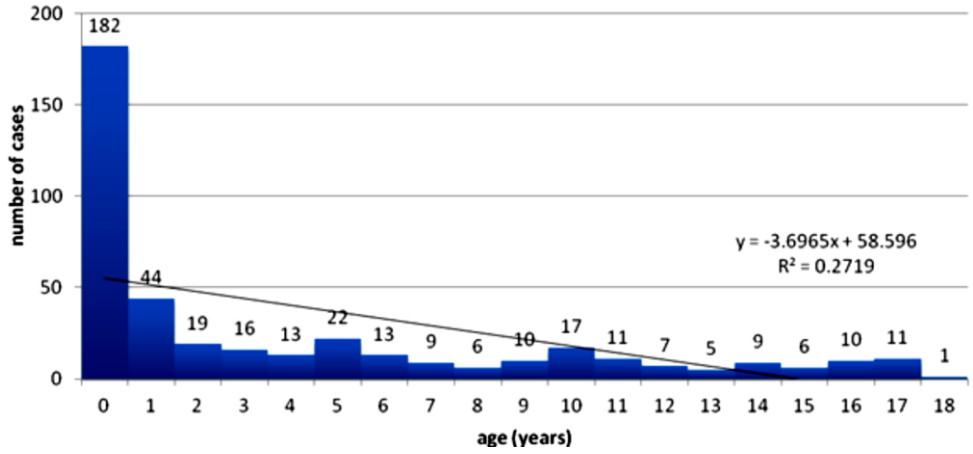

Figure 1.14. *Age histogram of the study group*

In the order of the frequency of isolates, another age group often involved in MDR pathogens was that of preschool children (2-5 years old), followed by the 6-11 years group, and 12-18 years group (Table 1.5).

Table 1.5. *Distribution of bacterial strains isolates in each standard pediatric age group*

Age group (years)	Bacterial strains	
	Number	%
0-1	226	54.98
2-5	70	17.03
6-11	66	16.06
12-18	49	11.93
Total	411	100%

Gender distribution revealed a predominance of boys (n = 230 isolates, representing 55.96% of the total), compared to girls (n = 181, representing 44.04%), with no significant statistical difference.

One of the major issues concerning the management level of HAIs was the distribution by the hospital wards from where the samples were collected. General pediatrics was most often involved with 99 samples (24.08%), while in the burn unit only 6 samples (1.45%) were collected (Table 1.6).

Table 1.6. *Distribution of bacterial isolates by hospital wards*

Ward	Isolates	
	Number	%
General Pediatrics	99	24.08
Intensive Care Unit	82	19.95
Surgery (general, orthopedics, neurosurgery)	61	14.84
Acute Therapy	47	11.43
Pneumology	33	8.03
Allergology	8	1.94
Cardiology	7	1.70
Burns	6	1.45
Toxicology	4	0.97
Pediatric rehabilitation	4	0.97
Nephrology	3	0.72
Hematology-oncology	1	0.24
Diabetes	1	0.24
Pediatric urology	1	0.24
Day-care hospitalization and ambulatory	32	7.85
Patients' admission room	22	5.35
Total	411	100%

The isolates with MDR microorganisms were collected from various pathological products, the most numerous, almost 1/4 of the total isolates, as expected, from pus (98 samples, representing 23.85%) of which 49 were taken from the wounds (11.92%), 42 from abscesses (10.22%), 5 from fistulas (1.21%) and 2 from sinuses (0.48%), as shown in Table 1.7.

Table 1.7. *Pathological products containing MDR bacteria isolates in the study group*

Pathological product	Frequency	
	Number	%
Plus – total	98	23.85
Wounds	49	11.92
abscesses	42	10.22
Fistulas	5	1,21
Sinuses	2	0.48
Hypo-pharyngeal aspirates	89	21.65
Conjunctival secretions	51	12.42
Ear secretions	39	9.48
Skin – cultures	29	7.06
Tracheal aspirates	29	7.06
Gastric aspirates	19	4.62
Sputum – microscopic ex.	14	3.42
Sputum – culture	13	3.16
Blood cultures	9	2.19
Peritoneal liquids	7	1.70
Catheter – tip	6	1.46
Stool culture	4	0.97
Tympanocentesis	2	0.48
Urine cultures	1	0.24
Others	1	0.24
Total	411	100%

The MDR microorganisms identified in cultures isolates from various pathological samples collected from the pediatric patients enrolled in our study group are expressed in Figure 1.15 and Table 1.7.

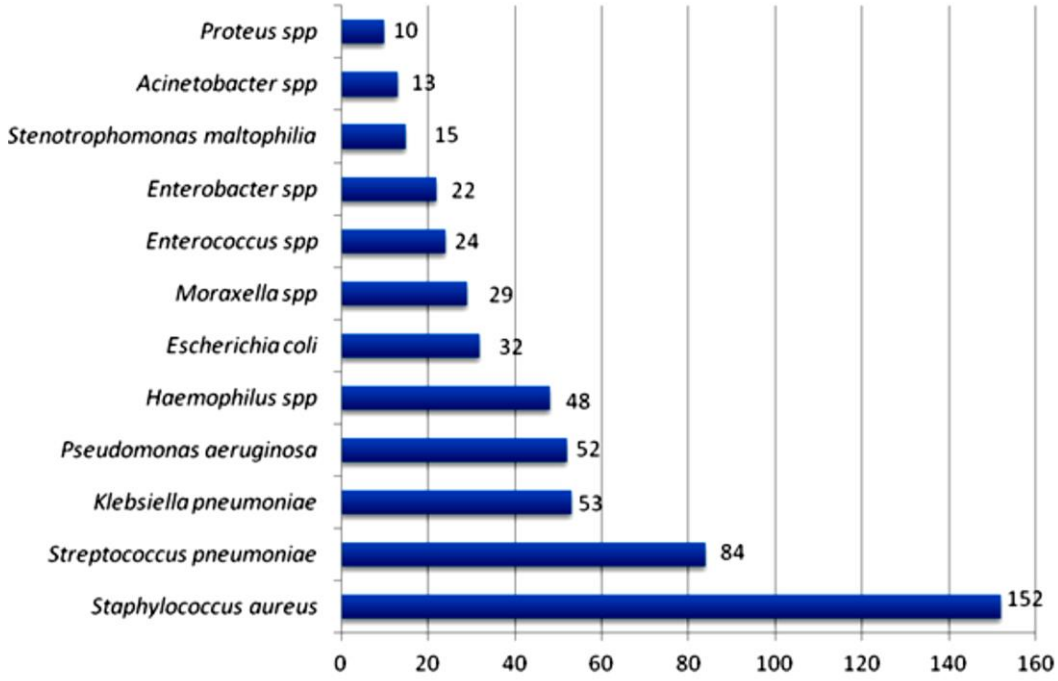

Figure 1.15. *Most frequent MDR microorganism identified in the pathological samples collected from the study group*

As expected, *Staphylococcus aureus* was the most frequent microorganism present in the collected pathological samples (isolated from 152 samples, representing 27.73%), followed by *Streptococcus pneumoniae, Klebsiella pneumoniae and Pseudomonas aeruginosa, Haemophilus spp, Escherichia coli.*

Antibiotic resistance is responsible for HAIs in over than 50% of the cases (Jones, 2001). Unfortunately, during the last decades, the development of new antibiotic molecules has not progressed in parallel with the increasing rates of common bacterial microorganisms' resistance, leaving fewer treatment options for MDR bacterial infections (Boucher et al., 2009; Spellberg et al., 2008).

Concerning our study, we assessed the efficacy of some antibiotic molecules on the main bacterial strains collected from the patients of the study group (Figures 1.16-1.20).

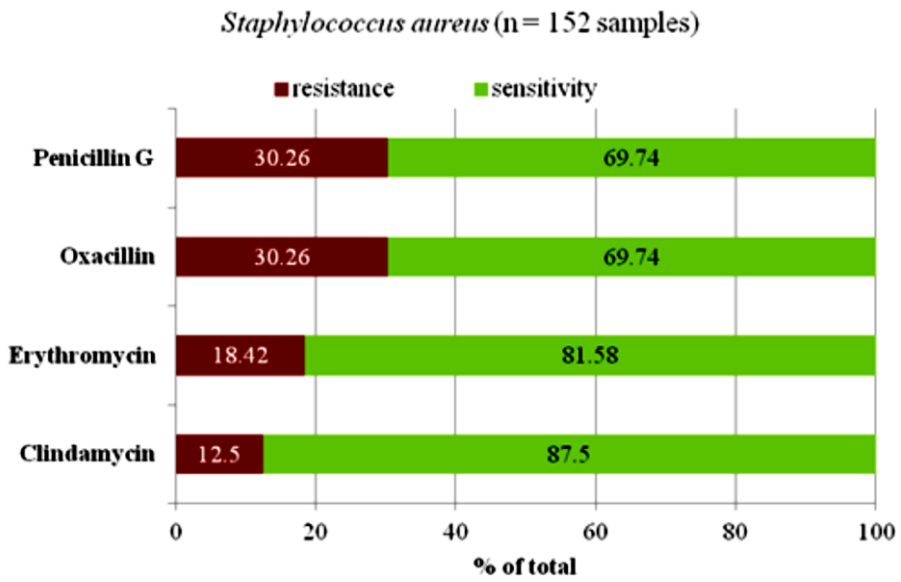

Figure 1.16. *Efficacy of commonly used antibiotic molecules on Staphylococcus aureus strains collected from the patients from the study group*

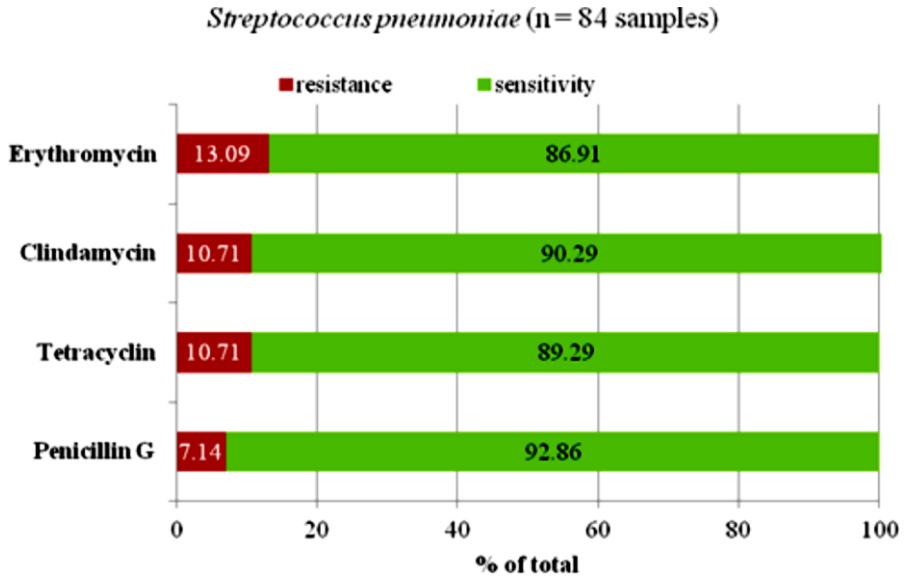

Figure 1.17. *Efficacy of commonly prescribed antibiotic molecules on Streptococcus pneumoniae strains collected from the patients from the study group*

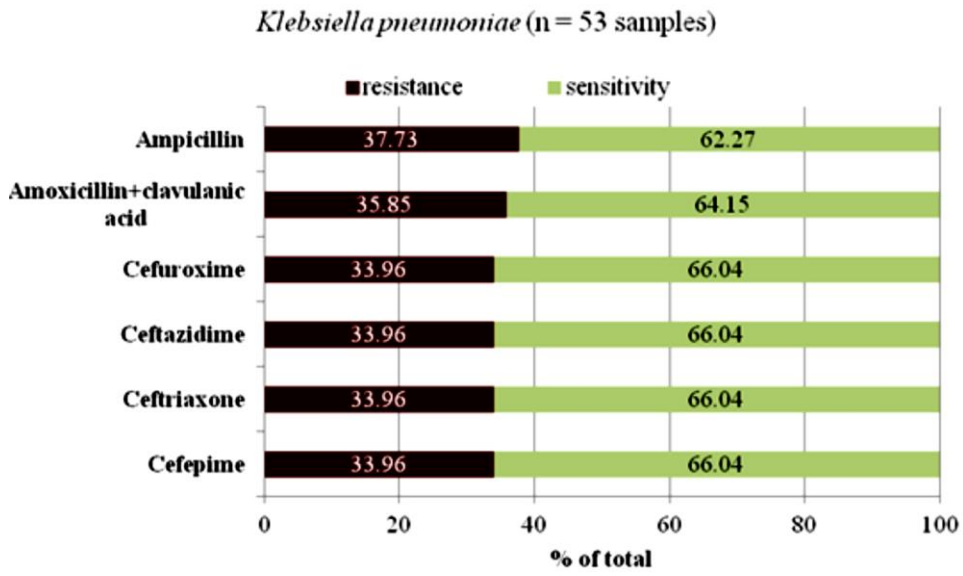

Figure 1.18. *Efficacy of commonly prescribed antibiotic molecules on Klebsiella pneumoniae strains collected from the patients from the study group*

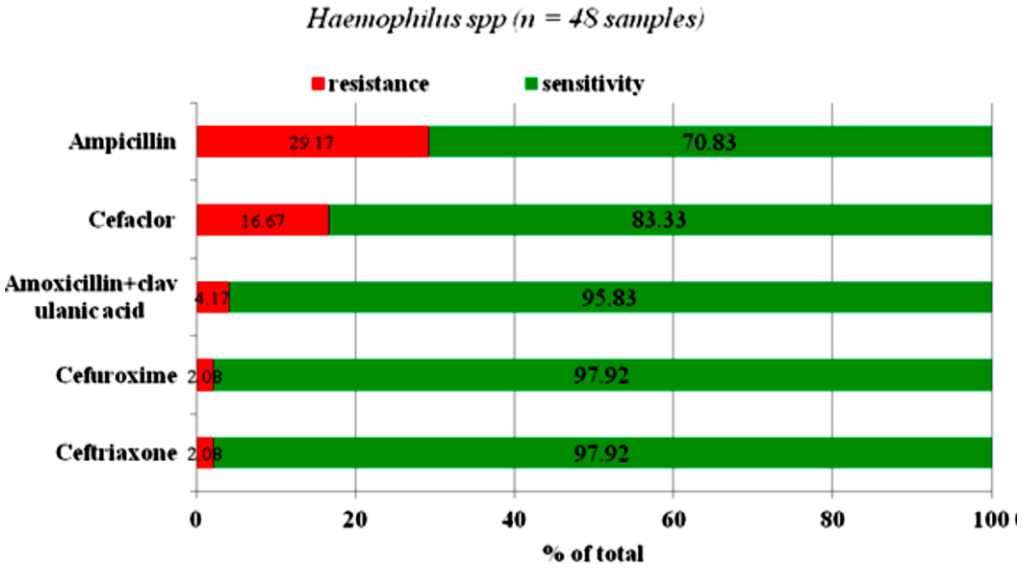

Figure 1.19. *Efficacy of commonly prescribed antibiotic molecules on Haemophilus spp strains collected from the patients from the study group*

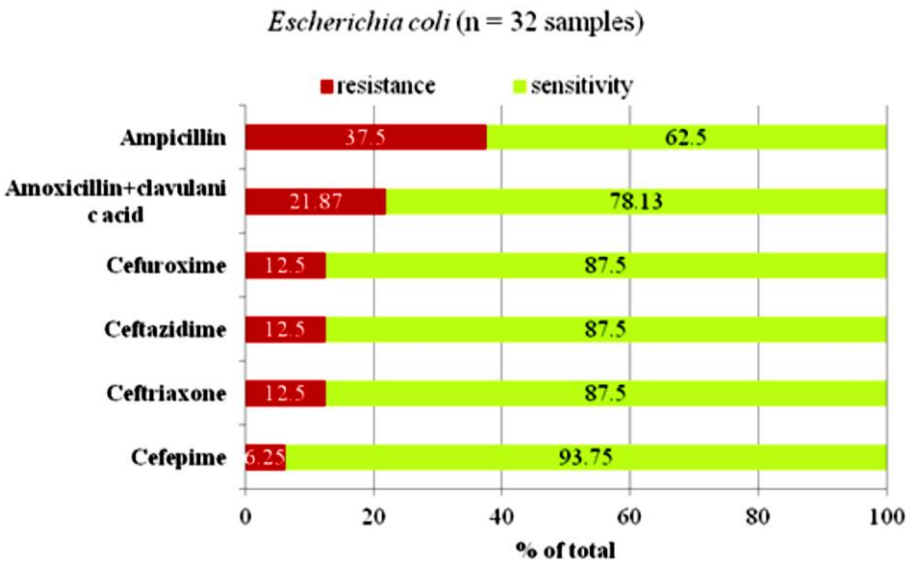

Figure 1.20. *Efficacy of commonly prescribed antibiotic molecules on Escherichia coli strains collected from the patients from the study group*

Penicillin G and oxacillin were inefficient in 46 *Staphylococcus aureus* strains samples (30.26% of 152 isolates), erythromycin in 28 isolates (18.42%), and clindamycin in 19 isolates (12.50%) (Figure 1.16).

On *Streptococcus aureus* strains penicillin G proved inefficient in 6 isolates (7.14% of a total of 84 isolates), erythromycin in 11 isolates (13.09%), clindamycin and tetracycline in 9 isolates (10.71%) (Figure 1.17).

A major problem was the antibiotic resistance of *Klebsiella pneumoniae* strains, which were resistant to ampicillin in 20 cases (37.73% of the 53 isolates), to amoxicillin + clavulanic acid in 19 cases (35.85%).

Klebsiella pneumoniae proved resistant to cephalosporins such as cefuroxime, ceftazidime, ceftriaxone, cefepime in 18 isolates (33.96%), in concordance to data published in previous studies performed in Romania and worldwide (Balici et al., 2016, Nicolini et al., 2014) (Figure 1.18).

On *Haemophilus spp* strains ampicillin was inefficient in 14 of 48 samples (29.17%) the combination of amoxicillin and clavulanic acid molecules proved inefficient in 2 samples (4.17%), while cefaclor in 8 strains (16. 67%) (Figure 1.19).

Regarding the antibiotic resistance of *Escherichia coli* strains, ampicillin was inefficient in 12 isolates (37.50%), amoxicillin + clavulanic acid in 7 isolates (21.87%), cephalosporins cefuroxime, ceftazidime, ceftriaxone in 4 isolates (12.50%), and cefepime in 2 isolates (6.25%) (Figure 1.20).

In a study conducted in Romania, Cucu A. et colab. (Cucu et al., 2014) reported the following antimicrobial resistance of the bacterial strains isolated from patients admitted to ICUs: 76.3% of Staphylococcus aureus isolates were resistant to erythromycin and 1.7% to chloramphenicol. Of the total number of strains of *Staphylococcus aureus*, 69.5% were methicillin-resistant and 35.1% were MRSA (resistant to all penicillins and 1st, 2nd, and 3rd generation cephalosporins).

For *Escherichia coli* isolates, 56.7% were resistant to ciprofloxacin, and 54.4% to imipenem sensitive ESBL strains. Over 66% of the *Pseudomonas aeruginosa* strains were resistant to all tested antibiotics except for colistin (sensitivity in 41.5% of the strains). *Klebsiella pneumoniae* isolates were ESBL - producing strains (resistance to all penicillin and

1st, 2nd, and 3rd generation cephalosporins) in 85.5% of the cases, and 77.0% were resistant to ciprofloxacin.

The same study reported different antibiotic resistance prevalence values for the same microorganisms in other hospital wards: 45.4% of *Staphylococcus aureus* strains were resistant to erythromycin, 19.5% of *Pseudomonas aeruginosa* isolates were resistant to piperacillin-tazobactam and 28.0% to ciprofloxacin, and the colistin susceptibility was only 37.5%.

The limitation of antibiotic as a self-medication, the use of narrow-spectrum antibiotics, restrictive strategies and guidelines at national level should be considered by Figure 1.18. Efficacy of commonly prescribed antibiotic molecules on *Klebsiella pneumoniae* strains collected from the patients from the study group Figure 1.19. Efficacy of commonly prescribed antibiotic molecules on *Haemophilus spp* strains collected from the patients from the study group Figure 1.20. Efficacy of commonly prescribed antibiotic molecules on *Escherichia coli* strains collected from the patients from the study group healthcare providers to decrease the prevalence of antibiotic resistance (Costan et al., 2008).

The antimicrobial resistance of bacteria such as *Escherichia coli*, *Klebsiella spp*. has increased in recent years, reported data showing significant differences from year to year for common prescribed antibiotic molecules, like gentamicin. However, no increase in ciprofloxacin resistance of Gram-negative bacteria was noticed in 2016, as it was stated in a study conducted in Romania by Voicu M. et colab. (Voicu et al., 2017). In the same study, the authors reported that cephalosporins, aminoglycosides and carbapenemes were less used in 2016 compared to previous years, and a decrease of the number of hospitalization days was also noticed. For the same period of 2016 (the first trimester, as in our study), the authors observed an increased consumption of quinolones due to the use of ciprofloxacin as antimicrobial therapy in Gram-negative bacterial infections (Voicu et al., 2017).

- ***Assessment of bacteria resistance according to antibiotic chemical structure***

For quantification of *E. coli* biofilm formation and antibiotic sensibility on glass and silicone surfaces was used epifluorescence microscopy. After 24 h of biofilm development silicone surfaces exhibits enhance biofilm formation compared to glass. Viable cells number remained constant during the first 3 hours for both materials and then the viability of biofilms formed on glass markedly decreased and a 7-log reduction was achieved after 7.5 h of treatment.

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

SEM images analyzed the morphological changes on the sessile cells exposed to antibiotic (Figure 1.21). The micrographs showed that the size of protrusions varies with features up to 10 μm and yet most of them exceed the *E. coli* cells size (Figure 1.21C). Bacteria cells adhered to silicone appeared to be extracellular polymeric substances compared to the cells observed on the glass surfaces (Figure 1.21A) which are distributed in aggregates or as individualized cells without adhesive material in their vicinity.

After 6 h ampicillin exposure, the amount of *E. coli* biofilm cells adhered to silicone and glass decreased (Figure 1.21B and Figure 1.21D respectively). The antibiotic-treated cells are more elongated on both materials compared to untreated cells and in the case of *E. coli* biofilm developed on glass (Figure 1.21B), the treated cells are longer than on silicone (Figure 1.21D).

In both tested materials, the cell wall of sessile cells showed no severe damage after 6 h of ampicillin treatment (Figure 1.21B and 1.21D).

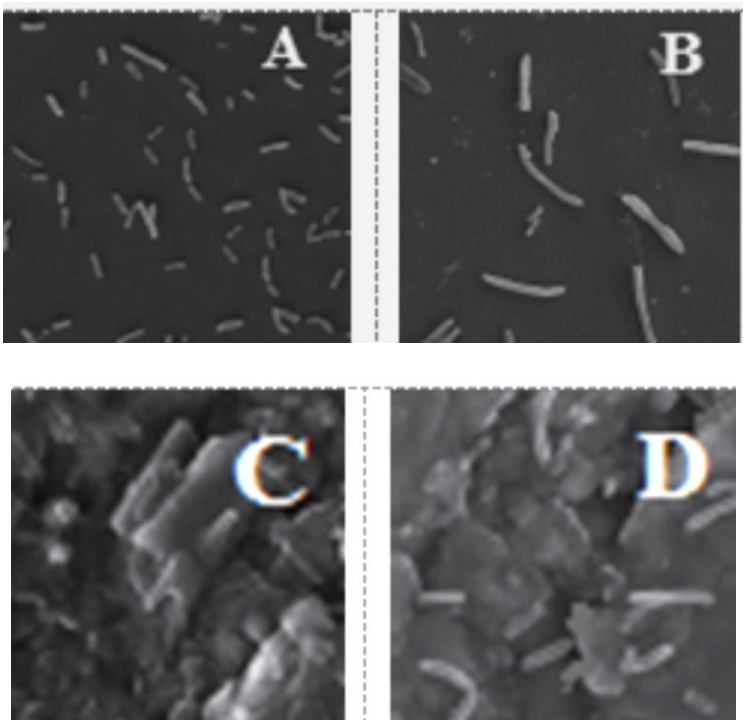

Figure 1.21. *SEM micrographs of 24 h biofilms not exposed to ampicillin formed on (A) glass and (C) silicone and after 6 h of exposure to ampicillin (B) glass and (D) silicone*

1.5.4 Conclusions

- ***Antibiotic molecules efficacy in pediatric bacterial infections***

The study revealed that penicillin G and oxacillin were inefficient in more than a third of *Staphylococcus aureus* strains collected from pathological samples in a pediatric hospital. *Streptococcus pneumoniae* was resistant to penicillin G and erythromycin; amoxicillin + clavulanic acid and 1st, 2nd and 3rd generation cephalosporins failed to combat *Klebsiella pneumoniae* infections; cefaclor was not efficient in *Haemophilus spp* isolates; *Escherichia coli* proved low sensitivity to ampicillin and 1st, 2nd and 3rd generation cephalosporins.

Continuous evaluation of antibiotic resistance of MDR bacterial strains, together with promotion of updated protocols and guidelines for appropriate antibiotic prescription, and development of new antibiotic molecules represent important objectives in reducing HAIs, expecting lower mortality rates, shorter hospitalization durations, and lower health care costs.

- ***Assessment of bacteria resistance according to antibiotic chemical structure***

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

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

Chapter 2. Gastroesophageal reflux disease in children

2.1 State of art

Our Greek and Roman ancestors, whose medicine was holistic, treated the patient as a whole and tried to prevent disease. Their approach to caring for children was empirical and based on observation. Children were considered “moist and warm” and their illnesses caused by the imbalance of humors. The aim of treatment in children was to restore the balance of the humors using herbal remedies (marshmallow, plantain, rhubarb). As early as the second century, Galen regarded breast milk as the ideal food for infants (Still, 1931). During the Renaissance, humoral thinking continued to exist looking for new causes of illness and approaches to therapy. Medical knowledge was disseminated throughout Europe, and the invention of the printing press in the 1450s facilitated the circulation of the works of ancient writers as well as new treatises on children's diseases, e.g., Paulo Bagellardo's Libellus de Egritudinibus Infantum (Bagellardo, 1472). The emphasis was on therapy, rather than diagnosis, with herbal remedies, along with observing the rules of hygiene, healthy living and nature’s healing power. Subsequently, the idea that disease is an independent entity, separable from the patient, allowed the compilation of disease case descriptions and taxonomies that included “causes” as well as “symptoms” (Weaver, 2018). The understanding of infectious diseases and nutritional disorders, child wellbeing, growth and development made significant progress between the 1850s and the 1950s, when pediatrics was born and the gastrointestinal tract, as an organ of nutrition, became of major interest to pediatricians (Nichols et al., 1991).

Ongoing research and the introduction of new diagnostic techniques has made it possible to develop new therapies for children with gastroenterological disorders that require close monitoring, particularly for their possible effect on growth and development (Walker-Smith and Walker, 2003).

The gastrointestinal tract derives entirely from the primitive bowel, a tube extended from the mouth to the anus, which is formed in the 3rd-4th week of intrauterine life through the invagination of the dorsal side of the vitelline sac into the embryo.

The development takes place as follows: from the endoderm derive the epithelium and the annex glands of the digestive apparatus, the mesoderm creates the lamina propria of the submucosae and of the muscular and serous tunics. Topographically, the primitive bowel is divided into three parts: anterior, middle and posterior, which is at the origin of the segments of the digestive tube.

The esophagus derives from the anterior part of the primitive bowel from which also derive: the oral cavity, the pharynx, the stomach, the proximal duodenum up to the ampulla of Vater, the liver, the gallbladder and the pancreas, all of them irrigated by the celiac trunk (Anghelescu, 1963).

At the cranial end, a ventral diverticulum which will become the trachea forms a groove in the foregut cavity floor about 3 weeks from the fertilization. As the structures elongates, ridges appear on the lateral walls of the foregut cavity and the diverticulum, which will unite about 5 weeks of age, forming separate tracheal and esophageal channels (DiMarino and Benjamin, 2002)

The esophagus is a musculo-membraneous conduct, supple and dilatable, which links the pharynx to the stomach, extending from the C6 vertebra (pharynx-esophageal junction) to the T11 vertebra (the cardia) (Angelescu, 2003).

The esophagus is not straight. It has curves in the sagittal and frontal plane. At birth it is 8-10 cm long and 0.5 cm in diameter, and then it grows 0.65 cm/year, reaching at an adult person 25 cm long and 2-3 cm in diameter (Behrman et al., 2007).

The esogastric junction (lower esophageal complex) is a physiological sphincter without anatomic individuality. The constituent elements of this area are: epiphrenic ampulla – radiological concept – an area of luminal expansion which appears above the vestibular sphincter in inspiration vestibular sphincter – corresponds to the lower esophageal sphincter (LES) esophageal vestibulum (esophageal ampulla) Z line – the line of epithelial transition – an anatomo-histological demarcation line between the stratified squamous epithelium of the esophagus and the columnar epithelium of the stomach – a very useful endoscopic mark (Behrman et al., 2007; Burlacu, 2006).

In normal conditions, the esogastric junction represents an anti-reflux anatomic device which prevents the reflux of the gastric acid content into the esophagus. The dysfunctions of the mechanism of gastroesophageal retention determine: the appearance of GERD with secondary peptic esophagitis up to stenosis, diaphragmatic hiatal hernias– when the esophageal hiatus is larger.

The esophageal innervation:

a) extrinsic innervation – sympathetic fibers, vagal nerves and recurrent laryngeal nerve (cerebrospinal)

b) intrinsec innervation – compose of myenteric Auerbach's plexus and submucosal Meissner's plexus (DiMarino and Benjamin, 2002).

The common nociception and central projection of the esophagus and trachea are responsible for the asthma-like of the GERD (Cannin and Mazzone, 2003).

Using imunohistochemistry studies it was possible to identify the sites of neurohormonal substances secretion (Aprodu et al., 2003): VIP – present since intrauterine period in plexuses and LES; P substance – in the muscular fibers and plexuses, determines the contraction of LES; Enkephalin – in the striated muscular fibers; Motilin – controls the LES.

The esophagus plays the role of: transport of the alimentary bolus from the oral cavity to the stomach to prevent the gastro-esophageal and esopharyngeal reflux, by means of the upper esophageal sphincter (UES) and LES.

UES maintains the proximal extremity of the esophagus and prevents the eso-pharyngeal reflux. The UES pressure is of 40-100 mmHg, and during the deglutition, UES transitorily relaxes itself (Haulica, 2000).

The esophageal transit of the alimentary bolus is carried out by means of two factors: peristaltic waves and gravity force.

The participation of the two factors depends on the position of the body (Angelescu, 2003):

1. in clinostatism the passage of the alimentary bolus is made only through peristaltic activity
2. in orthostatism, the gravity force also contributes to a variable extent.

The peristaltic waves at the esophagus level are:

1. primary waves – are originated in the pharynx, are in relation with the deglutition, they favor the propulsion of the alimentary bolus;
2. secondary waves – induced by the distension of the esophagus, chemical or mechanical irritants and don't correlate with the deglutition.

The prevention of the esophageal lesions determined by the gastric acid reflux is done by the secondary peristaltic contractions which perform the esophageal clearance.

The pressure exercised by the peristaltic waves get over the tonus of LES, they relax it. The tonus of that sphincter increases with age: at the young child and baby, the low muscular tonus makes the food pass fast "just like through a tube" and to fall into the stomach; at the

older child the pressure exercised by the alimentary bolus overcomes the muscular tonus and relax the cardia.

The main function of the lower esophageal sphincter is to prevent GER. LES doesn't always act as an anti-reflux barrier, even at healthy people, so after meal, there may appear a transitory relaxation of LES not preceded by a peristaltic one.

Gastroesophageal reflux (GER) is a common physiological process in children of all ages. In 2009, GER was defined by both the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN) and the European Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) as the passage of gastric contents into the esophagus with or without the presence of regurgitation and vomiting (Vandenplas et al, 2009). Reflux episodes occur more frequently in infants and are often physiological (Ciciora and Woodley, 2018). Most episodes are rare, brief and do not cause symptoms or complications (Springer, 2006). Gastroesophageal reflux disease (GERD) sets in when troublesome symptoms and/or complications (esophagitis, stenosis) related to GER occur (Vandenplas et al, 2009). The new 2018 guidelines used the same definitions for GER and GERD (Rosen et al, 2018).

Epidemiological studies have shown that gastroesophageal reflux occurs in about 50% of infants under 2 months of age, 60-70% of infants aged 3-4 months and 5% of infants up to 12 months of age (Nelson et al., 1997). A study in France reported that 24.4% of infants (0-23 months) had symptoms of GER, and the prevalence of GERD was 12.6% (Martigne et al. 2010). In the UK, 1700 children aged 1-17 years were diagnosed with GERD over 5 years (Ruigómez et al., 2010).

The diagnosis of GER and GERD is based on clinical symptoms and signs, medical history and physical examination, but these are non-specific, especially in infants (Vandenplas et al, 2009). Sometimes additional diagnostic investigations are needed to qualify and quantify GERD and to exclude other diseases than GERD (Rosen et al, 2018).

In the mid-1970s, prolonged reflux monitoring was introduced into practice, which expanded the knowledge on GER. Until then, reflux was recognized by the evidence of esophageal lesions at upper endoscopy. Subsequently, GERD was designated as reflux esophagitis. When the prolonged pH monitoring was introduced, the presence of reflux in healthy volunteers could be assessed and it was called "physiological reflux". Pathological reflux has been classified according to body position: orthostatic (the most common type), clinostatic and mixed. The original tool for monitoring the gastroesophageal reflux - esophageal pH-metry - identified only episodes of acid reflux. With evolution, by impedance-pH-metry, other types of refluxes have been acknowledged - acid, weak acid and alkaline reflux (Nasi et al., 2018).

Both pH-metry and multiple intraluminal impedance (MII) are now performed to assess the presence of reflux and the association between GER and symptoms. MII monitoring helps to differentiate between acid, weak acid and alkaline reflux episodes. Impedance pH-metry is an important diagnosis tool especially in infants, as in this age group GER episodes are more likely to be weakly acidic and/or alkaline, even in the absence of antisecretory therapy. There are studies in the literature that have reported that almost 45% of infants diagnosed with GERD by impedance pH-metry would have normal pH-metry (Vandenplas et al, 2009; Rybak et al., 2017).

As scientists and clinicians, we continuously contribute to the progress and evolution of science thus influencing its present and past.

This research aims to contribute to the description of intrinsic and extrinsic risk factors, to describing different correlations between gastroesophageal reflux disease and other pathologies and equally at establishing a correct diagnosis and treatment.

This line of research has been achieved through the publication of books, book chapters and articles, among the most significant:

Articles

1. **Lupu VV**, Miron IC, Lupu A, Moscalu M, Mitrofan CE, Munteanu D, Luca AC. The relationship between gastroesophageal reflux disease and recurrent wheezing in children. *Medicine*. 2021,100(47):e 27660. **ISI IF 1,889**
2. **Lupu VV**, Burlea M, Nistor N, Streanga V, Starcea MI, Paduraru G, Ghica DC, Mitrofan EC, Moscalu M, Ignat A. Correlation between esophageal pH-metry and esophagitis in gastroesophageal reflux disease in children. *Medicine.* 2018, 97(37): e12042. doi: 10.1097/MD.0000000000012042. **ISI IF 1,87**
3. Ignat A, Burlea M, **Lupu VV***, Păduraru G. Oral manifestations of gastroesophageal reflux disease in children. *Romanian Journal of Oral Rehabilitation*, 2017, 9(3): 40-43. ISI indexed
4. **Lupu VV,** Ignat A, Paduraru G, CiubaraA, Moscalu M, Marginean CO, Burlea M. Correlation between the different pH-metry scores in gastroesophageal reflux disease in children. *Medicine*, 2016; 95(26): e3804. doi: 10.1097/MD.0000000000003804. **ISI IF 1,804**
5. **Lupu VV**, Ignat A, Ciubotariu G, Ciubară A, Moscalu M, Burlea M. Helicobacter pylori infection and gastroesophageal reflux in children. *Diseases of the Esophagus*, 2016, 29(8): 1007-1012, doi: 10.1111/dote.12429. **ISI IF 2,571**
6. **Lupu VV**, Ignat A, Paduraru G, Mihaila D, Burlea M, Ciubara A. Heterotopic Gastric Mucosa in the Distal Part of Esophagus in a Teenager: Case Report. Medicine (Baltimore). 2015;94(42):e1722. **ISI IF 2,133**
7. A: **Lupu VV**, Ignat A, Paduraru G, Burlea M. Gastroesophageal reflux in newborns and infants (I). *Revista Română de Pediatrie,* 2015, LXIV(4): 387-392. B: **Lupu VV**, Ignat A, Paduraru G, Burlea M. Gastroesophageal reflux in newborns and infants (II). *Revista Română de Pediatrie*, 2016, LXV(1): 10-14.
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11. **Lupu VV**, Burlea M, Lupu A, Păduraru G, Uliliuc T, Diaconescu S. The gastroesophageal reflux disease in children – 5 years cases report. *RJOR*, 2012, 4(1): 31-34

Books and chapters

1. **Lupu VV**. Refluxul gastroesofagian la copil. *Ed. Amaltea*, București 2015, 147 pg., ISBN 978-973-162-149-4
2. **Lupu VV**, Ignat A, Păduraru G, Ciubotariu G, Burlea M. Actualități în boala de reflux gastroesofagian la copil, sub redacția Nicolai Nistor, Alina Costina Luca, Constantin C. Iordache, Ingrith Miron: Actualități în pediatrie, ed Junimea, Iași, 2014, ISBN 978-973-37-1798-0, pag 218-224
3. **Lupu VV**. Refluxul gastroesofagian - în Protocoale de diagnostic și tratament in pediatrie - coordonator Mircea Nanulescu, ed. Amaltea, București 2014, ISBN 978-973-162-127-2, pag. 67-70

2.2 Epidemiology of gastroesophageal reflux disease

2.2.1 Introduction

The gastro-esophageal reflux disease (GERD) is a frequent disease in the pediatric practice. It is assessed to be the most frequent esophagus disease in infants. It features abnormal and recurrent passage of the gastric acid content in the esophagus, having as result digestive, breathing and/or neurobehavioral manifestations. An infant showing recurrent vomiting must be immediately examined for gastro-esophageal reflux as well, if the infant growth is not satisfying (Behrman et al., 2007).

In infants, the recurrent regurgitations and vomiting are typical while in children, the heterogeneous symptomatology raises diagnosis-related issues. Signs such as recurrent wheezing, unexplained apnea crises, chronic cough, recurrent broncho-pneumopathies, and asthma crises are the consequence of complicated gastroesophageal reflux (Herbst, 2006).

The GERD diagnosis is even more laborious when atypical signs are present, which takes the disease out of the gastroenterology field, including it in the general pediatric pathology. The paraclinical investigations have the role of proving the presence of the gastroesophageal reflux and to highlight the lesions induced by the acid reflux on the lower esophagus. Direct and indirect tests are performed, their sensitivity and specificity varying depending on the method.

The esophageal pH monitoring or pH measurement is the investigation technique with the highest sensitivity and specificity in the gastroesophageal reflux detecting and quantification. In the GERD diagnosis, it was the "gold-standard" method, until impedance pH-metry appeared. PH-metry is based on the principle according to which, the passage of the acid gastric content into the esophagus during the reflux determine a decrease of the intraesophageal pH (Liburd and Hebra, 2005). It became a reference method after 1958, when it was recommended for the first time by Tutle and Grossman, and the first successful measurement was performed by Spencer in 1969. The method consists of the pH recording in the lower esophagus for a determined period: either 3-4 hours (short test), or 24 hours (long test) by the trans nasal placement of a microelectrode, 5 cm above the cardia, which is connected to a computerized device analyzing the different requested parameters, depending on which the graphic representation of the pH curves are made. The position of the electrode is radiological or monometrically controlled or it is established based on tables depending on the child's age and height. With small probes, the method can be used in the premature born infant, being well tolerated. The first electrodes were bipolar and made of glass, but of larger sizes, so they could not be used in children; after 1970, unipolar smaller, flexible electrodes appeared that can be easily introduced nasally, being used in children, as well. But the small glass made electrodes have relatively high impedance and are instable for long time use. More recently, small electrodes with antimony crystals appeared, with lower impedance, suitable to the children (Rudolph et al., 2001).

The method is able to supply information on the following parameters:

- the number of refluxates within 24 hours;
- the duration of the longest reflux;
- the number of refluxates taking more than 5 minutes;
- the average and the maximum duration of the esophageal clearance;
- the correlation between the acid "peaks" and the clinical symptoms: cough, regurgitation, apnea crises etc.

During recording, the main events are noticed:

- time and duration of lunches,

- sleeping time,
- the patient's change of position,
- crying time,
- active and passive exposure to tobacco;
- but, the previously mentioned symptoms which the patient or the subject is trained to monitor (Sondheimer, 2000).

In interpreting the results, the following parameters are considered:

- the total number of reflux episodes (normal – below 2 episodes in average per hour);
- the number of reflux episodes with a duration higher than 5 minutes (normal – below 8 episodes);
- the duration of the longest reflux episode (in minutes);
- the reflux index (RI) = the ratio between the total number of reflux episodes and the duration of recording (normal – below 4);
- the Euler score = x + 4 y, in which:

  x – no. of reflux episodes cu pH<4 longer than 1 minute;

  y – no. of episodes with pH<4 longer than 5 minutes.

A pathological reflux is discussed when:

- there are more than 2 reflux episodes in average per hour,
- the time summed up with pH<4 is above 5% of the total period in which the determination was made.

The duration of the pH monitoring depends on the desired information.

The short duration pH measurement is conducted in clinostatism, in fasting conditions, in order to avoid reflux frequent episodes occurring immediately postprandial. It is also called the acid reflux test and it highlights the presence of spontaneous or induced esophageal reflux.

The test is positive if within 30 minutes, two episodes of esophageal pH decrease occur. The disadvantage is that it does not report the alkaline reflux (Strobel et al., 1979).

The long duration or continuous, 24 hours pH measurement, allows prolonged monitoring and thoroughly details the events associated to reflux.

The indications of the esophageal pH measurement:

- gastro-esophageal reflux,
- dysphagia,
- recurrent vomiting,
- recurrent laryngitis,
- chronic cough and recurrent wheezing,
- growth failure,
- assessment of treatment results.

The counter indications of the esophageal pH measurement:

a) absolute:

- higher nasopharyngeal or esophageal obstructions,
- severe oromaxillofacial trauma,
- difficult to control coagulopathies.

b) relative:

- esophageal ulcerations,
- esophageal varices,
- recent surgeries in the esophagogastric area,
- low patient or subject compliance,
- impossibility of monitoring during testing.

Although it is an invasive investigation method, the pH-metry remains the most used diagnosis and assessment method in children with typical or atypical symptoms of GERD (Behrman et al., 2007).

Personal contribution – published paper:

1. **Lupu VV**, Burlea M, Lupu A, Păduraru G, Uliliuc T, Diaconescu S. The gastroesophageal reflux disease in children – 5 years cases report. *RJOR*, 2012, 4(1): 31-34

The aim of this study was to investigate the presence of GERD in children, both in the cases with typical symptoms of reflux and with atypical signs.

2.2.2 Material and method

The study involved 197 children with the suspicion of gastro-esophageal reflux diagnosis. The 24 hours esophageal pH-metry was performed for all of them in the V-th Clinic of Pediatrics, Emergency Clinical Hospital for Children "Sf. Maria" of Iasi, throughout 5 years (January 2006 – December 2010). The children were selected based on the typical reflux symptoms (recurrent regurgitations, recurrent vomiting, dysphagia) and on the atypical symptoms, particularly breathing signs (chronic cough, recurrent wheezing, recurrent higher airways infections, recurrent pneumopathies, bronchial asthma), cases which were admitted to the V-th and II-nd Clinic of Pediatrics. In the selected cases, 24 hours continuous esophageal pH-metry was performed with the Medtronic Digitrapper device and the results were interpreted based on the calculation of the Boix Ochoa score.

2.2.3 Results

The reflux was present at 146 children (74.11%), 95 males and 51 females.

For the age group 0–1-year, pH-metry was performed in 48 children, and the reflux was present in 34 cases (70.83%). Between 1-3 years old, out of 51 studied cases, 41 (80.39%) had reflux; between 3-10 years old, 55 children (71.05%) out of 76 had reflux; and over 10 years old, in 16 cases out of 22 studied, the reflux was demonstrated.

As the environment, most of the cases were from the urban area - 126 children, compared to the rural area - 71 children.

Over 50% of the children in the study presented only atypical reflux symptoms, especially respiratory. These children received antireflux treatment with a very good control of the symptoms.

2.2.4 Discussions

GERD is a frequent disease in the pediatric practice, but still underdiagnosed, and the Romanian and foreign literature has too little space for it. GERD has roots from the intrauterine period, important is that the family knows that the gastroesophageal reflux is more or less physiological in the first semester of life.

GERD manifests as a lot of comorbidities. The anemia, failure to thrive and recurrent respiratory infections makes the difference between the physiological and pathological reflux. The gastroesophageal reflux disease has a complex clinical picture, being often a diagnostic trap especially in respiratory diseases.

The modern investigation methods reveal the reflux disease associated with unspecific clinical symptoms, showing the real incidence of the disease.

Similarly, to the literature data, a prevalence of the males compared to the females was noticed, both in the cases selected in the study (123 boys, 74 girls) and the cases in which the reflux was highlighted upon the pH-metry determination (95 boys, 51 girls).

As the environment, most of the cases were from the urban area, compared to the rural area, explaining the more increased referral of the population in the cities to the specialist physician, mainly the cases with reflux atypical symptoms (i.e., respiratory signs) which do not respond to the classic symptomatic outpatient treatment and which requires the enlargement of the investigation area.

Regarding the age of the studied cases, they were divided on 4 age categories: 0-1 year, 1-3 years, 3-10 years and over 10 years. In the 0–1-year age group, the pH-metry was conducted in 48 children and the reflux was present in 34 cases (70.83%). Between 1-3 years, out of 51 analyzed cases, 41 children (80.39%) had proven reflux at pH-metry; between 3-10 years, 55 children (71.05%) out of the 76 studied children, showed reflux and over 10 years, in 16 cases out of the 22 analyzed, reflux was proved by pH-metry. The large number of cases studied in the 0–1-year age group is correlated to the increased incidence of recurrent regurgitations and vomiting in that age. After the age of 1, the atypical signs of reflux increased, in particular the respiratory signs. In 68% of the cases with pH-metry proven reflux, with atypical symptoms of reflux (particularly respiratory), the starting of anti-reflux specific treatment determined the improvement of the signs. The enrolment in the study group based on the presence of symptoms which raised the suspicion of GERD, proving the reflux by pH-metry in only 146 of the studied cases and its invalidation in 51 cases, shows the possibility of diagnosis traps, that is why the determination of the esophageal pH imposes in all suspected cases of having GERD.

2.2.5 Conclusions

The esophageal pH-metry has a decisive role in the GERD diagnosis, in particular in children with atypical signs of reflux.

The continuous pH monitoring for 24 hours is the preferable method, allowing for the careful assessment of the events associated with the reflux.

The esophageal pH-metry should be performed in all children in whom there is the clinical suspicion of pathological reflux.

The big advantage of the esophageal pH-metry is the possibility of out patient monitoring of children with GERD.

2.3 What are the limits between physiologic and pathologic gastroesophageal reflux in relation to age?

2.3.1 Introduction

Postprandial regurgitation is the most frequent sign of GER in infants. It is physiological in the first 3 months of life. Regurgitations usually disappear by 12 to 24 months. Risk categories are represented by: premature and dysmature infants, newborns who suffered from hypoxia or perinatal asphyxia, infants who benefitted from an “a demeure” gastric probe, infants with “gastric emptying retardation”, newborns operated for esophageal atresia, encephalopathic patients (Dusick, 1997).

The majority episodes of reflux are short and do not cause symptoms or complications.

GERD occurs when the reflux of the gastric contents causes symptoms that affect the quality of life, or pathological complications occur, such as weight stagnation, loss of appetite,

sleep disturbances, chronic respiratory disorders, esophagitis, hematemesis, apnea, life-threatening conditions (Czinn and Blanchard, 2013).

Personal contribution – published paper:

1. A. **Lupu VV**, Ignat A, Paduraru G, Burlea M. Gastroesophageal reflux in newborns and infants (I). *Revista Română de Pediatrie, 2015,* LXIV(4): 387-392
   B: **Lupu VV**, Ignat A, Paduraru G, Burlea M. Gastroesophageal reflux in newborns and infants (II). *Revista Română de Pediatrie, 2016,* LXV(1): 10-14

The aim of this review is to discuss actual theories concerning epidemiology, etiopathogenesis, clinical manifestations and paraclinical aspects in GER, as well as to point some of the new directions for GER prevention and therapy.

2.3.2 Epidemiology

The prevalence of the pathologic GER is not exactly established in the small child. According to the parents' statements, between 5 and 50% of the infants under 1 year old have numerous regurgitation episodes cause suspicion of pathologic GER (Orenstein et al., 1996; Vandenplas et al., 1996).

The authors of a research reported the presence of repeated regurgitations in approximately 5% of the infants between 2 and 4 months (Kelmanson et al., 2000). In general, the prevalence of reflux in infants and small children is influenced by the fact that the original diagnosis is for the most part based on the parents 'accounts. Parents are not always conscious of the symptoms and can overlook GER signs.

The authors of some studies have shown that there are a couple of infants with cow's milk protein allergy who present with vomiting and regurgitation, that are equivalent with GER symptoms (Iacono G et al., 1996; Borrelli et al., 2012).

Numerous factors such as the infant's sleeping position, activity (crying), feeding manner, food type and medication can influence the frequency and severity of reflux (Tobin et al., 1997; Badriul and Vandenplas, 1999).

It seems that the incidence of regurgitations is similar in breastfed infants and formula-fed infants (Jiang et al, 2001).

Genetic factors might have a role to play in the etiology of GER. For infantile GER there is reported a gene 9q22-9q31, so it seems to be an increased concordance of GER in monozygotic twins compared with dizygotic twins (Orenstein et al., 2002).

2.3.3 Etiopathogenesis

Lower esophageal sphincter dysfunction, esophageal dysfunction and gastric dysfunction are the pathogenic mechanisms allowing the gastric content to reflow into the esophagus.

The lower esophageal sphincter (LES) dysfunctions caused by mechanic dysfunctions (shortening of the intraabdominal segment of the esophagus), LES dystonia (changes in anatomic relations, conditions of the sphincteral and esophageal muscles in systemic diseases – scleroderma, Sjögren syndrome, diabetes mellitus, myxedema, systemic erythematous lupus, polymyositis), food (chocolate, pepper, fats, carbonated drinks, citrus fruits, tomatoes).

Furthermore, the lower esophageal resting pressure can be changed by medication; reduced by anticholinergics, theophylline, caffeine, nicotine, alcohol, dopaminergic drugs, epinephrine, E1 and E2 prostaglandins, certain hormones (glucagon, secretin, cholecystokinin, progesterone, estrogen); increased by: cholinergic drugs, dopamine receptor antagonists, serotonin, histamine, norepinephrine, phenylephrine, gastrin.

Abnormal esophageal clearance is caused by esophageal dysfunction. 4 mechanisms are responsible for its efficiency: the motor activity of the esophagus (peristaltic waves), motility disturbance causes GER; the gravitational force - if absent, the clearance is delayed; the saliva secretion – due to its bicarbonate content it buffers the acid reflow from the stomach, the decrease in saliva secretion being caused by: tobacco, anticholinergic medication, radiotherapy, xerostomia; the secretion of the esophageal (mucous and non-mucous) glands.

Gastric dysfunction is caused by: increase in the gastric volume (overfeeding), gastric distension (aerophagia, prolonged overfeeding), increase in the intraabdominal pressure (obesity, coughing effort, sneeze, defecation), delayed gastric emptying (abnormal antral motility), duodenal-gastric reflux, gastric hypersecretion (causes perturbations of the normal esophageal peristalsis).

2.3.4 Clinical manifestations

Non-complicated GER requires a functional or physiological process in a healthy child, without subjacent systemic anomalies.

Clinical manifestations vary with age. Regurgitation is present in nearly all cases and is the most frequent symptom. Although GER is frequently present at birth, regurgitation may occur in the second or third week of life, when oral intake is increased.

Healthy infants can have high prevalence of reflux symptoms, such as daily regurgitations (40%), mild respiratory symptoms, crying for more than one hour a day (17%), arching (10%), or daily hiccups (36%) (Orenstein et al., 1996). The GER that is complicated by excessive crying, irritability, sleep perturbations, lack of appetite, stationary weight curve or respiratory complications can usually be clinically diagnosed without an extended evaluation. In 85% of the infants with non-complicated GER, it is self-limited, and it usually disappears between the age of 6 and 12 months (Sondheimer, 2003).

The GERD is a pathologic process in infants manifested through difficult weight gain, esophagitis, hidden hemorrhages, anemia, persistent respiratory symptoms or complications and neurobehavioral changes. GERD appears in approximately 1 out of 300 infants (Herbst, 2000).

A child with GERD has more than five reflux episodes a day, regurgitates more than 28 g/episode, refuses feeding, has apnea episodes, has a stationary weight curve and irritability (Reust and Blake, 2000). The differentiation between GER and GERD in infants is based on clinical manifestations (Table 2.1).

Table 2.1. *The difference between GER and GERD (Jung, 2001)*

GER	GERD
Regurgitations with normal weight curve	Regurgitations with weight loss or stationary weight curve
No signs or symptoms of esophagitis	Persistent irritability, dysphagia, food refusal; hematemesis, melena, hyposideremia, anemia
No significant respiratory symptoms	Apnea and cyanosis, sleep disorders, wheezing or stridor, aspiration or recurrent pneumonia, chronic cough, dysphonia
No neurobehavioral symptoms	Abnormal posture (Sandifer syndrome)

The symptoms or complications of reflux in infants include: food refusal, stationary weight curve, irritability, sleep disorders and respiratory symptoms. Furthermore, GERD in infants can be associated with cough, suffocation, wheezing or symptoms of the upper respiratory tract. Despite this, the GERD diagnosis based on symptoms can be difficult, especially because medication does not always improve the symptoms.

Digestive manifestations

The digestive manifestations are represented by symptoms that suggest esophageal pain (Stanciu, 1993):

● ***Regurgitations***

Physiological regurgitations appear in the first weeks of life and disappear towards the age of 12 to 18 months. The pathologic ones are abnormal induration and in number per 24 hours, are favored by posture changes or by the situations that increase intraabdominal pressure. In infants allergic to the cow milk protein, regurgitations and vomits cannot be differentiated from the ones specific to physiological GER (Orenstein et al, 2009; Nielsen et al., 2004). In such infants, the presence of vomits decreases significantly (usually in approximately 2 weeks) after the elimination of cow milk from their diet, and reintroducing it causes the reappearance of the symptoms (Iacono et al., 1996).

The pathologic regurgitation is the expression of the pathologic GER, but it may also appear in other etiologic circumstances, therefore requiring a differential diagnosis with: 1) congenital esophageal obstructions: esophageal stenosis, esophageal atresia with or without fistula, hiatal hernia; 2) acquired esophageal lesions: esophagitis, strictures, pseudodiverticula, esophageal foreign bodies, retroesophageal abscess (Georgmanescu, 1997).

Regurgitations of the pathologic reflux are abnormal as regards duration, number/24 hours and are favored by posture or by the situations that increase the intra-abdominal pressure.

According to the taste of the regurgitated material perceived in the mouth the acid character (sour taste) or the alkaline character (bitter taste) of the reflux may be appreciated (Pascu and Grigorescu, 1996).

● ***Vomiting***

Vomits are accentuated by crying or by prone position, by situations in which the intra-abdominal pressure increases and, in most cases, it is followed by regurgitations (Georgmanescu, 1997).

● ***Rumination (merycism)***

Rumination is a rare but severe form of chronic regurgitation

Rumination (merycism) is repetitive regurgitation of food from the stomach, followed by its chewing and re-swallowing or expulsion from the mouth. Its debut is between months 3 and 6 of life. Rumination in infants is a rare but serious condition, which can develop when there is no mutual interaction between the child and his caregiver. The child learns to bring the gastric content back into his mouth as a self-stimulation means and in order to satisfy his needs that are not satisfied by his caregiver (Malcolm et al., 1997).

The differential diagnosis with other pathologies associated to vomits or regurgitations is necessary (Table 2.2) (NICE guideline, 2015).

***Table 2.2**. Alarming symptoms suggesting diseases other than GER (NICE guideline, 2015)*

Signs and symptoms	**Possible diagnosis implications**	**Recommended actions**
Gastrointestinal diseases		
Frequent strong vomits (in the form of a projectile)	They may suggest hypertrophic pyloric stenosis in infants up to 2 months old	Surgical intervention
Bile vomits	They may suggest intestinal obstruction	Surgical intervention
Hematemesis except for the blood swallowed (epistaxis, nipple cracks in the case of breastfed infants)	It may suggest esophagus, stomach or upper intestinal hemorrhage	Specialty examination
The debut of regurgitations and/or vomits after 6 months or persisting after 1 year	The late debut suggests another cause than reflux (for instance, urinary tract infection)	Specialty examination Microbiological urine test
Blood in stool	It may suggest a variety of conditions such as bacterial gastroenteritis, cow milk protein allergy (CMPA), or an acute surgical condition	Specialty examination Microbiological stool test
Abdominal distension, sensitivity or palpable mass	It may suggest intestinal obstruction or another acute surgical condition	Surgical intervention
Chronic diarrhea	It may suggest CMPA	Specialty examination
Systemic diseases		
Influenced general state Fever	Various infections	Specialty examination Clinical evaluation Microbiological urine test
Bulging of the fontanelle	It may suggest high intracranial pressure (meningitis)	Specialty examination
The quick increase in the cranial circumference (more than 1 cm a week) Aggravation of morning vomits	It may suggest high intracranial pressure (hydrocephaly or brain tumor)	Specialty examination
Altered receptivity (lethargy or irritability)	It may suggest meningitis	Specialty examination
Infants and children with a high risk of atopy	It may suggest CMPA	Specialty examination

Dysphagia is rare, as it is the expression of esophagitis lesions. This is suspected in the infant or child who refuses the bottle or the teaspoon.

Dysphagia emerges in association with oral or esophageal anatomic anomalies, neurological and motor disorders, oral or esophageal inflammatory diseases. GERD is quoted as a reason of dysphagia or odynophagia, but there are no studies on children to demonstrate this relation, as the symptomatology did not improve after the anti-reflux therapy.

Digestive hemorrhages are manifested by small hematemesis.

Sialorrhea is triggered by the esophageal-salivary reflex when the re-flown gastric content comes into contact with the esophageal mucous membrane.

Respiratory manifestations

Respiratory manifestations are expressed by chronic cough, obstructive apnea crises, wheezing, chronic or recurrent pneumonia, cyanosis attacks accompanied by stridor, hiccup, dysphonia, aspiration pneumonias, recurring obstructive bronchitis or recurring otitis media.

The association with GERD of the respiratory manifestations has been explained in time through 3 theories: micro aspiration of acid refluxate into the respiratory tract, suggested by the anatomic relation between the digestive and the respiratory tracts; the common embryonic origin of the esophageal and pulmonary nervous fibers - vagal mediated bronchospasm; the pulmonary manifestations can be the cause of reflux – inverse relation.

A study on infants based on the impedance-pHmetry and cardiorespiratory monitoring reported a 30% association between short episodes of apnea and reflux (Wenzl et al., 2001).

Recurrent pneumonia and interstitial pneumonia can be complications of reflux, probably following the failure of the respiratory tract protection mechanisms to protect the lungs from the gastric aspirate (Boesch et al., 2006).

Children with respiratory complications are generally considered the most susceptible to benefit from anti-reflux surgery when medical treatment fails, but additional studies are necessary to confirm this hypothesis (Vandenplas et al., 2009).

Neurobehavioral manifestations

Neurobehavioral manifestations have been associated with "visceral hyperalgesia" (Ciofu and Ciofu, 2002) or with pain perception and can be characterized by: sleep disorders; crises of agitation and crying; arching and rigidity, hyperextension of the neck; generalized irritability; convulsions or pseudo-psychiatric behavior, occasionally.

In older children, they are particularly associated with Sandifer syndrome, which comprises a complex of signs, such as the extension of the head, torticollis, twisted neck, opisthotonos posturing, occasionally with facial asymmetry, which occurs as a specific response of the head to reflux. Furthermore, they can be associated with hiatal hernia, and in 50% of the cases, lesions of esophagitis are encountered (Turcanu et al., 1988).

Nutritional consequences

They are the consequence of a prolonged and severe gastroesophageal reflux disease. Therefore, the following may occur:

- weight and height growth disorders, when vomiting and nourishment refusal (due to the esophagitis lesions) are long-termed, "the failure to grow" (Ciofu and Ciofu, 2002);
- signs of chronic dehydration, even states of nonacid chronic ketosis, in persistent, untreated forms;
- microcytic hypochromic anemia, due to chronic vomiting or digestive hemorrhages (Burlacu, 2005).

2.3.5 Paraclinical aspects

Generally, the medical history told by the parents is sufficient to diagnose reflux, especially if the problems appear regularly and causes discomfort.

Usually, GERD is not investigated or treated in infants without regurgitations and showing only one of the following: inexplicable feeding difficulties (for instance, food refusal), unusual behavior, growth difficulty, chronic cough, dysphonia, only one pneumonia episode (NICE guideline, 2015).

The growth chart and feeding history are also of help, but occasionally additional tests are recommended.

Esophageal tests that may be helpful in diagnosis are the barium esophagogram, gastroesophageal scintigraphy, and prolonged esophageal pH–monitoring. Regarding esophageal pH-monitoring, it can directly correlate acid reflux episodes with wheezing or other symptoms of bronchospasm. The 24 hours esophageal pH-monitoring is the most used method to diagnose the acid reflux. The sensitivity of the esophageal pH-monitoring is higher than 85% and its specificity is 95%. Sensitivity is improved by using impedance pH-monitoring, but this technology has not yet been standardized to a level which would satisfy the definition of a test suitable for routine clinical practice (Ahmed T et al, 2005).

Approximately two thirds of the patients affected with asthma responded favorable to anti-reflux therapy.

As regards the GERD diagnosis, the AJG (The American Journal of Gastroenterology) Guides from 2013 recommend:

1. A presumptive GERD diagnosis can be set in typical cases with regurgitations. Empirical medical treatment with a proton-pump inhibitor (PPI) is recommended in this case.

2. The eso-gastro-duodenal barium swallow examinations is not to be done to diagnose GERD.

3. Upper digestive endoscopy is not necessary in the presence of typical GERD symptoms. Endoscopy is recommended in the presence of alarming symptoms and for the screening of patients with high risk of complications. It is not indicated to repeat endoscopy in patients lacking Barret's esophagus or in the absence of over-added symptoms.

4. Biopsies from the distal esophagus are not recommended as a routine in diagnosing GERD.

5. Esophageal manometry is recommended for postoperative evaluation, but it is not to be used in diagnosing GERD.

6. Outpatient monitoring of esophageal reflux is indicated before considering endoscopy or surgical treatment in patients with non-erosive reflux disease, as part of the evaluation of patients' refractory to the PPI therapy and when the GERD diagnosis is uncertain. The outpatient monitoring of the esophageal reflux is the only test that can evaluate the association of reflux with other symptoms.

7. The screening for the *Helicobacter pylori* (*H. pylori*) infection is not recommended in GERD. The eradication of the *H. pylori* infection is not a routine necessity as part of the antireflux therapy (Philip et al., 2013). Outpatient monitoring of gastro-esophageal reflux (pH-metry or impedance-pH-metry) is a test allowing the tracking of abnormal esophageal exposure to acid, the frequency of reflux and the symptoms associated with the reflux episodes. PH-metry is the investigation technique of the highest sensitivity (77-100%) and specificity (85-100%) in detecting and quantifying acid GER. The impedance-pH-metry increases the sensitivity for the detection of reflux to almost 90% (Sifrim et al, 2004).

Esophageal impedance is a technique used to detect the movement of (acid and alkali) fluids, solids and gases in the esophageal lumen. This can be used to measure the volume, the speed and physical length of the esophageal bolus. Impedance-pH-metry has the same indications as pH-metry, being useful especially in evaluating intermittent symptomatology such as cough, dysphagia or apnea. Thus, for the past few years it has been used to investigate how GER and GERD correlate with apnea, cough and neurobehavioral disorders. PH-metry and impedance-pH-metry are recommended in infants suspected of recurrent aspiration pneumonia, inexplicable apnea, upper airways infections, dental erosions associated with neurodisability, frequent otitis media, possible need to perform a fundoplication (NICE guideline, 2015).

Upper digestive endoscopy allows the direct examination of the esophageal mucous membrane. The macroscopic lesions associated with GERD are represented by esophagitis, erosions, exudate, ulcerations, strictures, hiatal herniation, areas of esophageal metaplasia and

sometimes polyps. Sensitivity, specificity and the predictive positive value vary from 29% to 86%, from 21% to 83% and from 80% to 82% respectively, if we are to compare the pH-metry results (Chen et al., 1992; Aksglaede et al., 2004).

2.3.6 GERD treatment

The treatment options in physiological GER and in GERD include: lifestyle changes, pharmacological therapy and surgery. Lifestyle changes in infants with physiological GER include feeding and positional changes. Parents' education, guides and moral support are always necessary and usually enough for infants with physiological GER symptoms. In young infants with functional GER, regurgitations and vomits spontaneously disappear until the age of 1 year old.

Parents have to be informed of the physiological nature of the condition and of the distinction between physiological and pathological reflux. Parents need to be listened to and comforted. We have the obligation to understand their anxiety, and tell them about the natural history of the disease. Parents have to be informed completely and to the point because most procedures will be performed by the parents.

Diet-related measures

Approximately 50% of the healthy infants aged 3 to 4 months have at least one regurgitation a day (Martin et al., 2002). Both breastfed infants and formula-fed infants have a similar frequency of physiological reflux, but the length of the reflux episodes established by the pH monitoring can be shorter in breastfed infants (Osatakul et al., 2002; Barak et al., 2006).

A study done on infants showed that feeding a large amount of milk caused regurgitation, probably due to the increase in the frequency of the transient LES relaxation and a reduced volume of milk decreased the reflux frequency (Khoshoo et al., 2000). The severe reduction of the food amount for a long time can deprive the newborn of the necessary caloric energy and can affect weight gain. Infants with inappropriate weight due to the losses caused by regurgitations can benefit from the caloric energy increase by means of special formulas when the volume or frequency of feeding is lower as part of the therapy (Vandenplas et al., 2009).

Products that thicken meals or thickened formulas (Gelopectose 3-5%, Gumilk 2%) and anti-reflux milks lead to the decrease in the number of regurgitations; similarly, rich foods for infants such as cereal formula seem to reduce GER. They increase viscosity and form a fine flocculate easy to evacuate from the stomach, remaining as a suspension in the gastric fluid (Georgescu, 2005).

Lunches thickened with rice cereal are associated with a decrease in the number of vomiting episodes, but the reflux index is not improved (Rudolph et al., 2001). There are studies reporting that thickened formulas can reduce the frequency of regurgitations and the total volume per vomit. Furthermore, they can reduce the child's crying time and increase the sleeping time (Jiang et al., 2001). Other studies show that thickening lunches can aggravate cough episodes and/or other postprandial respiratory symptoms (Oyen et al., 1997). The rice cereals used increase the caloric density of the formula and can cause constipation. Milk thickening is associated with a higher risk for necrotic enterocolitis among premature infants.

In addition, milk formulas that are evacuated faster from the stomach are used. The richer the milk formulas are in casein, the slower is their evacuation. Casein flocculates in the acid gastric environment (Georgescu, 2005).

The tobacco environment will be suppressed as it decreases the LES pressure. Moreover, all sources of abdomen compression must be avoided: wrapping the infant or clothes that are too tight, inappropriate anti-herniation bandage or sudden handling that can cause regurgitations or vomits (Georgescu, 2005).

Consequently, in formula-fed infants with frequent regurgitation, the following approach is recommended: the feeding history is established, the meal amount is reduced only if they are excessive for the child's weight, smaller and more frequent lunches are provided (at the same time maintaining the same amount of daily milk) except if lunches are already small and frequent, then thickened formulas are added (for example, the ones containing rice starch, corn starch or carob flour) (NICE guideline, 2015).

In breastfed infants who continue to manifest regurgitations despite evaluation and counselling, alginate therapy is considered for 1 to 2 weeks. If the alginate therapy is beneficial, it is maintained, but it is suspended at certain intervals to check if the infant has recovered (NICE guideline, 2015).

In formula-fed infants, if this approach is not successful, the thickened milk formulas are removed from feeding and an alginate therapy is administered for 1 to 2 weeks. Similar to the breastfed infants, if the alginate therapy is beneficial, it is maintained, but it is suspended at certain intervals to check if the infant has recovered (NICE guideline, 2015).

Medication suppressing acidity such as proton pump inhibitors (PPI), H2 receptor antagonists is not administered to treat infants with regurgitations as isolated symptoms (NICE guideline, 2015).

Postural recommendations

Originally, it was noticed that positioning the baby in ventral decubitus with the head higher leads to less frequent and shorter reflux episodes, but without significant effects on the duration of the reflux (Orenstein and Whitington, 1983). Subsequently, the possibility of sudden death in this position reduced enthusiasm. In many countries a campaign against ventral positioning of infants was even launched due to the sudden death risk. This positioning can be useful when the infant is awake. This way, placing the awake infant in ventral position with the head on one side, inclined at 30-45^{o} is efficient in controlling regurgitations and/or vomits and implicitly reflux (Vandenplas et al., 1997).

PH-metry helped prove that the ventral position decreases exposure of the esophagus to the acid refluxate in comparison to the dorsal position (Bhat et al., 2007).

The supine position and the semi-sitting position are to be avoided, placing the infant in the car seat or in any sitting position after meals is to be avoided, as it can exacerbate the GER, by increasing intra-abdominal pressure.

Consequently, the postural therapy is a therapeutic manner useful in small children, by placing them on a plane inclined at 35-40^{o} in dorsal decubitus under surveillance.

Pharmacological treatment

The purpose of the pharmacological therapy in GERD is: to improve the reflux symptoms, to prevent major complications and surgery, to prevent the recurrence of the disease. Pharmacotherapy is indicated in pathologic GER or when the conservative treatment (postural therapy and diet) did not have the expected result.

Therapy with PPI or H2 receptor antagonists is not recommended for the treatment of infants with regurgitations as an isolated symptom.

A 4-week treatment with PPI or H2 receptor antagonists is considered for patients who cannot account their symptoms (infants), with numerous regurgitations and difficult feeding (for example, in infants refusing food or with suffocating episodes), unusual behavior or growth difficulties.

If the symptoms do not disappear or reappear after the treatment with PPI or H2 receptor antagonists is stopped, an upper digestive endoscopy is considered.

When the PPI or the H2 receptor antagonists are chosen, their availability for the age in question, the parents' preference and the purchase price are taken into account.

PPI is the most efficient treatment that suppresses hydrochloric acid, binds covalently and deactivates the proton pumps in the parietal cells (pumps H+/K+ - ATPase). Due to their anti-secretory effect, they are the preferred medication in the treatment of GER, as they entail the disappearance of the reflux symptoms in over 90% of the cases (Hassall, 2005).

This empirical treatment is maintained during 4 to 8 weeks and is a true diagnosis test that is simple and cost-efficient, including for those with extra digestive symptoms (Walker et al., 2004).

Maximum efficiency is obtained when they are administered ½ hour before breakfast so that the peak of plasma concentration may coincide with the meal; if the treatment is administered in two daily intakes, the second dose is administered ½ hour before the evening meal. PPI are represented by benzimidazoles: Omeprazole, Pantoprazole, Lansoprazole, Esomeprazole, Rabeprazole.

French authors reported high toxicity for PPI: headache, vertigo, sleepiness, cutaneous reactions, mental confusion, hematological changes, hepatitis (possibly in fulminant form), digestive and electrolytic disorders, medication interactions (Castot et al, 1993).

The basic indication of the PPI treatment remains the severe esophageal lesion (erosion and esophageal ulceration) or Barrett's esophagus. The administration of PPI to those with non-acid reflux does not influence the volume and frequency of the reflux episodes in comparison to those with acid reflux.

The emergence during the PPI treatment of nocturnal "acid leaks" (i.e., nighttime reflux episode) is controlled by doubling the PPI dose or by associating a H2 receptor blocker intermittently, to prevent tachyphylaxis, which develops quickly under continuous administration.

H2 receptor antagonists reduce the acid secretion by inhibiting the H2 histamine receptors in the gastric parietal cells. In a study on infants, ranitidine (2 mg/kg/dose) reduced the time in which the pH was < 4 by 44% when they were administered twice a day and by 90% when they were administered three times a day (Sutphen and Dillard, 1989). Among the members of the series (Cimetidine, Ranitidine, Famotidine, Nizatidine), the most studied and used in pediatrics are Ranitidine and Nizatidine (Paul et al., 2001). H2 receptor blockers are still used as first-line therapy in mild and medium esophagitis, for 6 to 8 weeks in mild esophagitis and for 3 to 4 weeks for moderate forms of esophagitis. H2 receptor antagonists' therapy is efficient, but it should not be used on a long term. They can cause tachyphylaxis in six weeks' time and can increase the risk of liver disease and gynecomastia.

Metoclopramide, domperidone and erythromycin are not administered to treat GER or GERD, considering their potential in side effects (NICE guideline, 2015).

Prokinetics can reduce the symptoms of GERD by improving the contractility of the esophageal body, increasing the LES pressure and increasing the gastric emptying rate. Despite this, the benefits of these agents cannot overcome negative effects such as: sleepiness, agitation and extrapyramidal reactions. There is no sufficient proof to support the routine use of prokinetics in GERD in children.

Surgical treatment

For the surgical treatment (fundoplication), an upper digestive endoscopy is performed for biopsy purposes. pH-metry or impedance pH-metry can also be performed before surgery.

After the GERD diagnosis is confirmed, surgical therapy is considered for patients:

- for whom medical management was not successful (severe regurgitations uncontrolled by treatment or important side effects following the treatment);
- for whom surgery is an option despite the efficient treatment (life quality, the administration of medication during the patient's entire life, the cost of medication is taken into consideration);

- who develop GERD complications (Barrett's esophagus, peptic stenosis) (Spechler and Goyal, 1996; Lagergren et al., 1999);
- who show extra-digestive manifestations (dysphonia, chronic cough, aspiration pneumonia) (Rakita et al., 2006; Meyer et al., 2004; Lindstrom et al., 2002).

In addition, in infants with severe GERD, fundoplication is performed when the diet-related measures have proved inefficient, in case feeding thickened formulas by nasogastric tube continues for a long time (NICE guideline, 2015).

The Nissen fundoplication is the preferred method, being very effective in controlling vomits. It is performed in classic surgery, and lately more and more centers choose to do the intervention laparoscopically, which is superior to the classic techniques, due to better visibility and its less invasive and aggressive character.

Generally, anti-reflux surgery has been more attentively evaluated in adults than in children. Among the children who had surgery, those with neurological disorders suffered from complications twice more often, morbidity was three times higher, and a second surgery was necessary 4 times more often than in those without neurologic disorders (Smith et al., 1992; Spitz et al., 1993).

Fundoplication in early childhood has a rate of failure higher than fundoplication performed later in childhood (Diaz et al., 2005).

2.3.7 Evolution of GERD in newborns and infants

The consequences of reflux can become obvious from the very neonatal and small infant period. At this age, the emetic form, the hemorrhagic form and respiratory forms or forms of neurologic expression settle into shape.

Hematemesis emerges before the age of 10 days of life (through esophagitis often associated with gastroduodenitis) and is the main cause for hemorrhage at this age, being aggravated by hypovitaminosis K; thus, prophylaxis with vitamin K administered systematically during the first hours of life becomes useful, and in some countries, it is maintained until the age of 3 months (for example in Italy).

Moreover, a pathologic GER can generate paroxysmal attacks, which often have a dramatic course, with cyanosis and hypotonia, respiratory distress, laryngeal dyspnea or apnea crises, needing reanimation (sometimes marks sudden death); manifestations are hard to correlate with reflux, but the positive answer to anti-reflux therapy and the pH-metry tests confirm the cause.

In addition, at the same age, other consequences of reflux are not negligible either, such as the respiratory ones: repeated bronchiolitis or pneumopathies, nocturnal cough and wheezing episodes, which – in evolution – turn into chronic respiratory disease, and nutritional consequences: weight stagnation, hypochromic anemia.

2.3.8 Prognostic in GERD

The prognostic for infants with a history of physiological or functional GER is very good, even with spontaneous and full healing.

GERD raises problems where the manner of treatment, the response to therapy and time plays a decisive role.

The following can deem unfavorable prognosis factors:

- delayed diagnosis, in the phase of severe esophagitis, esophageal stenosis or Barrett's esophagus;
- encephalopathic children or children with other associated neurologic disorders or behavioral disorders; considering that infants with psychomotor retardation are affected

by GER in over one third of the cases. These develop extended esophagitis lesions generating short-term anemia, and long-term esophageal stenosis lesions;

- other associated diseases such as: scleroderma, mucoviscidosis;
- Incorrect treatment, with discordance between the lesion type and the type of treatment used;
- the patients' or parents' lack of compliance;
- resistance to treatment (for example, nonresponsive to the PPI treatment).

GERD is a condition with a good prognostic, the therapeutic results reported by all the data in literature are favorable. Every case must and needs to be individualized.

2.3.9 Conclusions

It is important to establish accurately the physiological or pathological character of gastroesophageal reflux to know the subsequent therapeutic approach.

Reduced compliance to laborious procedures (pH-metry, impedance pH-metry and upper digestive endoscopy) makes the clinical examination and parents' accounts sufficient to diagnose reflux.

In the case of newborns and infants, it is important to apply the diet-related and postural measures necessary when regurgitations and vomits appear.

2.4 Respiratory symptoms in gastroesophageal reflux disease

2.4.1 Introduction

The main role of the esophageal tract is of leading the bolus by means of the common action of the buccal, pharyngeal, and esophageal muscle formations and indirectly of the laryngeal ones. Another important role of it is its secretory function: the esophagus secretion has a mucous consistency and plays a major role in lubricating the surface of the esophageal mucous membrane, favoring the progression of the bolus; it is vagally mediated; the mucus is produced by numerous acinar glands located in the submucous membrane.

Reflux is physiological during the first 3 months of life, manifested very early, usually during the first 6 weeks of life (Ciofu and Ciofu, 2002). For most cases, regurgitations disappear by the age of 12-24 months, and their persistence after this age raises the problem of the reflux consequences – reflux esophagitis, and the inclusion of cases in the gastroesophageal reflux disease group (Walker et al., 2004).

The population with a risk of GERD includes: 1) premature and dysmature babies; 2) newborns who suffered from hypoxia or perinatal asphyxia; 3) babies who benefited from a gastric tube "a demeure"; 4) babies with a delayed gastric emptying (primary or secondary); 5) newborns operated for esophageal atresia with or without fistula; 6) cystic fibrosis; 7) encephalopathic children (Geormăneanu, 1997).

The GERD is a frequent entity in pediatrics, which is still under-diagnosed ("the disease of the third millennium"). The polymorphic clinical symptoms are dominated not only by post-feeding vomiting, but in many cases also by respiratory symptoms (from night cough to apnea episodes, recurrent wheezing, aspiration pneumonia and even sudden death syndrome).

Personal contribution – published paper:

1. Burlea M, **Lupu VV***, Paduraru G, Diaconescu S, Ignat A. Respiratory symptoms in children with gastroesophageal reflux disease. *Bulgarian Medical Journal*, 2013, 7(1): 83-86

The aim of this review was to discuss actual theories concerning respiratory manifestations in GER, as well as to highlight the cases of this complex disease.

2.4.2 Etiopathogenesis

Three pathogenic links participate in variable proportions in the appearance of the pathological gastroesophageal reflux (Ciofu and Ciofu, 2001): dysfunction of the lower esophageal sphincter (LES), esophageal dysfunction, and gastric dysfunction.

Dysfunction of the Lower Esophageal Sphincter

Transitory relaxation is the major mechanism producing GER, while basal (underlying) relaxation of the lower esophageal sphincter (LES) is the minor mechanism producing gastroesophageal reflux (Ciofu and Ciofu, 2001; Georgescu, 2005).

The transitory relaxation of the LES can be spontaneous and usually emerges after the normal peristaltic movements. Literature studies attempted to individualize factors responsible for the transitory relaxations of the LES, but the data obtained are insufficient. More studies assert the involvement of a vagal reflex – a type of neural mechanism with a starting point at the level of mechanoreceptors of the gastric wall stimulated by the gastric distension (Walker et al., 2004).

The production of abnormal pressure at the LES level or changes in the LES length or position creates the premises of the emergence of the gastroesophageal reflex:

- Mechanic dysfunctions of the LES – the shortening of the intraabdominal segment of the esophagus (Bufo et al., 1997) – determines the change in the LES normal pressure, therefore it can no longer maintain its tonus in certain conditions: changes of the body position, situations in which the intraabdominal pressure increases, favoring reflux;
- Dystonia of the LES – appear in case of: 1) changes in the anatomic relations of the structures ensuring the eso-cardiac-tuberosity fixation (phrenic esophageal ligament and gastrophrenic ligament) (Burlacu, 2005); 2) conditions of the sphincter and esophageal muscles in systemic diseases that change the LES peristalsis and pressure: scleroderma, Sjogren syndrome, diabetes mellitus, myxedema, SLE, polymyositis; 3) different incoordination, vagotomy, sclerotherapy (Pascu and Grigorescu, 1996).
- Hormones, peptides, pharmacological agents (Burlea, 1999) – acting by: 1) increasing the pressure of the lower esophageal sphincter – acetylcholine, histamine, encephalin, bombesin, motilin, alpha-adrenergic agonists; 2) decreasing the LES pressure – alpha-adrenergic agonists, serotonin, progesterone, glucagon, dopamine, VIP, AMPc.

Esophageal dysfunction

It is represented by the insufficiency of the esophageal clearance leading to esophagus lesion. The clearance defines the capacity of the esophagus of removing the reflowed material and, thus, of shortening its contact with the esophagus mucous membrane.

Four mechanisms ensure the efficiency of the esophageal clearance:

- the motor activity of the esophagus (peristaltic waves) – the gastric reflowed material that reaches the stomach during transitory relaxation of the LES is resent to the stomach by means of the peristaltic waves (Robinson, 1994). Perturbations of the esophageal motility determine GER.
- the force of gravity – in orthostatism, contributes to resending the gastric reflowed material back to the stomach; in supine position or during sleep, in the absence of gravity, esophageal clearance is delayed, determining the stagnation of the reflowed material in the esophagus and the irritation of its mucous membrane.

- the salivary secretion – due to its salivary bicarbonate content, buffers the acid reflowed from the stomach, eliminating its irritating effect on the esophagus mucous membrane.
- the secretion of the esophageal glands (mucous or non-mucous) – plays a protective role on the esophageal mucous membrane.

Gastric dysfunction

It is another pathogenic link (Pascu and Grigorescu, 1996) contributing to the production of GER through:

- the increase in the gastric volume – in overfeeding or poor functioning of the gastric emptying (delayed emptying), it determines repeated transitory relaxations of the LES, favoring reflux;
- gastric distension – through aerophagia or prolonged overfeeding, leading to the shortening of the LES length in the intraabdominal segment;
- the increase in the intraabdominal pressure – in values exceeding the basal pressure of the lower esophageal sphincter, determining its relaxation;
- delayed gastric evacuation – in over 40% of the GER patients, gastric evacuation is prolonged; patients with gastric stasis have more abundant GER and more severe esophageal lesions;
- the duodenal-gastric reflux – seems to be the basis of more severe lesions in case of mixed acid and alkaline gastroesophageal reflux, through the combined action of HCl, pepsin, pancreatic enzymes and biliary acids;
- gastric hypersecretion – the increase in the acid concentration aggresses the esophageal mucous membrane during the transitory relaxations of the LES; likewise, acidity determines perturbations of the normal esophageal peristalsis (study of the endoesophageal perfusion with 0.1N diluted hydrochloric acid done by Triadafilopoulos in 1991 (Triadafilopoulos and Castilio, 1991). *H. pylori* favors the appearance of several severe gastric diseases (including Barret esophagus and esophageal adenocarcinoma), and, on the other hand, exercises a protective role against the gastroesophageal reflux disease (Walker et al., 2004).

2.4.3 Respiratory manifestations

The respiratory manifestations are determined by the aspiration of the gastric content in the respiratory ducts and are better represented in children in comparison with adults. The following may occur (Ciofu and Ciofu, 2002; Burlea, 1999; Walker, 2004; Behrman et al., 2004): chronic cough; obstructive apnea crisis; wheezing; chronic or recurrent pneumonia; a simple chronic hoarseness; spasms of cyanosis at which are added stridor, hiccup, dysphonia; aspiration pneumonia; crises of bronchial asthma; recurrent obstructive bronchitis; recurrent otitis media; rhinopharyngitis and repeated pharyngitis.

The presence of fluids in the airways can cause bronchospasm. Most commonly unconscious, macro-aspiration can cause the respiratory tract to close, which can be associated with lesions such as hemorrhagic pneumonitis and non-cardiac pulmonary edema (Marik, 2010). On the other hand, micro-aspiration (reflux theory) can induce bronchospasm directly by stimulating the larynx through the tracheal receptors (Allen and Leclair, 2009). It is also known that the esophagus and trachea have common embryonic origins, hence the theory that acidification of the distal esophagus causes vagal stimulation resulting in bronchoconstriction, unrelated to micro-aspiration (Mathew et al., 2004).

The prevalence of the respiratory manifestations associated with GER is variable. Therefore, in a personal study, Navarro (Navarro, 2000) establishes a quantification of the

respiratory symptoms associated with GER: chronic or recurrent respiratory manifestations – 31%; paroxysmal respiratory manifestations – 10%, including cyanosis spasms – 5%, apnea – 3%, and sudden death – 2%, and he concludes with the fact that there is not always the case of a causal relationship between the respiratory manifestations and the reflux.

The GER prevalence, that was obtained in a study developed on 39 patients diagnosed through a 24-hour monitoring of the pH, was 60% in cases of gastrointestinal affections, 48.6% in cases of respiratory affections, and 75% in a mixed group; the conclusion of the study was that the coexistence of gastrointestinal and respiratory symptoms in patients with GER may be related with severe reflux (Uzun et al., 2012).

Recurrent wheezing represents more than one episode of wheezing within the last 12 months. Recurrent wheezing attacks impairs the quality of life for the patient and his/her family and represent the most common causes of emergency department visits and hospitalizations.

The most common causes of recurrent wheezing are: asthma, gastroesophageal reflux disease, foreign body aspiration, bronchopulmonary dysplasia, bronchiolitis obliterans, an immunodeficiency, primary ciliary dyskinesia, vocal cord dysfunction, cardiac etiologies structural abnormalities (Ducharme et al., 2014).

The authors of a recent study concluded that anemia and GER are risk factors for recurrent hospitalizations for wheezing and should be treated. Another study concluded that silent GER is common in infants with daily wheezing and controlling GER improves morbidity and decreases the need for daily asthma medications (Ozdogan et al., 1999).

Wheezing is the most common symptom associated with asthma in children, but this is also common in non-asthmatic children. A study concluded that only 30% of preschoolers with recurrent wheezing are eventually diagnosed with asthma at the age of six years. There are studies reported that about ½ - 1/3 of infants and children with early-childhood wheezing develop persistent asthma later. It is important to make an early and correct diagnosis and provide the proper treatment to infants with wheezing for the prevention of airway remodeling (Taussig et al., 2003).

Wheezing in early childhood is often in association with a viral respiratory disease. Many unnecessary investigations are conducted, inadequate treatment is provided and the child need hospitalizations and considerable healthcare costs.

Misdiagnosis has led the therapy with inhaled corticosteroids, resulting in some children developing significant steroidal side effects.

There are some studies which confirmed the association between silent GER and food sensitization in infants with recurrent wheezing without food allergy. The presence of silent GER might contribute to early food sensitization and then to the development of atopic asthma in childhood. In a study, the authors showed that in the silent GER group, 12.2% of patients had food sensitization and in the non-GER group, 20.5% had food sensitization, without significantly different between the two study groups. They obtained no difference between GER parameters of subjects who had positive specific IgE to food and those negative (Sung et al., 2010).

The GER diagnosis may be confirmed by an upper endoscopy with esophageal biopsy, esophageal manometry, by monitoring the intraluminal impedance, with a 24-hour monitoring of the esophageal pH or by monitoring the intraluminal impedance combined with a 24-hour monitoring of the esophageal pH (Vandenplas, 2009; Moraes - Filho et al., 2010; Francavilla et al., 2010). None of these methods has all the characteristics necessary to be considered a "golden standard".

To explain the GER association with respiratory manifestations various hypotheses were issued:

- the micro aspiration of acid repressing particles in the respiratory tree, suggested by the anatomic relation between the digestive and respiratory paths (Mendelson, 1946);
- common embryonary origin of the esophageal and pulmonary nervous fibers – bronchospasm through vagal relation;
- the pulmonary manifestations may be a reflux cause – reverse relation.

The association of GER with bronchial asthma is well proven both in the case of a child, as well as in the case of an adult. Two recent systematic assessments indicated the fact that the prevalence of the GER symptoms is considerable higher in adults and children with asthma than in those without asthma (Thakkar et al., 2010).

The elimination of a possible reflux in a child with repeated pneumopathies, chronic cough, recurrent wheezing, repeated crises of bronchial asthma, became a frequent practice (Walker et al., 2004).

The amelioration of the respiratory symptomatology through medical therapy or anti-reflux surgery is an extra argument that certifies the GER interrelation – respiratory manifestations, mainly asthmatiform ones (Sontag, 2000).

The assessment of the respiratory answer to proton-pump inhibitors (PPIs) in a batch of 37 children with obstructive sleep apnea syndrome and GER lead to the conclusion that in these children, the reduction of the obstructive respiratory events following the short-term treatment with PPIs may suggest, in some children, a causal relation between apnea and reflux (Wasilewska et al., 2012).

Esophageal tests that may be helpful in diagnosis are the barium esophagogram, gastroesophageal scintigraphy, and prolonged esophageal pH–monitoring. Regarding esophageal pH- monitoring, it can directly correlate acid reflux episodes with wheezing or other symptoms of bronchospasm. The 24 hours esophageal pH-monitoring is the most used method to diagnose the acid reflux. The sensitivity of the esophageal - pH-monitoring is higher than 85% and its specificity is 95%

Sensitivity is improved by using impedance pH-monitoring, but this technology has not yet been standardized to a level which would satisfy the definition of a test suitable for routine clinical practice.

Approximately two thirds of the patients affected with asthma responded favorably to anti-reflux therapy (Ahmed and Vaezi, 2005).

2.4.4 Treatment

The objectives of the treatment are related to the treatment of the digestive and extra-digestive manifestations, healing of the lesions, if they are present, prevention of reoccurrence of the disease and its complications.

These objectives are reached by applying general measures and pharmacologic and surgical therapy.

General measures are mandatory for all patients and consist in educating parents (the physiological and benign nature of the GER during lactation must be explained). They must distinguish between physiological and pathological GER. The recommended posture is the 30-40^{o} proclive one. Dietetic measures are required both for little children and older children.

The pharmacological therapy consists in the association of prokinetic (Domperidone, Cisapride), anti-acid and anti-secretory medication (proton-pump inhibitors: Omeprazole, Pantoprazole, Lansoprazole, Esomeprazole). At present, the PPI therapy is the most widely accepted medical therapy for children and adults.

Nevertheless, PPI did not succeed to prove benefits in terms of control of the bronchial asthma in children with GER in most of the well-conceived studies (Martinez, 2012). A study has reported two cases of children with asthmatic symptoms that were difficult to treat and

which, eventually, proved to be related to the GER. The two children were treated with anti-reflux procedures and became asymptomatic. Moreover, literature was also reviewed to reveal this complex disease (Zhi - Wei et al., 2012). On the other hand, another recent study disagrees with the repeated use of anti-reflux medication in the treatment of bronchial asthma weakly controlled in childhood, reporting that the PPI treatment was not only inefficient, but the side effects were frequent, including an increased prevalence of symptomatic respiratory infections (Blake and Teague, 2013).

The preferred surgical procedure is represented by Nissen's fundoplication. Surgical measures can be followed by both immediate and distant complications.

2.4.5 Conclusions

GERD is a frequent entity in pediatrics, which is still under-diagnosed ("the disease of the third millennium").

Clinical, rather polymorphic symptoms are dominated not only by post-feeding vomiting, but also by respiratory symptoms, in many other cases (from night cough to episodes of apnea, recurrent wheezing, aspiration pneumonia and even the sudden death syndrome).

GERD therapy is triple: general measures, pharmacological and/or surgical therapy.

The initial drugs (prokinetic agents) were lately replaced by proton-pump inhibitors, as these are considered medicines which can determine the disappearance of reflux symptoms in most of the cases.

2.5 Oral findings in gastroesophageal reflux

2.5.1 Introduction

As mentioned above, GERD can present with classical symptoms. Digestive symptoms (regurgitation, vomiting, merycism) or non-digestive (respiratory, neurobehavioral and/or nutritional consequences) may occur. Respiratory symptoms include: chronic cough, obstructive apnea episodes, wheezing, chronic or recurrent pneumonia, chronic dysphonia, cyanosis episodes, aspiration pneumonia, bronchial asthma, and recurrent otitis media. Neurobehavioral symptoms include sleep disturbances, agitation and crying episodes, neck hyperextension, arching and rigidity or irritability. Also, nutritional consequences such as growth impairment or hypochromic microcytic anemia may occur. Classic symptoms of GER may be absent in some patients. Dentists can frequently diagnose a systemic disease by analyzing the oral cavity, especially the gastroesophageal reflux disease (GERD) with a minimal expression of digestive symptoms. The presence of acid reflux in the oral cavity can cause dental erosion, especially at the palatine surface level (Chen et al., 2016). Dental erosion is defined as the progressive loss of tough dental tissues caused by a chemical (Pindborg, 1970). This has been associated with the ingestion of acidic foods, bulimia, rumination and GERD (Asher and Read, 1987; Jones and Cleaton-Jones, 1989; Schroeder et al., 1995; Barron et al., 2003). The authors of a study performed on 112 children reported a significant incidence of dental erosions in patients with GERD compared to the control group, both in primary teeth and in permanent teeth (Farahmand et al., 2013). Typical oral symptoms in GERD are dental caries, dry mouth, burning sensation, halitosis, mucosal erythema of the palate and uvula.

Personal contribution – published paper:

1. Ignat A, Burlea M, **Lupu VV***, Păduraru G. Oral manifestations of gastroesophageal reflux disease in children. *Romanian Journal of Oral Rehabilitation*, 2017, 9(3): 40-43

The aim of this review was to establish actual theories concerning non classical manifestations in GER, as well as how to approach them.

2.5.2 The protective role of saliva

Saliva plays an important role in the protection of the oral cavity against mechanical, thermal, microbial and chemical damage (endogenous and exogenous acids) (Mihailopol et al., 2011). It provides all the raw ingredients necessary to repair the tough dental tissue by remineralization and has a buffering action both in the resting phase and in the stimulation phase (Edgar et al., 2004). Saliva allows for effective mastication, swallowing and speech. Saliva also has antibacterial and antifungal properties that control the nature of oral biofilm, and thus has a protective role. Acid acts most often on the structure of the tooth in situations where saliva is compromised from a qualitative and quantitative point of view. However, even in situations where saliva is not compromised, low pH acidic media cause a rapid demineralization of the tooth surfaces.

The authors of several studies have found a significant association between GERD, hyposalivation and "dry mouth" (xerostomia), which is commonly associated with the oral burning sensation (Campisi et al., 2008).

The main method for preventing the endogenous erosion of teeth is the elimination of the primary cause by the GERD treatment.

2.5.3 Oral symptoms associated with GERD

Dental erosions

The association between dental erosion and GERD was reported for the first time in 1933 (Ranjitkar et al., 2011). Dental erosion (dental corrosion) is the loss of tooth surface caused by chemical or electrolytic processes of non-bacterial origin, usually involving acids (Ranjitkar et al., 2011; Imfeld, 1996). The etiology of dental erosions is multifactorial. The acids may be of endogenous (intrinsic) or exogenous (extrinsic) origin. Gastric acids of intrinsic origin are present in the gastric acid reflux, while those of extrinsic origin come from food or drugs (Campisi et al., 2008). Erosion caused by acids originating from external sources is different from the one caused by gastric acid reflux. In this case, we can notice the deterioration of the labial tooth surface of the front teeth, with a decreasing severity in the posterior area (Picos et al., 2013).

Dental erosion begins at the surface of the dental tissue, where the decalcification acid or chelating agents destroy the film, which causes the dissolution of the organic substratum of the teeth and the demineralization of their surface. The damaged dental area is exposed to all the processes that occur in the oral cavity: chewing, swallowing, brushing (Holbrook et al., 2009).

The "critical pH" for enamel demineralization is about 5.5. It is permanently buffered by the action of calcium and phosphate ions present in the saliva and in the blood stream (Holbrook et al., 2009).

Also, antacids and proton pump inhibitors decrease the production of acid in the stomach, and implicitly the gastric reflux. Thus, the potential for tooth erosion may vary depending on the composition and pH of the reflux, the buffering mechanisms, the amount of saliva, or the time between reflux and dental brushing.

The authors of a study performed on 249 patients (children and adults) reported a significant association between molar erosion and gastric reflux shown by performing upper gastrointestinal endoscopy, esophageal manometry, and 24-hour esophageal pH monitoring (Tolia and Vandenplas, 2009). The authors of another study performed on children in 2009 found a higher prevalence of dental erosion in children with GERD compared to the control

group (Petruzzi et al., 2012). Tofan N et al. presented a case of a young adult patient with dental erosion produced by GERD associated with frequent consume of acid foods (Tofan et al., 2016).

Oral mucosal lesions

Oral mucosal lesions can result from direct contact with the gastric acid reflux. There are reports in the literature that a burning sensation of mouth, aphthae-like lesions and dysphonia appear in patients with upper digestive tract diseases (Järvinen et al., 1988). The authors of a study noticed a significant association of GERD with the palate and uvula mucosal erythema (Di Fede et al., 2008).

However, the described lesions are not specific to patients with GERD. Therefore, a differential diagnosis should be made with oral candidiasis, changes in salivary flow or drug-induced xerostomia. Also, diet changes and oral hygiene status should be noted.

Bruxism

Bruxism is characterized by the stereotypical rhythmic movement of the masticatory muscles, which causes the teeth to grind and clench (AASM, 2005). It is known that it is made more serious by stress, sleep disturbances, GERD and medication (Mengatto et al., 2013). Bruxism can occur during sleep, but also when the patient is awake. Certain published studies have reported the presence of bruxism in patients with night-time GERD, this association being most commonly found in Caucasians (Miyawaki et al., 2003). This association being most commonly found in Caucasians (Miyawaki et al., 2003).

2.5.4 The paraclinical diagnosis of GERD

To determine the presence of GER, one can use: esophageal pH monitoring, impedance-pH monitoring or scintigraphy. GERD-induced lesions can be determined by performing upper digestive endoscopy.

Esophageal pH monitoring for 24 hours cannot currently be considered as the gold standard for the diagnosis of GERD because its primary limitation is that it only detects gastric acid reflux. In addition, it has a low tolerability. Impedance-pH monitoring can detect acid, weakly acidic and alkaline reflux episodes, but this technique is limited by high costs and by the fact that there is no effective treatment for weakly acidic and non-acid reflux, so the clinical relevance of measuring these types of refluxes remains questionable (Lupu et al., 2016). Boix-Ochoa score is the most accurate score used for the interpretation of pH-monitoring in children for GERD diagnosis (Lupu et al., 2016).

2.5.5 GERD treatment in children

The management of GERD nowadays consists of the control of the symptomatology and its major impact on the quality of life, unlike the previous period, where healing the esophageal mucosal lesions was the main purpose. The evaluation of the response to proton pump inhibitors (PPIs) is required if we consider the correlation of GERD with gastritis of infectious etiology and symptomatic overlap that raises differential diagnosis problems (Lupu, 2015).

The main therapeutic goals are: healing the lesions when present, the treatment of the non-digestive symptoms, the prevention of recurrences and of the complications of the disease. These goals are achievable by: general measures, medical treatment, and surgical treatment (Lupu, 2015). The treatment of acid reflux is also needed in order to stop the potential for tooth erosion. Proton pump inhibitors (PPIs) are the most effective in the treatment of GERD in children. Drugs which may cause or exacerbate hyposalivation and which may lead to xerostomia should be avoided. Patients should avoid the consumption of acidic foods that may

exacerbate dental erosions associated with acid regurgitation. Tooth brushing and the chewing of food and sugar-free gum should be avoided for approximately two hours after the regurgitation episode in order to allow for the restoration of the salivary film and the subsequent remineralization of the tooth surface (Campisi et al., 2008).

2.5.6 Conclusions

GERD-induced dental erosions progress slowly and are often subtle, so the examination of the oral cavity by a dentist is often necessary. The late diagnosis and, implicitly, the late treatment of GERD can lead to significant deterioration of the teeth and of the mastication system. The successful treatment of GERD requires a multidisciplinary approach involving gastroenterologists, ENT specialists and dentists.

2.6 Diagnostic investigations in gastroesophageal reflux disease in children

2.6.1 Introduction

Barium transit, ultrasound and scintigraphy are techniques that can investigate the presence of reflux over a short period of time and especially after lunch. Barium transit is useful to detect malformations of the gastrointestinal tract: hiatal hernia, malrotation, pyloric and duodenal stenosis, esophageal narrowing, achalasia, extrinsic esophageal compression (Gonzalez Ayerbe et al, 2019). Ultrasound depends on the examiner and it is not recommended for GERD diagnosis. Although sensitivity is about 95%, compared to pH-metry, ultrasound has a specificity of only 11% (Westra et al, 1990; Jang et al 2001). Scintigraphy is also not indicated in the diagnosis of GERD, with a sensitivity of 69% and a specificity of 78% (Patra et al, 2011).

Esophagitis implies inflammation or damage to the esophageal mucosa. Esophagitis symptoms include: pyrosis, epigastralgia, dysphagia or odynophagia (Habbal et al., 2019). The most common causes of esophagitis are: GER, radiation, infections, medications and eosinophilic esophagitis. Upper gastrointestinal endoscopy (UDE) with biopsies is used to assess lesions of the esophageal mucosa, to detect complications (erosive esophagitis, stenoses and Barrett's esophagus) or for the differential diagnosis of GERD (eosinophilic esophagitis). Studies have reported that the sensitivity of erosive esophagitis in GERD diagnosis ranges from 15% to 71%, and the sensitivity of microscopic esophagitis in GERD diagnosis ranges from 83% to 88% (Ravelli et al., 2006; Cucchiara et al., 1993).

Esophageal pH-metry was initially introduced in 1969 and it was considered the gold standard for the diagnosis of GERD since the 1980s (Chen et al., 2016).

The pH-metry is based on the principle that the passage of acid gastric contents into the esophagus during the reflux causes a decrease in the esophageal pH. The method consists of recording the pH in the lower esophagus over a period of time, with an electrode placed 5 cm above the cardia, which is connected to a portable, battery powered, pH meter. The pH can be written on paper or transferred to a computer, which analyzes various required parameters, resulting in a graphical representation of esophageal pH curves (Christopher et al., 2003).

The test is considered positive if the pH falls below 4 for a period longer than 5 seconds (Chiou et al., 2011). After the computer interpretation of data recorded, it can obtain 3 scores: Boix-Ochoa (n <11.99), DeMeester (n <14.72), and Johnson-DeMeester (n <22). The scores were calculated in the same time by the program.

These studies stimulated the research in the field.

Personal contribution – published papers:

1. **Lupu VV**, Burlea M, Nistor N, Streanga V, Starcea MI, Paduraru G, Ghica DC, Mitrofan EC, Moscalu M, Ignat A. Correlation between esophageal pH-metry and esophagitis in gastroesophageal reflux disease in children. *Medicine,* 2018, 97(37): e12042. **ISI IF 1,87**
2. **Lupu VV,** Ignat A, Paduraru G, Ciubara A, Moscalu M, Marginean CO, Burlea M. Correlation between the different pH-metry scores in gastroesophageal reflux disease in children. *Medicine,* 2016; 95(26): e3804. **ISI IF 1,804**

The aim of these studies was to determine the best method of diagnosis and to establish the correlation between esophageal pH-metry and esophageal lesions in gastroesophageal reflux disease in children.

The literature recommends for pediatric age the use of Boix- Ochoa score. Being able to calculate other scores also (DeMeester and Johnson-DeMeester), we proposed to study possible correlations and to determine their sensitivity and specificity.

2.6.2 Material and methods

- ***The link between esophageal pH-metry and esophagitis***

The study was conducted on a group of 72 children, aged over 4 years old, admitted in a pediatric gastroenterology regional center in Northeast Romania, diagnosed with GERD by 24 hours esophageal pH-metry, which also underwent the upper digestive endoscopy. Results were interpreted using the Boix–Ochoa score.

The indications for 24 hours pH monitoring involved a diagnostic uncertainty.

To determine the pH, the authors used Medtronic Digitrapper pH 100, SN 37660, with Polygram Net TM pH Testing Application and Zinetics 24 and ComforTec by Sandhill multiuse catheters. The sensor was positioned 5 cm above the lower esophageal sphincter. Continuous pH recording was performed for 24 hours.

The Boix–Ochoa score was used to calculate the following distal pH variables: number of acid refluxes longer than 5 minutes, longest acid reflux, fraction of total time pH below 4, fraction of upright time pH below 4, fraction of supine time pH below 4, and fraction of prone time pH below 4.

All study patients underwent upper gastrointestinal endoscopic examinations. The endoscopic findings of reflux esophagitis in the lower esophagus were classified according to the Los Angeles classification system.

- ***The link between the different pH-metry scores***

We performed a retrospective study on 234 children of both sexes, 1 month and 18 years old, both from rural and urban area, admitted in the same pediatric gastroenterology regional center in Northeast Romania, over a period of 5 years. They underwent 24-hour esophageal pH-metry. The indications for 24-h pH monitoring involved a diagnostic uncertainty. The patients had atypical symptoms, especially respiratory, unresponsive to usual therapy, such as cough, hoarseness, sore throat, atypical chest pain, and asthma. The cases which did not match with the imposed criteria (the children with a diagnostic certainty) were excluded from the study.

The pH-metry was conducted and the results were interpreted by a pediatrician. The Boix-Ochoa score, as a gold standard, was used to determine the positive and negative predictive value of DeMeester and Johnson-DeMeester scores.

2.6.3 Results

- ***The link between esophageal pH-metry and esophagitis***

GERD and esophagitis degree

A total of 72 children aged more than 4 years old were investigated. Upper endoscopy evaluation revealed only grade A and B esophagitis (Table 2.3). Grade C and D esophagitis, also Barrett esophagus were not found in any patient. Around 47 patients (65.28%), meaning 2/3 of all, had the aspect of grade A esophagitis and, only 1/3 (34.72%) have lesions of grade B esophagitis, meaning 25 patients.

Table 2.3. *Esophagitis grade in GERD*

N=72 Esophagitis	**No. cases**	***%***
Grade A	47	65.28%
Grade B	25	34.72%
Total	72	

Correlation of pH-metry with the endoscopic lesion

Using the Boix–Ochoa score there is a strong correlation between the pH-metry values and the grade of esophagitis. The average score is practically double (56.75) at grade B esophagitis compared to 25.37 that is found in grade A esophagitis (Table 2.4); also, with a high level of confidence revealed by the ANOVA test (Table 2.5).

Table 2.4. *Statistical indicators of the Boix–Ochoa score regarding the grade of esophagitis*

Esophagitis	**Average Boix-Ochoa Score**	**Average**		**Std. Dev.**	**Std. er.**	**Min**	**Max**	**Q25**	**Average**	**Q75**
		-95%	**+95%**							
Gr. A	25.37	14.55	36.19	26.21	5.24	0.50	105.00	7.90	21.20	26.10
Gr. B	56.75	36.42	77.08	28.42	8.99	30.70	125.60	38.90	44.70	66.20

Table 2.5. *Test for average the Boix–Ochoa score values versus grade of esophagitis*

Boix-Ochoa Score	**F** *(95% confidence interval)*	**P**
ANOVA test	9.765825	0.003693

The graphical representation of the Boix–Ochoa score values regarding the grade of esophagitis, clearly proves, that in GERD with grade B esophagitis the Boix–Ochoa score is statistically significantly higher, compared to the value obtained in GERD with grade A esophagitis (F=9.76, P=.0036, 95%CI) (Figure 2.1).

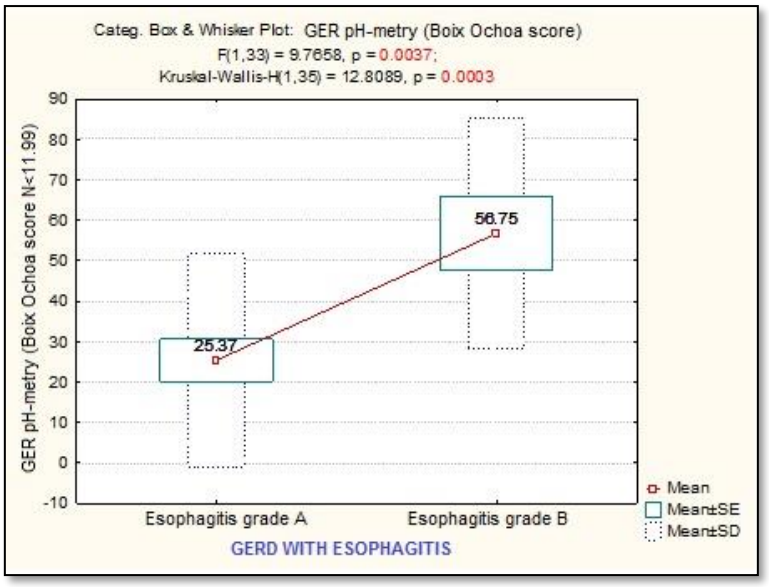

Figure 2.1. *Statistical indicators of Boix–Ochoa score regarding the grade of esophagitis*

The same result is obtained using the Pearson correlation test, which shows a very strong correlation between the grade of esophagitis and the Boix–Ochoa score (Table 2.6).

Table 2.6. *Pearson correlation test—grade of esophagitis versus the Boix–Ochoa score*

Pearson	**r** *(correlation factor) (95% confidence interval)*	**p**
Esophagitis grade vs. Boix-Ochoa score	0.54778	0.004

The correlational analysis proves the fact that the Boix–Ochoa score values can predict, for sure, the grade of esophagitis in GERD (r=0.547, P=.004, 95%CI) (Figure 2.2).

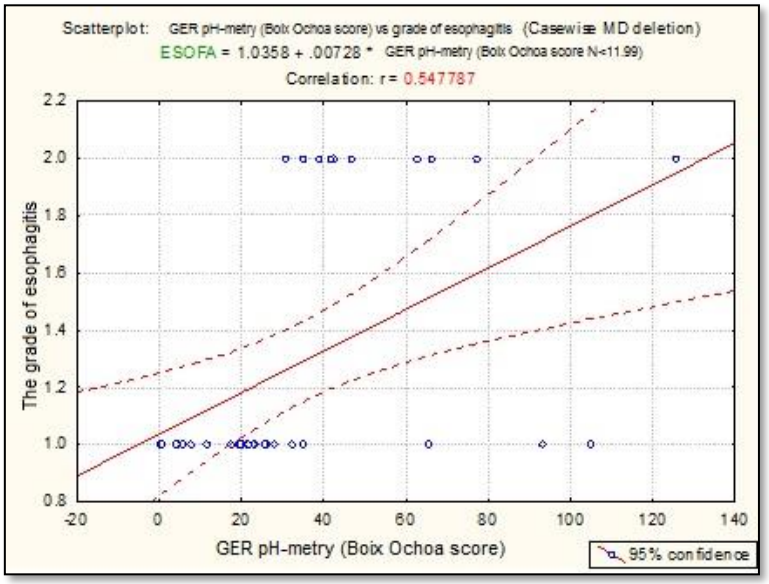

Figure 2.2. *Regression line esophagitis grade vs. Boix-Ochoa score*

The large area under ROC curve (AUC—area under the ROC Curve) (AUC=0.9111; 95% CI: 0.8692–0.9530, P<.001) shows the high diagnostic value of grade A esophagitis based on the Boix–Ochoa score (Figure 2.3). The calculated reference value for Boix–Ochoa score in case of grade A esophagitis was 19.4, with a sensitivity of 93.3% and specificity of 78.6%. The positive predictive value was 81.3% and the negative predictive value 92.2%. The large area under ROC curve (AUC—area under the ROC curve) (AUC=0.949; 95% CI: 0.9204–0.9777, P<.001) shows the high diagnostic value of grade B esophagitis based on the Boix–Ochoa score (Figure 2.4). The calculated reference value for Boix–Ochoa score in case of grade B esophagitis was 53.9, with a sensitivity of 94.4% and specificity of 82.98%. The positive predictive value was 84.7% and the negative predictive value 93.7%.

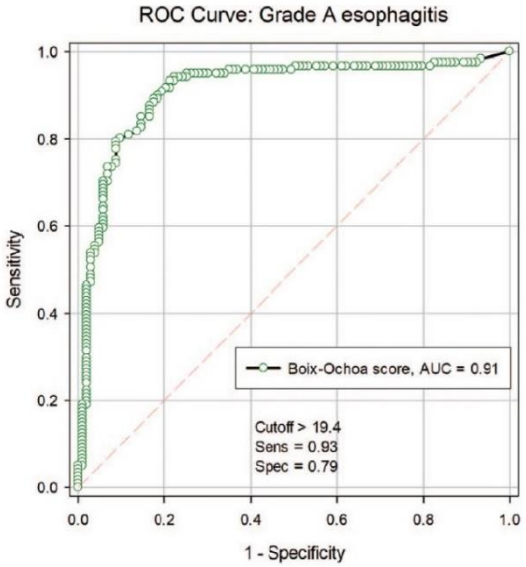

Figure 2.3. *ROC curve for grade A esophagitis. ROC=receiver operating characteristics*

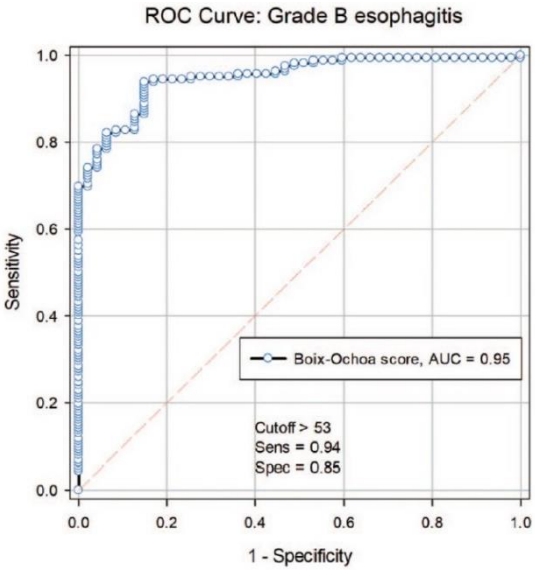

Figure 2.4. *ROC curve for grade B esophagitis. ROC=receiver operating characteristics*

- ***The link between the different pH-metry scores***

PH-metry interpretation by the Boix-Ochoa score (n<11.99), DeMeester score (n<14.72), and Johnson-DeMeester score (n<22)

In the past 5 years, out of 234 children (1 month and 18 years old) of both sexes, both from rural and urban area, 172 (73.50%) had a positive Boix-Ochoa score and 62 (26.50%) had a normal Boix-Ochoa score (<11.99). As regards to the DeMeester score, in 149 children (63.68%) it was positive and in 85 (36.32%) it was normal (<14.72).

According to the Johnson-DeMeester score, gastroesophageal reflux was associated in 120 cases (51.28%), and it was not associated in 114 cases (48.72%) (Table 2.7). The scores were calculated in the same time by the program.

Table 2.7. *Ph-metry interpretation by the Boix-Ochoa score, DeMeester score and Johnson-DeMeester score*

Boix-Ochoa score positive	Boix-Ochoa score normal	DeMeester score positive	DeMeester score normal	Johnson-DeMeester score positive	Johnson-DeMeester score normal
172 (73.50%)	62 (26.50%)	149 (63.68%)	85 (36.32%)	120 (51.28%)	114 (48.72%)
Total 234					

There were not any adverse events registered after pH-metry. In a group of 72 patients, GERD was also confirmed, based on upper gastrointestinal endoscopy.

Correlation of the Demeester score versus the Boix-Ochoa score

The correlation of the DeMeester score with the Boix-Ochoa score is very high (r=0.978, P<<0.01, 95% CI) (Table 2.8). The results show good values of sensitivity (Se) and specificity (Sp) of the DeMeester test. When the DeMeester score was applied, 86.63% of the cases confirmed with GERD by the Boix-Ochoa score were also confirmed by the DeMeester score, and only 13.37% were false-negative (Table 2.9). It is also ascertained that the DeMeester score did not show any false positive case; therefore, all negative cases were correctly diagnosed (Table 2.10). In conclusion, when applying the DeMeester test, we take the risk of potential false-negative results (Sp>Se, Sp=98.4%).

Table 2.8. *Results of the Pearson correlation test between DeMeester score and Boix-Ochoa score*

Pearson	**r** *(correlation coefficient) (95% confidence interval)*	**p**
DeMeester score vs. Boix Ochoa score	0.9781	0.00

Table 2.9. *Congruence of pH-metry versus DeMeester score*

		DeMeester -		**DeMeester +**	
		No. of cases	**%**	**No. of cases**	**%**
pH-metry	+	23	13.37%	149	86.63%
	-	62	100%	0	
Total			**234**		

Table 2.10. *DeMeester score sensitivity and specificity*

Sensitivity (%)	86.62%
Specificity (%)	98.43%
Positive predictive value (%)	99.33%
Negative predictive value (%)	73.25%
Accuracy (%)	90.21%

Correlation between the Johnson-Demeester score versus the Boix-Ochoa score

The correlation of the Johnson-DeMeester score with the Boix-Ochoa score is very high (r=0.94, P<<0.01, 95% CI), but it is slightly lower in comparison with the DeMeester score (Table 2.11).

The results show very good values of the specificity of the Johnson-DeMeester test, which proves the summing of the false negative results by considering the results of this table (Table 2.12). Technically, this test increases the risk of the presence of gastroesophageal disease, which may, however, not be detected after the Johnson-DeMeester test. In comparison with the DeMeester test, the Johnson-DeMeester test has a higher risk of false-negative results (Table 2.13).

Table 2.11. *Results of the Pearson correlation test between the DeMeester score and the Boix-Ochoa score*

Pearson	**r** *(correlation coefficient) (95% confidence interval)*	**p**
Johnson-DeMeester score vs. Boix Ochoa score	0.9409	0.000

Table 2.12. *Congruence of the results of the pH-metry versus Johnson-Demeester score*

		Johnson-Demeester **-**		***Johnson-Demeester*** **+**	
		No. of cases	**%**	**No. of cases**	**%**
pH-metry	**+**	52	30.23%	120	69.77%
	-	62	100.00%	0	
Total		**234**			

Table 2.13. *Johnson-DeMeester score sensitivity and specificity*

Sensitivity (%)	69.76%
Specificity (%)	98.41%
Positive predictive value (%)	99.17%
Negative predictive value (%)	54.38%
Accuracy (%)	77.44%

It should be noted that the motivation of the pH-metric investigation was rather the associated pathology than the digestive symptomatology. Our results assessed, based on Boix-

Ochoa, Demeester, and Johnson-DeMeester scores, show a high degree of specificity and sensitivity when applied to our patients. We do not have indeterminate results, missing data, and outliers of the index tests.

2.6.4 Discussions

- ***The link between esophageal pH-metry and esophagitis***

PH-metry is often performed, despite its major limitation, the inability to detect nonacid reflux (pH above 4). This test is not accurate for the detection of reflux episodes in patients undergoing acid suppressive treatment (Van Wijk et al., 2009). The results of pH-metry are affected by the ingestion of acidic foods and drinks (Van Wijk et al., 2009).

We performed upper digestive endoscopy in all children because this allows direct visual examination of the esophageal mucosa. This may be a useful tool to assess GER in children with other signs and symptoms suggestive of GERD such as apnea or apparent life-threatening event, reactive airways disease, recurrent pneumonia, asthma, dental erosions or Sandifer syndrome. In our study, we found only grade A and B esophagitis, without any other type of lesions. The fact that, there were no more advanced esophagitis lesions can be hypothetically explained by the age of the patients and the duration of the disease from its debut until endoscopy. Also, the previous treatment attempts with PPIs (more than last 3 months) may have influenced this situation.

The identification of esophagitis with upper gastrointestinal endoscopy has specificity 90%–95% for GERD (Richter, 2003) but has a poor sensitivity of around 50% (Dent, 1999).

All our patients received initially home treatment for reflux esophagitis for 6–8 weeks with proton pump inhibitors (PPIs) (esomeprazole or pantoprazole). High doses of PPIs are necessary in patients with higher grades of esophagitis or when there are conditions that can determine severe GERD (Goldani, 2012). In our study, if the symptoms of children have not completely resolved, the treatment has been extended for a longer period of time (another 6–8 weeks). If the symptoms of children have not completely resolved, the treatment has been extended for a longer period of time (another 6–8 weeks). When patients are with high grades of esophagitis at first upper digestive endoscopy, or they have persistent symptoms after an adequate treatment, or in case of atypical symptomatology, we can follow up performing another endoscopy (Goldani, 2012).

Study limits are dictated by monitoring body position in young children in a hospital ward, which presents considerable practical difficulties, as few children maintain any position for very long. Also, pH-monitoring alone cannot diagnose alkaline reflux.

- ***The link between the different pH-metry scores***

Impedance-pH monitoring is limited by its high cost and the lack of normative data of multichannel intraluminal impedance-pH in the pediatric population (Shin, 2014). However, as long as there is no effective medical therapy for weak acid and nonacid reflux, the clinical relevance of measuring these types of refluxes remains debatable (Lundell et al., 1999).

The sensitivity and specificity of a method depend only on the test qualities (and potentially on the examiner), whereas the predictive values do not provide information on the test itself, but on the situation after the exploration, being related to the clinical context (pretest probability), and also the native characteristics of the method (sensitivity and specificity).

The more sensitive the test is, the better the negative predictive value is (the degree of certainty that a patient with a normal test does not have the disease is higher), and the more specific the test is, the better the positive predictive value is; therefore, a patient with an abnormal test will definitely have the disease. Accuracy shows us how close we are to the

actual value of the test. Accuracy is the extent to which the measurement or the estimation based on certain measurement presents the actual value of the measured characteristic.

When pH evaluation is performed over a prolonged period, it presents high sensitivity and specificity indices (Sherman et al., 2009). The Boix- Ochoa methodology is considered to be the most appropriate for application to the pediatric age group (Dent et al., 2005).

The DeMeester score has its limitations, with reported sensitivities ranging from 60% to 100% (Shin, 2014), through to indexes as low as 28% (Van Wijk et al., 2009). The inconstant sensibility of the examination made its methodology doubtful, and then some points were considered if they were normal: gastroparesis or functional heartburn, the presence of nonacid reflux, the possibility that pH probe might have missed distal acid reflux, noxious effect of the nasal catheter could have limited both eating and activity and resulted in a false-negative test, and the influence of electrode location in the result of esophageal prolonged pH monitoring (Richter, 2003).

The sensitivity of Johnson-Demeester score for the typical symptom heartburn is 68% and its specificity is 63% (Vakil et al., 2006). In our study, we obtained a sensitivity similar to that of literature 69.76% and a higher specificity of 98.41%, but we applied pH-metry only for atypical symptoms. The 24-hour esophageal pH composite score has proved to be a durable standard to identify and quantify GERD by measuring esophageal acid exposure (Dent, 2009).

With an accurate score and more experience in pH monitoring, we would be able to identify children at risk to develop severe complications, control them, and aid in the selection of those patients requiring surgical intervention.

Study limits are dictated by monitoring body position in young children in a hospital ward (not in their normal environment at home), which presents considerable practical difficulties, as few children maintain any position for very long. Also, the pH monitoring alone, cannot diagnose alkaline reflux. Another limitation would be that we compared the scores only for reflux with atypical symptoms.

2.6.5 Conclusions

- ***The link between esophageal pH-metry and esophagitis***

Upper digestive endoscopy performed in patients with gastroesophageal reflux disease shows the constant presence of esophagitis at all patients. There were only grade A and B esophagitis due to the fact that they are young patients with a relative short history of the disease. The correlation tests show a perfect parallel between the pH-metry scores and the endoscopic lesion. The correlation is so accurate, that the pH-metry scores can be sufficient to prove the disease and the grade of esophagitis, the upper digestive endoscopy being reserved only for the cases that does not respond to the medical treatment or have other complications.

- ***The link between the different pH-metry scores***

As considered until now, the Box-Ochoa score is the most accurate score to be used in pediatrics for the GERD diagnose. The use of the different scores - Box-Ochoa, Demeester, and Johnson-Demeester - showed a high sensitivity and specificity of the pH-metric measurements applied to the study lot. Regarding the Demeester score, we obtained a sensitivity of 86.62% and a specificity of 98.43%. The Johnson-Demeester score had a higher risk of false-negative results and we found a sensitivity of 69.76% and a specificity of 98.41%.

2.7 Studies regarding gastroesophageal reflux disease in children

2.7.1 Introduction

- ***The role of Helicobacter pylori infection in gastroesophageal reflux***

H. pylori infection plays a major role in the pathogenesis of many gastrointestinal diseases such as chronic gastritis, peptic ulcer disease, gastric mucosa – associated lymphoid tissue lymphoma and the development of gastric cancer. However, its role in gastroesophageal reflux disease (GERD) without esophagitis and in reflux esophagitis is not fully understood.

There are many important issues to be elucidated regarding the effect of *H. pylori* eradication on reflux esophagitis or GERD. Several reports have shown beneficial effect of *H. pylori* on acid reflux by alkalinization of gastric secretions caused by the bacteria (Fallone et al, 2000; Schwizer et al., 2001). Contradictory results have been reported an association of *H. pylori* eradication with the development of GERD or reflux esophagitis symptoms (Take et al, 2009; Cremonini et al., 2003).

Symptoms like heartburn, acid regurgitation, dysphagia in older children or agitation, and food refusal in toddler and infant are usually sufficient to confirm the diagnosis of GERD and start the treatment. The most common test used to confirm GERD is ambulatory 24-hour esophageal pH monitoring.

A satisfactory therapeutic response to proton pump inhibitors (PPI) in GERD induced us the idea to study whether GERD correlates with infectious etiology of some forms of gastritis, especially since there is an overlap of symptoms that raise issues of differential diagnosis. There is some evidence that the combination of *H. pylori* and chronic acid suppression can lead to atrophic gastritis, a precancerous condition in the stomach (García Rodríguez et al., 2006). It has been demonstrated that interactions between bile acids, pH, and *H. pylori* it is associated with the occurrence of corpus-predominant gastritis after PPI therapy in *H. pylori*-positive patients with GERD (Mukaisho et al., 2014). It is term PPI therapy should be tested for *H. pylori* infection. If the infection is present, the bacteria should be eradicated (Hagiwara et al., 2011).

- ***Correlation between wheezing and gastroesophageal reflux disease***

Recurrent wheezing is defined as more than one episode of wheezing within the last 12 months (Hermann et al., 2005). Wheezing represents a common disorder characterized by a continuous high-pitched expiratory sound produced by an air flux that becomes turbulent flowing thorough the reduced airway caliber (Brand et al., 2008). It's estimated that about one third of school-age children manifest the recurrent wheezing during the first 5 years of life (Mallol et al., 2010). The most frequent cause of recurrent wheezing in children is asthma, but other causes should be considered in the differential diagnosis. Recurrent wheezing attacks impair the quality of life for the patient and his/her family and represent one of the most common causes of emergency department visits and hospitalizations (Ozdogan et al., 2015). The rate of presentation to emergency departments and hospitalization was reported to be 16 % and 12 % (Bisgaard and Szefler, 2007). The most common causes of recurrent wheezing are: asthma, gastroesophageal reflux disease, foreign body aspiration, bronchopulmonary dysplasia, bronchiolitis obliterans, an immunodeficiency, primary ciliary dyskinesia, vocal cord dysfunction, cardiac etiologies and structural abnormalities (Ducharme et al., 2014).

The authors of a study concluded anemia and gastroesophageal reflux (GER) are risk factors for recurrent hospitalizations for wheezing and should be treated (Ozdogan et al., 2015). Another study concluded that silent GER is common in infants with daily wheezing, and

controlling GER improves morbidity and decreases the need for daily asthma medications (Sheikh et al., 1999).

- ***Another cause of reflux-like symptoms***

Heterotopic gastric mucosa (HGM) of the esophagus was first defined in 1805 by Schmit during a post-mortem examination (Tang et al., 2004). HGM of the esophagus is mostly localized in the upper esophagus and termed inlet patch (Chong, 2011). An inlet patch is a congenital anomaly and most of these are largely asymptomatic. It usually cannot be diagnosed easily. Rarely, it can also be found in other parts of the esophagus (Von Rahden et al., 2004; Borhan-Manesh and Farnum, 1991). HGM is reported in up to 10% of the general population among numerous reports and epidemiologic studies which have been essentially performed in adult population (Tanpowpong and Katz, 2011). Pediatric data are still limited. There are case reports showing that this injury may play a role in the development of stenosis, ulcer, perforation or esophagotracheal fistula related to its capability of hydrochloric acid secretion (Azar and Soweid, 2009; Rosztoczy et al., 2012). Malignant progression of HGM occurs in a stepwise pattern, following the metaplasia-dysplasia-adenocarcinoma sequence (Noguchi et al., 2001; Komori et al., 2010). These complications may be exacerbated by *Helicobacter pylori* (Gutierrez et al., 2003).

This type of gastric heterotopia can cause reflux-like symptoms and need differentiation from other pathologies of the esophagus.

We reported a case of esophageal gastric heterotopia that was in the distal esophagus and needed differentiation from Barrett's esophagus.

Personal contribution – published papers:

1. **Lupu VV**, Miron IC, Lupu A, Moscalu M, Mitrofan CE, Munteanu D, Luca AC. The relationship between gastroesophageal reflux disease and recurrent wheezing in children. *Medicine*. 2021,100(47):e 27660. **ISI IF 1,889**
2. **Lupu VV**, Ignat A, Ciubotariu G, Ciubară A, Moscalu M, Burlea M. Helicobacter pylori infection and gastroesophageal reflux in children. *Diseases of the Esophagus*, 2016, 29(8): 1007-1012, doi: 10.1111/dote.12429, **ISI IF 2,571**
3. **Lupu VV**, Ignat A, Paduraru G, Mihaila D, Burlea M, Ciubara A. Heterotopic Gastric Mucosa in the Distal Part of Esophagus in a Teenager: Case Report. *Medicine*. 2015;94(42):e1722. **ISI IF 2,133**

Some studies suggest that Helicobacter pylori (H. pylori) infection would be a protective factor for the gastroesophageal reflux. The aim of this study was to explore this fact.

We proposed also to explore the relationship between wheezing and gastroesophageal reflux disease and to evaluate the outcome after appropriate treatment.

The aim of the case report was to present a rare case of a teenage girl with HGM located in the distal esophagus, associated with chronic gastritis and biliary duodenogastric reflux and to underline that endoscopy combined with biopsies is a mandatory method in clinical evaluation of metaplastic and non-metaplastic changes within HGM of the esophagus.

2.7.2 Material and methods

- ***The role of Helicobacter pylori infection in gastroesophageal reflux***

A correlational study between GERD and gastritis with *H. pylori* was conducted on a group of 72 children, admitted in a pediatric gastroenterology regional center in Northeast Romania, diagnosed with gastroesophageal reflux by 24-hour continuous esophageal pH monitoring, which underwent upper endoscopy with gastric biopsy. Results were interpreted using the Boix-Ochoa score.

We used as control group 62 patients with suspicion of GERD, but who did not have gastroesophageal reflux proved by pH-metry.

Some exclusion criteria were applied: previous therapy to eradicate *H. pylori*, PPIs treatment in the last 3 months, concomitant consumption of aspirin and nonsteroidal anti-inflammatory drugs, patients with endoscopic evidence of active gastrointestinal bleeding, presence of esophageal stricture or esophagitis secondary to systemic diseases or any past history of gastric or esophageal surgery.

Twenty-four-hour esophageal pH monitoring

To determine the pH, we used the Medtronic Digitrapper pH 100, SN 37660 with Polygram Net TM pH Testing Application and Zinetics 24 multi-use and ComforTec by Sandhill catheters. The sensor was positioned 5 cm above the lower esophageal sphincter. Continuous pH recording was performed for 24 hours. Meal periods were excluded.

Current consensus shows that the total percentage of time the pH is below 4 is the most useful single discriminator between physiologic and pathologic reflux (Pandolfino and Vela, 2009).

The Boix-Ochoa score was used to calculate the following distal pH variables: number of acid refluxes longer than 5 minutes, longest acid reflux, fraction of total time pH below 4, fraction of upright time pH below 4, fraction of supine time pH below 4 and fraction of prone time pH below 4. The Boix-Ochoa score is developed for infant/pediatric usage. A normal score is a score below 11.99.

During the study, the children took no medications that could interfere with the results and consumed an unrestricted diet.

Endoscopy

All study patients underwent upper gastrointestinal endoscopic examinations. Intravenous sedation was given and standard upper gastrointestinal endoscopy, using the Olympus and Pentax video pediatric gastroduodenoscopes was performed to identify evidence of macroscopic abnormalities. Endoscopy was performed under general anesthesia in children aged below 10 years.

The endoscopic findings of reflux esophagitis in the lower esophagus were classified according to the Los Angeles classification system. Esophagitis was graded by endoscopy: grade A, one (or more) mucosal break no longer than 5 mm, that does not extend between the tops of two mucosal folds; grade B, one (or more) mucosal break more than 5 mm long that does not extend between the tops of two mucosal folds; grade C, one (or more) mucosal break that is continuous between the tops of two or more mucosal folds, but which involves less than 75% of the circumference; grade D, one (or more) mucosal break which involves at least 75% of the esophageal circumference (Lundell et al., 1999).

Biopsies taken from the gastric antrum and corpus were used for rapid urease testing and for bacteriologic and histologic examination. For a conclusive bacteriologic examination of gastric biopsies, the sampling was made before any treatment with antibiotics, bismuth or

PPI. Patients were considered *H. pylori* positive if, at least two of the four biopsy specimens had positive results. The same criteria for *H. pylori* diagnosis were used in each group.

One biopsy specimen from the antrum (2 cm from the pylorus) was used for the rapid urease test. The fragment of gastric mucosa is introduced into the environment, immediately after the biopsy. If gastric mucosa contains bacteria of the species

H. pylori, the urease hydrolyzes urea to ammonia and carbon dioxide, the pH becomes alkaline, and the environment turns red. The results were interpreted after 24 hours. Any other biopsy from the corpus for the rapid urease test was not necessary because the sampling was made before any treatment with PPI.

One biopsy specimen from the antrum and one from the corpus were used for culture of *H. pylori*. Biopsy specimens were transported to the laboratory immediately after the endoscopy. *H. pylori* was cultured by rubbing gastric biopsy specimens into Columbia with agar supplemented with Skirrow's supplement (containing vancomycin, trimethoprim, and polymyxin B) and lysed horse blood. The plates were incubated under micro aerobic conditions at 35°C for 4 to 7 days.

A biopsy specimen from the antrum for histopathologic examinations was fixed in buffered 4% formalin overnight and was embedded in paraffin. Two sections were stained with hematoxylin-eosin and one section was stained by the modified Giemsa procedure and examined. The slides were microscopically examined for the bacterial density.

Statistical analysis

Data management and statistical analyses were performed using the „STATISTICA" program.

- ***Correlation between wheezing and gastroesophageal reflux disease***

We performed a retrospective study on a group of 85 children, 1 month – 18 years old, with two or more recurrent hospitalizations resulting from wheezing within the last 12 months, admitted in a pediatric gastroenterology regional center in "St. Mary" Children Emergency Clinical Hospital, Iasi, Romania. They were evaluated for the presence of gastroesophageal reflux by 24-hour continuous esophageal pH monitoring and the results were interpreted using the Boix Ochoa score.

Exclusion criteria were: foreign body aspiration, a previous diagnosis of sleep apnea, bronchopulmonary dysplasia, cystic fibrosis, bronchiolitis obliterans, an immunodeficiency, primary ciliary dyskinesia, vocal cord dysfunction, proven food sensitivity, cardiac etiologies and structural abnormalities. Also, children with an acute respiratory tract infection within one month of being screened for the study were excluded.

Inclusion criteria	**Exclusion criteria**
1 month – 18 years old two or more recurrent hospitalizations resulting from wheezing within the last 12 months	foreign body aspiration a previous diagnosis of sleep apnea bronchopulmonary dysplasia cystic fibrosis bronchiolitis obliterans an immunodeficiency primary ciliary dyskinesia vocal cord dysfunction proven food sensitivity cardiac etiologies and structural abnormalities acute respiratory tract infection within one month of being screened for the study

The diagnosis was based on the anamnesis, clinical and paraclinical examination. Previously requested investigations and current hospitalization records were investigated.

We used Boix-Ochoa score (N<11.99) to evaluate gastroesophageal reflux in these children. The test is considered positive if the total percentage of time the pH is below 4 is the most useful single discriminator between physiologic and pathologic reflux (Pandolfino and Vela, 2009; Lupu et al., 2016; Lupu et al., 2018). For the interpretation of results, we used following parameters: the total number of reflux episodes (normal – under 2 episodes an hour on an average); the number of reflux episodes that last for more than 5 minutes (normal – under 8 episodes); the duration of the longest reflux episode (in minutes); the reflux index (RI) = the ratio between the total number of reflux episodes and their duration (normal – under 4); the Euler score = x + 4y, in which: x – number of reflux episodes with pH < 4 longer than 1 minute and y – the number of episodes with pH < 4 longer than 5 minutes.

Medtronic Digitrapper pH 100, SN 37660, with Polygram Net TM pH Testing Application and Zinetics 24 and ComforTec by Sandhill multi-use catheters were used to measure the pH. Esophageal pH monitoring is based on the principle that the passage of the acid gastric content into the esophagus during reflux generates a decrease in the intraesophageal pH. The method consists in measuring the pH in the lower esophagus for 24 hours by means of an electrode placed 5 cm above the cardia and connected to a portable pH-meter on batteries. The pH can be printed on paper or transferred to a computer, which analyses different required parameters, depending on which the intraesophageal pH curves are graphically represented (Christopher et al., 2003).

The device is calibrated in 2 solutions with pH 1 and 7 before each use. Before the procedure no food or drink allowed at least 6 hours for children over 1-year-old, at least 3 hours for infants (Jamieson et al., 1992). Antacid therapy should be discontinued or at least 6 hours before, the anti H2 receptor 3 days before, the proton pump inhibitor 7 days before and the prokinetics 48 hours before (Kleiman et al., 2008).

The child is placed in the left lateral decubitus (for infants and young children), the examiner is to the right side of the patient, and in a seated position for children over 5-6 years old. The lubricated electrode is inserted, nasal up to 5 cm above the cardia. The electrode is connected to the pH meter, the caregiver is explained about the function and operation of the device and then, the recording is started. Each patient or caregiver was trained to record in a table any symptoms occurred, the time and body position (supine, standing) and, at the same time, using the button provided on the device (Lupu et al., 2016; Lupu et al., 2018).

All patients with positive score received treatment with proton pump inhibitors and they were evaluated again after 2 months. Statistical Package for Social Sciences (SPSS) software 20 was used for the statistical data processing. For the correlation analysis, the Pearson parametric correlation was used and the correlation coefficients were calculated for a confidence interval (CI) of 95%. The logistic regression offers a useful means for the modelling of the dependence of a dichotomous response variable on one or several explanatory variables called "predictors", which can be categorical or continuous. The risk is mathematically modelled in the form of an equation as a predictor variable.

Informed consent was obtained from all patients or from their family, and the "St. Mary" Children Emergency Hospital Ethics Committee's approval was obtained for publishing this study.

- ***Another cause of reflux-like symptoms***

We studied a case of a Caucasian teenage girl, 16 years old, followed up for the last 3 years, was initially admitted in the V-th Pediatric Gastroenterology Clinic of „St Mary" Children's Emergency Hospital, Iasi, Romania for postprandial epigastric pain.

Over the three years, her physical exam revealed good generally status, abdominal pain localized in the left hypochondrium and/or in epigastrium, without any other pathological elements at examination. The informed consent was obtained each time the patient was hospitalized.

2.7.3 Results

- ***The role of Helicobacter pylori infection in gastroesophageal reflux***

In 19 of 72 patients (26.39%) *H. pylori* was present and in 53 patients the bacteria were not detected (Table 2.14).

Table 2.14. *The frequency of H. pylori in GERD*

N = 72 esophagitis	Number of cases	%
With *H. pylori*	19	26.39
Without *H. pylori*	53	73.61
Total	72	

Involvement of H. pylori in GERD

It was analyzed in comparison (making contingency table), the frequency of *H. pylori* in GERD in study group and in the control group. Thus, in the study group consisting of 72 patients with GERD were 19 positive results with a percentage of 26.39% and in control group composed of 62 children were 10 cases with *H. pylori* infection with a percentage of 16.13% (Figure 2.5).

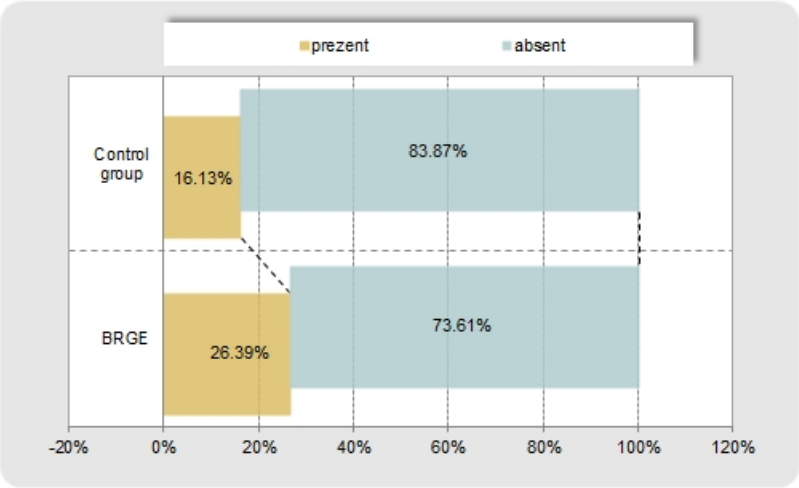

Figure 2.5. *The frequency of H. pylori in GERD and in the control group*

The presence of *H. pylori* was not found to be a causal factor in GERD, which is demonstrated by insignificant differences in the presence of *H. pylori* in children with GERD compared with the frequency of *H. pylori* in the control group ($\chi 2 = 0.065$, P = 0.7974, 95% confidence interval [CI]) (Table 2.15).

Table 2.15. *The estimated parameters in the association GERD with H. pylori*

	Chi-square χ^2	**p** ***95% confidence interval***
Pearson's chi-squared - χ^2	0.0658681	0.79745
Correlation coefficient (Spearman Rank R)	0.016778	0.79850

The calculation of chance or risk parameters did not bring additional items, the estimated value is insignificant (Table 2.16).

Table 2.16. *Parameters estimation of chance and risk in the occurrence of GERD versus H. pylori*

	Estimated Value	*95% confidence interval*	
		Minimum	**Maximum**
PARAMETERS of chance			
Odds ratio (OR)	0.51	0.12	0.94
PARAMETERS of risk			
Risk ratio (RR)	0.34	0.24	0.87

The involvement of H. pylori in GERD based on the grade of esophagitis

Trying an association between the presence of *H. pylori* and the grade of esophagitis it can be observed the existence of an inverse relationship. If the presence of *H. pylori* is lower, the grade of esophagitis is greater (34.04% for grade A vs. 12% for grade B of esophagitis) (Figure 2.6).

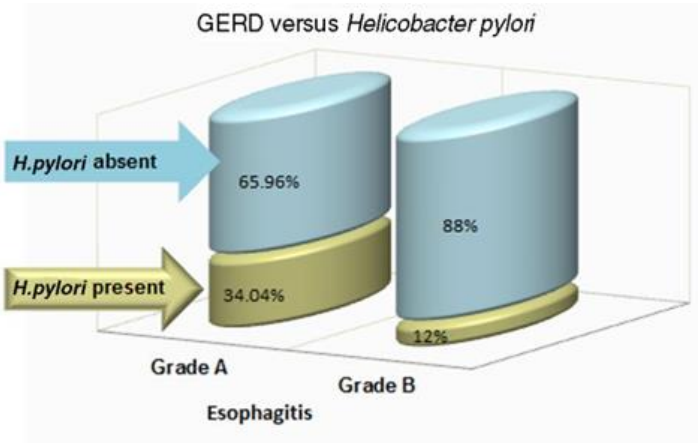

Figure2. 6. *The grade of esophagitis in GERD versus H. pylori*

The presence of *H. pylori* was found to be significantly associated with the presence of grade A esophagitis of GERD and not with grade B esophagitis ($\chi 2 = 54.69$, $P << 0.05$, 95% CI) (Table 2.17). It appears that the presence of bacteria would slow down the progression of esophagitis from grade A to B and then to more severe forms. The presence of *H. pylori* is beneficial, and it does not aggravate the grade of esophagitis in GERD. The results seem to confirm the protective role of *H. pylori* in the evolution of GERD. However, further studies are needed to clarify this situation.

Table 2.17. *Estimated parameters in the association of H. pylori with esophagitis in GERD*

df=1	**Chi-square χ^2**	**p** *95% confidence interval*
Pearson's chi-squared - χ^2	54.69916	0.00000
Correlation coefficient (Spearman Rank R)	0.64294940	0.00000

df = degrees of freedom

Boix-Ochoa score vs. the presence of H. pylori in GERD

Analysis of Boix-Ochoa score according to the presence of *H. pylori* in patients with esophagitis clearly demonstrates that the presence of the bacteria in GERD determines a significantly lower Boix-Ochoa score than the values recorded in GERD when *H. pylori* is not present (F = 8.13, P = 0.0015, 95% CI) (Tables 2.18, 2.19).

Table 2.18. *Statistic indicators of Boix-Ochoa score based on H. pylori*

H. pylori	Mean Boix-Ochoa score	Mean		Standard deviation	Standard error	Min	Max	Q25	Median	Q75
		-95%	+95%							
Present	29.92	22.92	43.36	25.30	4.96	12.36	105.00	19.50	27.15	42.50
Absent	44.26	34.90	70.68	42.79	14.26	21.65	125.60	23.90	32.50	59.00

Table 2.19. *Test for comparing average values of score Boix-Ochoa versus H. pylori*

Boix-Ochoa score vs. *H. pylori*	**F** *(95% confidence interval)*	**p**
ANOVA test	8.13	0.001535

- ***Correlation between wheezing and gastroesophageal reflux disease***

From 85 children (52 males, 33 females) with recurrent wheezing (Table 2.20), 71 (83.5%) had gastroesophageal reflux proven by a positive Boix Ochoa score, while 14 (16.5%) had a negative score (Table 2.21).

Table 2.20. *Patient's characteristics*

Age	No. cases	%	Sex distribution	
0 - 6 mo	7	8.23%	**female**	2
			male	5
6 mo - 1 year	9	10.59%	**female**	3
			male	6
1 - 3 years	51	60.00%	**female**	20
			male	31
4 - 7 years	9	10.59%	**female**	5
			male	4
7 - 12 years	8	9.41%	**female**	3
			male	5
12 - 18 years	1	1.18%	**female**	0
			male	1
Total	**85**			

Table 2.21. *Association of GERD with recurrent wheezing*

		Recurrent wheezing	
		No. cases	**%**
Study group (GERD)	+	71	83.5%
	-	14	16.5%
Total	**85**		

All the 71 children with recurrent wheezing and gastroesophageal reflux disease were submitted to postural therapy and to specific diet for their age. The treatment administrated consisted in proton pump inhibitors (PPI) - Omeprazole or Esomeprazole. The usual doses were used with daily administration for 2 months.

After 2 months of treatment with PPI, the pH was measured again. The Boix Ochoa score remained positive for 15 patients (21.13%). For those patients we recommended another 2 months of treatment with PPI.

Correlational analysis showed the presence of a significant correlation between GERD and recurrent wheezing ($\chi2=6.88$, p=0.0086, 95%CI) (Table 2.22).

Table 2.22. *Estimate parameters in the association GERD - recurrent wheezing*

	Chi-square $\chi2$	**p 95% confidence interval**
Pearson's chi-squared test - $\chi2$	6.888757	0.00868
Coefficient of correlation (Spearman Rank R)	0.4135213	0.00854

The correlational analysis and the parameters estimation of chance and risk in the occurrence of GERD versus wheezing (Table 2.23) makes wheezing a reason for searching and objectification of GERD.

Table 2.23. *Parameters estimation of chance and risk in the occurrence of GERD vs. wheezing*

	Estimated value	*95% confidence interval*	
		Minimum	**Maximum**
PARAMETERS of chance			
Odds Ratio (OR)	2.41	1.18	4.98
PARAMETERS of risk			
Relative Risk (RR)	1.23	1.07	1.43

- ***Another cause of reflux-like symptoms***

Laboratory tests: complete blood count showed normal results.

The upper gastrointestinal (UGI) endoscopy revealed the presence of multiple pseudo-polypoid formations with slightly eroded tip, measuring approximately 0.5 cm, in the lower 1/3 of esophagus (Figures 2.7 a, b); stomach with hypertrophic folds, with nodular purpuric congestion and erosions covered with hematic deposit in the antral region, sero-mucous fluid stasis of bile (Figure 2.8), duodenum with diffuse congestion without ulceration, friable mucosa.

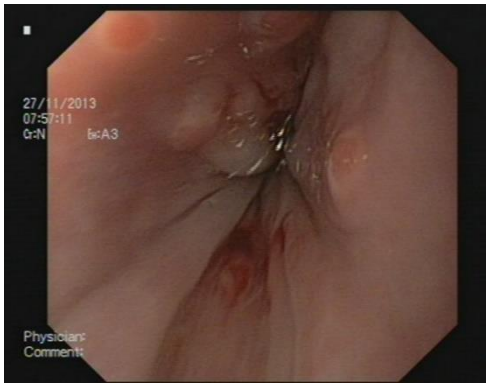

Figure 2.7a: *Gastric heterotopia in the distal part of esophagus*

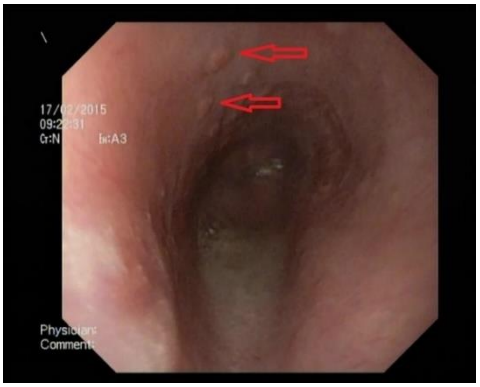

Figure 2.7b: *Gastric heterotopia in the distal part of esophagus*

Figure 2.8: *Nodular purpuric gastritis with hypertrophic folds; biliary reflux*

Histopathologic examination of the biopsy samples taken from the pseudo-polypoid formations showed acid-secreting, oxyntic-type, glandular, gastric epithelium, attached to the esophageal epithelium, without intestinal metaplasia or dysplasia. There were a few micro-hemorrhagic outbreaks and a discrete inflammatory infiltrate (Figures 2.9, 2.10, 2.11). Our histopathological examination did not show intestinal metaplasia or goblet cells (Alcian Blue Stain), which are important for the diagnosis of Barrett's esophagus. 5 biopsy samples were taken, 3 from inlet patches which revealed gastric epithelium and 2 down (distal) from these injuries where squamous epithelium was found. These inlet patches were clearly separated from the stomach by squamous epithelium. The test was negative *for Helicobacter pylori*.

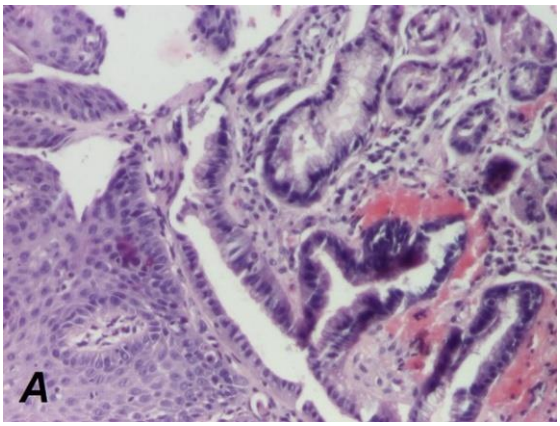

Figure 2.9: *Histopathologic examination HEx100: gastric epithelium attached to the esophageal epithelium*

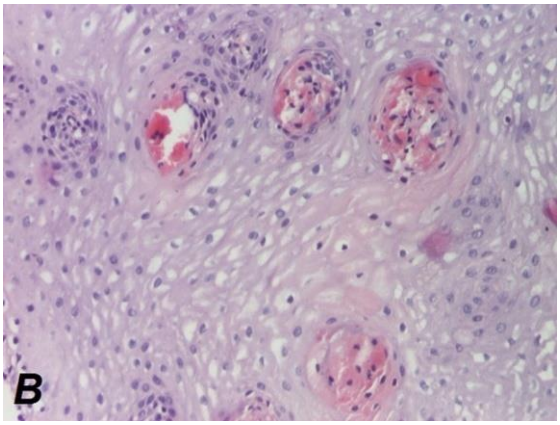

Figure 2.10: *Histopathologic examination HEx100: inflammation and micro-hemorrhages in the esophageal epithelium*

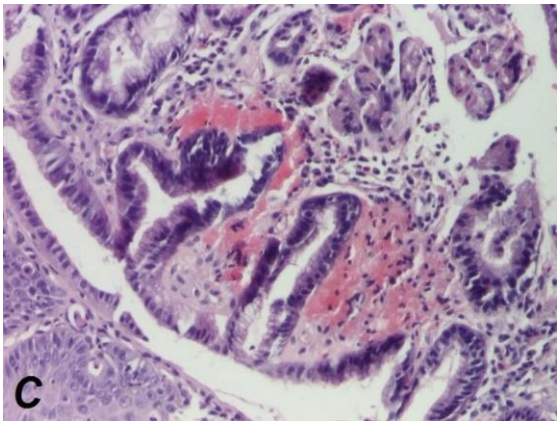

Figure 2.11: *Histopathologic examination HEx100: inflammation and micro-hemorrhages in the gastric epithelium*

At colonoscopy, the mucosa of rectum, sigmoid and descending colon appeared normal, without evidence of diverticulosis or polyps.

2.7.4 Discussions

- ***The role of Helicobacter pylori infection in gastroesophageal reflux***

H. pylori has been shown to increase the risk of gastritis, peptic ulcer and precancerous lesions in the stomach. The role *H. pylori* infection plays in the esophagus remains doubtful. Epidemiologic studies have shown the incidence of *H. pylori* infection in patients with GERD from 30 to 90% and the average is approximately of 35% in most series (Savary and Miller, 1978). Both conditions affect a large proportion of the population and they may occur either independently or concomitantly (Malfertheiner and Peitz, 2005).

It was suggested that *H. pylori* could contribute to GERD through different mechanisms: a decrease of lower esophageal sphincter pressure and impairment of gastric filling, the development of antral gastritis which increases acid production (Kusano et al., 2004).

Gastric acid secretion, therefore, is the key factor in the relationship between *H. pylori* and GERD. In patients who develop chronic atrophic gastritis as a consequence of

H. pylori infection, gastric acid is suppressed and so acid would no longer appear to be produced in a critical amount for the induction of GERD (Koike et al., 2001; Yamaji et al., 2001). A study in a large patient group suggests that, even a corpus-predominant gastritis would exert a protective effect against GERD development (El Serag et al., 1999). Studies from Japan in patients with atrophic gastritis reported increased acid production following *H. pylori* eradication and induction of GERD in a subset of patients (Haruma et al., 1999; Koike et al., 2001). The protective potential of *H. pylori* has been demonstrated in studies that discovered more virulent strains to be less prevalent or even absent in severe forms of GERD. Cag A carrying strains were suspected to protect from Barrett's adenocarcinoma (Vaezi et al., 2000).

On the contrary, other studies found that *H. pylori* eradication did not lead to alterations in the gastroesophageal reflux pattern and the bacteria status in patients with GERD did not impact on the grade of esophageal acid exposure (Zentilin et al., 2003; Peters et al., 1999).

The authors of a study found no influence of *H. pylori* infection either on pH-metry results or on endoscopic findings (Gisbert et al., 2001). In a recent study, out of 184 GERD patients, 46% were *H. pylori*-infected while 54% were *H. pylori*-negative with no statistic difference regarding presence and severity of reflux esophagitis between patients with and without *H. pylori* infection (Grande et al., 2014). In our trial consisting of 72 patients with GERD, 26.39% were positive and 73.61% were negative for *H. pylori* infection.

The authors of a study, report that the prevalence of reflux esophagitis, like a biomarker for GERD, among *H. pylori*-positive children regardless of their age and gender was twice as high as among *H. pylori*-negative patients: 81.3% vs. 38.1%; there was no difference in the apparent severity of reflux esophagitis between *H. pylori*-positive and negative patients (Moon et al., 2009). A study reported a correlation between *H. pylori* infection and a reduction in the severity of reflux esophagitis. These findings suggest an inverse correlation between *H. pylori* infection and the risk of esophagitis (Fallone et al., 2000). In our study, regarding the correlation between the presence of *H. pylori* and the grade of esophagitis we obtained the existence of an inverse relationship.

24-hour pH monitoring cannot be regarded as a definitive gold standard for GERD diagnosis. The main limitation of the 24-hour pH monitoring is its low tolerability (Sarani et al., 2002). Patients report that pH-metry frequently induces unpleasant side effects lasting for most of the day. North American Society of Pediatric Gastroenterology, Hepatology, and Nutrition and the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition guidelines on pediatric GERD established that multichannel intraluminal impedance-pH monitoring detects acid, weakly acid and nonacid reflux episodes and it is superior to pH monitoring alone for evaluation of the temporal relation between symptoms and GERD (Vandenplas et al., 2009). This technique is limited by its high cost and the lack of normative data of multichannel intraluminal impedance-pH in the pediatric population (Shin, 2014). However, as long as there is no effective medical therapy for weak acid and nonacid reflux, the clinical relevance of measuring these types of refluxes remains debatable (Wenzl et al., 2012).

When pH evaluation is performed over a prolonged period, it presents high sensitivity and specificity indices (Jamieson et al., 1992). The Boix-Ochoa methodology is the most appropriate for application to the pediatric age group (Boix-Ochoa et al., 1980).

The clinical reality is that there is a large population of children with *H. pylori* infection and concomitant GERD. There is inconclusive evidence that more severe forms of GERD have a lower prevalence of *H. pylori* infection or are infected with less virulent strains. From all current debates concerning the clinical management of *H. pylori* infection

in patients with GERD, eradication treatment is recommended in those who require long-term PPI (Malfertheiner et al., 2002).

The limitations of our study include the fact that the data reflect a single clinical center with a low prevalence of *H. pylori* in children with GERD.

- ***Correlation between wheezing and gastroesophageal reflux disease***

Task Force proposed to use the terms episodic wheezing to describe children who are wheezing intermittently and are well between episodes, but there is poor agreement on definitions of preschool wheezing disorders (Stocks et al., 2001).

Wheezing is the most common symptom associated with asthma in children, but this is also common in non-asthmatic children (Weinberger and Abu-Hasan, 2007). A study concluded that only 30% of preschoolers with recurrent wheezing are eventually diagnosed with asthma at the age of six years (Taussig et al., 2003).

It has been reported that the frequency of GER in 85 infants with recurrent wheezing was 48.2% (41 children had positive pH monitoring results) (Sung et al., 2010).

Two studies reported that about 1/2 - 1/3 of infants and children with early-childhood wheezing developed persistent asthma later (Martinez et al., 1995; Wennergren et al., 1997). It is important to make an early and correct diagnosis and provide the proper treatment to infants with wheezing for the prevention of airway remodeling (Sung et al., 2010).

Wheezing in early childhood is often observed in association with a viral respiratory disease (Jackson et al., 2008, Heymann et al., 2005). Sometimes unnecessary investigations are conducted, inadequate treatment is provided and the child may need hospitalizations resulting in considerable healthcare costs. Misdiagnosis has also led to therapy with inhaled corticosteroids, resulting in some children developing significant steroidal side effects.

Several studies have reported that boys have an increased risk of early persistent wheezing (Garcia-Marcos et al., 2010). A recent study reported the presence of recurrent wheezing in 64% of the boys (Ozdogan et al., 2015). In our study we found similar results, 61.2% of the patients with recurrent wheezing were boys.

There are some studies which confirmed the association between silent GER and food sensitization in infants with recurrent wheezing without food allergy. The presence of silent GER might contribute to early food sensitization and then to the development of atopic asthma in childhood. In a study, the authors showed that in the silent GER group, 12.2% of patients had food sensitization and in the non-GER group, 20.5% had food sensitization, without significant difference between the two study groups. There was no difference between GER parameters of patients who had positive specific IgE to food and those who were negative (Weinberger and Abu-Hasan, 2007).

There are studies that suggest that the most patients with an association between cough and gastroesophageal reflux have predominantly biliary reflux, while wheezing is most often preceded by acid reflux (Kunsch et al., 2011; Blondeau et al., 2008; Ford et al., 2006).

The presence of fluids in the airways can cause bronchospasm. Most commonly unconscious, macro-aspiration can cause the respiratory tract to close, which can be associated with lesions such as hemorrhagic pneumonitis and non-cardiac pulmonary edema (Marik, 2010). On the other hand, micro-aspiration (reflux theory) can induce bronchospasm directly by stimulating the larynx through the tracheal receptors (Allen and Leclair, 2009). It is also known that the esophagus and trachea have common embryonic origins, hence the theory that acidification of the distal esophagus causes vagal stimulation resulting in bronchoconstriction, unrelated to micro-aspiration (Mathew et al., 2004).

Regarding the correlation between GER and wheezing, there is not necessarily an etiological link between the two conditions. Thus, recent studies have proposed biomarkers to

identify GER-associated respiratory infections, parameters from the bronchoalveolar lavage: the lipid-laden alveolar macrophages and the rate of neutrophilic inflammation. It has also been shown that these parameters correlate with GER severity, but their specificity remains debatable (Sacco and Silvestri, 2006; De Baets and Aarts, 2010).

There are different methods used to diagnose GERD: 24 hours esophageal pH-monitoring, esophageal manometry, impedance – pH-metry, ultrasonography, including the intraluminal one, and upper gastrointestinal endoscopy (Sifrim and Blondeau, 2006). These methods have different specificity and sensitivity. The 24 hours esophageal pH-monitoring is the most used method to diagnose the acid reflux. The sensitivity of the esophageal - pH-monitoring is higher than 85% and its specificity is 95% (Simpson, 1995).

Limitations of the study consist in that the esophageal - pH-monitoring cannot detect weakly acid and nonacid reflux episodes, also that the data reflect the experience of a single clinical center.

- ***Another cause of reflux-like symptoms***

HGM is a congenital defect that is usually no larger than 1 cm. HGM can be smaller than 1 cm or larger than 5 cm (UyanIkoğlu et al., 2011). The inlet patch is considered a congenital anomaly. Inlet patches represent the incomplete transformation from columnar to squamous epithelium during embryonic development (Maconi et al., 2000). The process is starting from the middle third of the esophagus and extending proximally and distally. The last esophageal segment re-epithelialized is the most proximal.

HGM develops on incomplete re-epithelialization. The persisting columnar cells at birth is usually proximally over the upper third of the esophagus or, much less frequently, distally over the esophago-gastric junction. The preferential localization of HGM in the cervical esophagus is explained by the temporal difference in the stratified re-epithelialization of both ends of the esophagus (Chong, 2013).

It is generally accepted that Barrett's esophagus is an acquired metaplastic change due to chronic gastroesophageal reflux. Another proposed theory on the development of HGM involves metaplastic transformation of the squamous lining to columnar from chronic acid injury as seen in Barrett's esophagus (Ayidan et al., 2001).

The origin of esophageal HGM has not been finally determined as yet, and further investigations are needed to resolve these questions (Von Rahden et al., 2004).

Most inlet patches are solitary and extend longitudinally. In the patients with multiple patches, they tend to be small (Day et al., 2003). In our case, we have described them as multiple pseudo-polypoid formations with slightly eroded tip.

Most inlet patches are clinically asymptomatic (Shu-Jung et al., 2015). The underlying factor of the symptoms, clinical findings and complications is the secretion of acid. Only in 10% of cases do they produce clinical symptoms, such as chest and throat pain, dysphagia, globus sensation, shortness of breath, chronic cough and hoarseness (Basseri et al., 2009).

In our case, the patient presented with postprandial epigastric pain only. In our patient, HGM was associated with discrete inflammation, chronic gastritis and biliary duodenogastric reflux. The HGM was grade I according to the proposed clinicopathological classification (Table 2.24) (Von Rahden et al., 2004).

Table 2.24. *Clinicopathologic Classification of Esophageal HGM*

HGM 1	**Asymptomatic**
HGM II	Symptomatic without morphologic changes (dysphagia/odynophagia)
HGM III	Symptomatic with morphologic changes (benign complications: strictures, ulcers, webs, stenoses, fistula)
HGM IV	Intraepithelial neoplasia (dysplasia) (low-grade/ high-grade)
HGM V	Invasive adenocarcinoma
Suffix	
a.	¼ inlet patch (macroscopically visible patch of HGM)
b.	¼ microscopic foci (only microscopically visible HGM)
HGM = heterotopic gastric mucosa	

Ectopic gastric mucosa can occur anywhere along the gastrointestinal tract. For this reason, we performed the colonoscopy which was normal.

Columnar epithelium in the lower esophagus is generally related to Barrett's esophagus. Barrett's esophagus is widely considered to be a consequence of longstanding acid induced injury commencing as an erosive esophagitis and progressing over years to columnar and intestinal metaplasia of the squamous epithelium. In our case, the patient had biliary duodenogastric reflux, without esophagitis. It needed a histopathological confirmation, i.e., the presence of intestinal metaplasia (Spechler et al., 2011). Our histopathologic examination revealed just a discrete inflammatory infiltrate, without intestinal metaplasia or dysplasia. In Barrett's esophagus, columnar epithelium extends from the stomach up to the gastro esophageal junction into the esophagus (Spechler et al., 2011). It looks like a continuation of gastric mucosa. In our case, columnar epithelium was not the continuation of gastric mucosa; it was clearly separated from the stomach by squamous epithelium. There were multiple pseudo-polypoid formations, which can indicate that all lesions are congenital and not reflux-related as in Barrett's esophagus. Furthermore, over the last 3 years since we have followed up the patient, the injuries have remained the same in number and pathology. Based on studies from the literature, we reevaluated the findings in our patient as gastric heterotopia and not Barrett's esophagus.

A strong affinity of Helicobacter pylori to colonize gastric type mucosa has been demonstrated (Alagozlu et al., 2010). In our case, colonization of HGM and of stomach with Helicobacter pylori was not observed.

This type of gastric heterotopia can cause reflux-like symptoms, which resolved very well with proton pump inhibitor treatment (Zeynel, 2014). No treatment is required for asymptomatic HGM.

Strictures are treated with serial dilatation and should be biopsied to rule out malignancy.

It can be discussed about an ablation of the gastric heterotopia's area. We did not try it because of the lack of support in the literature. Neoplastic transformation in gastric heterotopia is rare. In contrast to Barrett's esophagus, gastric heterotopia has not been accepted as a premalignant lesion (Von Rahden et al., 2004). But the inlet patch has been accepted as a premalignant lesion.

We were following the evolution by endoscopy every six months and multiple esophageal biopsies once a year to monitor any change in HGMs.

2.7.5 Conclusions

- ***The role of Helicobacter pylori infection in gastroesophageal reflux***

The presence of the *H. pylori* is not an important factor in the gastroesophageal reflux. This is demonstrated by insignificant differences in the presence of *H. pylori* in children with GERD (26.39%) compared with the frequency of *H. pylori* in the control group (16.13%). On the other hand, its relationship with esophagitis appears to be inverse ratio. The fact that the *H. pylori* presence is statistically greater in the grade A esophagitis could confirm the hypothesis that the bacteria would slow down the development of the esophagitis. Also, the presence of *H. pylori* determines lower Boix-Ochoa score, so less acid reflux episodes.

- ***Correlation between wheezing and gastroesophageal reflux disease***

Recurrent wheezing is a solid reason for evaluating the presence of a gastroesophageal reflux by 24-hour continuous esophageal pH-metry. The bronchial spasm triggered and maintained by the aspiration of the acid refluate remains the most plausible explanation of this relationship and association. Adequate treatment of gastroesophageal reflux also resolves the recurrent wheezing.

- ***Another cause of reflux-like symptoms***

We are presenting a case with multiple pseudo-polypoid formations of gastric heterotopia that are located in the distal part of the esophagus. HGM should not be overlooked in the diagnostics. Endoscopy combined with histopathological and microbiological analysis of biopsies is definitely a mandatory method in clinical evaluation of metaplastic and non-metaplastic changes within HGM of the esophagus.

2.8 Ethical considerations in pediatric gastroenterology

2.8.1 Introduction

Following the pioneers of endoscopic digestive exploration, P. Bozzini (1796) and his *lichtleiter* (light conductor), A.J. Desormeaux who, in 1835 uses the term endoscope for the first time, and A. Kussmaul, who in 1868 does the first human gastroscopy, the use of optical instruments adapted to these procedures is improved by successive innovations by using a closed tube (Rosenheim and Schindler in 1885), of an open tube (Ch. Jackson in 1907), respectively of a semi-flexible examination device (Schindler and Wolf in 1932). Decisive modernization of these instruments belongs to B. Hirschowitz, who in 1958 and 1961 succeeds in clinically applying the "transport of images" in endoscopy through optical fibers. Nowadays, the transmission, the magnifying and the electronic processing of the images is being permanently improved, according to the multiplication of the interventional techniques and also as a result of a hard competition between the producers of this technology (Gilger, 2001; Cardanel and Mougenot, 2006).

The development of the areas of exploration in the esophagus, stomach and duodenum, respectively rectum and sigmoid to the entire colon and then to the lower intestine, the biliary and pancreatic ducts was the consequence of perfecting the technical tools and accessories, of the evolution from diagnosis to therapeutic endoscopy, of the extension of the indications to an even more various pathology, as well of the introduction of these methods in younger children, new-born and premature.

Pediatric endoscopy inaugurated by Kremer, Ottenjann (1970), Kremer (1974), initially with adult devices, subsequently with tools adapted to pediatric size, has continuously evolved,

both by researching more new anatomy territories and by varying the spectrum of pathology approached and successfully treated (Cardanel and Mougenot, 2006; Kawai et al., 1970; Ottenjann, 1970; Cremer et al., 1974). All of this led to the creation of an increasing experience for more and more specialists and to the knowledge, the diagnosis and the treatment of numerous illnesses related to pediatrics. It has therefore become the subject of numerous publications and scientific events that have contributed to the development of a new field of medical expertise – pediatric digestive endoscopy – where interventional aspects tend to overcome the exploration and diagnosis ones (Chang et al., 1983).

Endoscopic procedures are a major and indispensable component, through their precision and volume of information offered to the diagnosis, treatment and monitoring; they have to be performed by an expert who knows both digestive pathologyat children and the practice of various specific gestures and manoeuvres. We should underline that these techniques are invasive, suppose risks and the possibility of complications and failures.

These include: the anesthetic technique, the increased complexity of the procedures, especially the therapeutical ones and finally the ones determined by the field and pathology specific to their age. In such conditions we should mention the series of procedures practiced in present, as well as their indications and contraindications.

Esophagoscopy: extraction of foreign bodies, diagnosis and assessment of caustic wounds – dilatations, strictures, tumors – biopsies, placement of stents, screening and monitoring of Barrett esophagus, evaluation of reflux esophagitis, the assessment of dysphagia, odynophagia and non-cardiac thoracic pains (Ament et al., 1988; Kim, 2010).

Gastro(duodeno)scopy, the oldest and most frequently used procedure used in various circumstances: foreign bodies, dissolution of bezoars, digestive hemorrhages (hematemesis, melena), abdominal pains including suggestive systemic signs for an organic pain (weight loss, anemia, fever), nausea and persistent vomiting, precocious satiety, anorexia or refusal of food, refractory iron deficiency anemia, growth delay, assessment of some imagistic modifications; recently, PEG placement (Kim, 2010; Benaroch and Rudolph, 1994).

Enteroscopy, an investigation that registered the most spectacular modernizations ("pushed" technique with one or double balloon or with the capsule) is useful when mentioning the origin of certain digestive bleedings, in Crohn's disease, celiac disease, intestinal polyposis, but also when placing feeding tubes or rarely in monitoring the evolution of an intestinal transplant (De Riddes et al, 2012; Iddan et al., 2000; Aabachen et al., 2003).

(Recto)colonoscopy is also indicated at children for the diagnosis and the control of hemorrhagic episodes (melena, hematochezia), chronic diarrhea, anaemic and painful syndromes clinically significant, but unexplainable, the diagnosis and the monitoring of polyposis (biopsy, polypectomy) and more rarely when dilating certain stenosis, the assessment of surgical lesions or of intestinal transplants (Habri Gama et al, 1979; Rossi, 1988).

Advanced explorations are rare in children: ERCP for cholestasis syndromes and pancreatic pathology, EUS for pancreatic masses and for rare tumors of the upper digestive tract. Contraindications of the endoscopic exploration in pediatric practice are provided by cardiovascular collapse, respiratory distress or neurological deterioration, perforation or intestinal obstruction, peritonitis and extreme prematurity, recent food ingestion, hypoglycemia in diabetic children. According with a geometric progression of the number and difficulty of these exams and with a spectacular lowering of the age at which they are practiced, it has been described the existence of possible complications even in the expert execution (≈ 1%) related either to sedation / anesthesia or to the procedure itself (Rothbaum, 1966). General anesthesia – considered safe and effective, is recommended for young ages as well as conscious sedation is preferred at older ages; each may generate specific incidents: O2 desaturation, respiratory depression, apnea, hypotension, bradycardia and even cardiovascular collapse and death (0.06%) (Hussein, 2006). Other complications can be minor nausea, transient hypoxia and

swallowing, bleeding episodes more or less important, infections (*Salmonella, Mycobacterium, H. pylori*), and perforation, fistula (after PEG placement) or capsule retention. The presentation of the possibilities and indications but also of the technology of these explorations defines their invasive nature and argues obligation and importance of obtaining an informed consent from parents, legal tutors of children but also the child's agreement – especially in those with appropriate physical and intellectual development (different age groups) that become active decision makers in authorization of diagnostic, therapeutic (and even research) practice on their person. Obtaining informed consent in pediatric affording new understanding in ethical and legal standards. It is still defined as a willing agreement or acquiescence given by a person of discernment that is not obtained by fraud and is consistent between the internal and the declared will of the patient containing both ways: voluntary option (never presumed) and the need for authorization both legal and institutional effective. Discernment is the individuals' ability to understand, appreciate and judge their actions (intellectual moment) and anticipating their consequences to decide on the optimum (volitional moment). Consent becomes an action (and finally a document) derived from the ethical principle of respect for patient's autonomy and self-determination, respectively the right to decide on procedures and treatments offered.

The fundamental human rights have been provided by national and international regulations (Vicol, 2010). The life itself, which means autonomy and freedom, has received the status of immutable and inalienable social value, the paternalist character itself of medicine, in which the physician decides, and the patient executes, would be profoundly modified (UNESCO, 2005).

The evolution of society and particularly of medical sciences in the last century led to updating of principles and their adjustment to the new values (Buta and Buta, 2008). Therefore, the two main ethical values: life and freedom are transformed in two ethical principles that flow from these values and namely the respect for life and the respect for individual's autonomy.

In time, this evolution has led to the change of patient-physician relations and society-health system, achieving the character of a contractual relation (Hoerni and Benezech, 1994). In the case of the society, this establishes a contract with the health system based on some rules provided by the law, while in the case of the physician-patient relation, the patient entrusts the physician with personal and intimate information regarding his/her health condition and life. In his turn, the physician must provide the patient with all the information regarding his/her suffering and the possibility to recover the health condition from the perspective of the medical procedure, along with the risks that it entails (Hoerni and Benezech, 1994). Thus, it created a partnership relation, in which the statute of patient is the one of well-informed beneficiary, partnership which takes the form of the informed consent, and the physician's statute is the one of the health care providers, whose services the patient can accept or not. Thus, the physician – patient relation, that had a paternalist character, is gradually transformed into a contractual relation with mutually binding rights and obligations, fully informed regarding the stages of the medical procedure and its consequences (Mason and Mc Call, 1999).

The doctrine of informed consent includes the principle of benefit – only those practices that can bring good to the patient are indicated as well as the principle of fairness – the same measures for the same disease in each patient. Differences exist from doctor to doctor and according to specific departments on the quantity and quality of information content and especially on the way in which they are exposed. The involvement of the medical staff is nonuniform sometimes superficial, contradictory and even chaotic. In children the legal concept evolved particularly in some European countries and the USA assuming – in addition to a better understanding of how the doctor must work with parents – obtaining the minor patient's agreement, which is essential. Parental consent is rather an informal explicit permission to which is added mandatory the consent of a minor patient. It is accepted that since

the age of 7 a child can understand the purpose of exploration, at 10 years the risks and the right of refusal and after 14 years the moral and intellectual maturity, the ability to understand, the abstract thinking and the hypothetical assessment approach the adult ones allowing responsible decisions (Committee of Bioethics, 1995; Shield and Baum, 1994).

The prevalence of erosive esophagitis in children with GERD symptoms reported in different studies was 10-50% (Nelson et al., 2000; El-Serag et al., 2002). Erosive esophagitis was defined as visible breaks of the esophageal mucosa detected by esophagogastroduodenoscopy (Vandenplas et al., 2009). This is the reason why sometimes we should perform endoscopy, to exclude such type of lesions and for this we need an informed consent.

Personal contribution – published paper:

1. Tighici E, Burlea M, Lupu V, **Lupu VV**. Ethical aspects in pediatric gastroenterology. *Romanian Journal of Bioethics*, 2012, 10(3): 116-123. **ISI IF 1,00**
2. Diaconescu S, Olaru C, Gimiga N, Ciubotariu G, **Lupu VV**, Ciubara A, Galos F, Burlea M. Informed consent in pediatric gastrointestinal endoscopy. *Revista românå de pediatrie*, 2013, LXII(4): 345-406

The aim of this review was to approach the ethical problems of the medical procedure and implicitly the physician – patient relationship in the view of modern medicine, particularly in the field of Pediatrics and with an emphasis on pediatric gastroenterology. In this context, it is mainly pointed out the digestive endoscopy with its features from the perspective of the medical bioethics.

2.8.2 The informed consent and its features in pediatrics

In our country the obtaining of an informed consent is stipulated by Law No. 46/21 January 2003 on patients' rights to receive information about health services and their mode of applying as well as about the identity and professional status of health care provider. The patient has the right to receive complete information about his health, the benefits of medical treatments and interventions (including exploratory procedures) that can help restore or improve his health and to be or not to be informed about his disease if the revealing would cause distress. The informing should be so complete as to allow a conscious patient to take a decision and the refusal must be respected but as with consent, the assumed responsibility must record in writing by the patient, while for children by parents or legal tutors. The terms of legislation cannot cover many circumstances encountered in clinical practice and the legal and ethical dilemmas or even conflict situations. Thus, from the start can be a lack of mutual empathy between parents and physician and lack of social code, intellectual, cultural and even language barriers. The relationship between parents and children can be affected by excessive authoritarianism or tolerance, by the parents' doubts about the right and the ability of children to decide or by differences of opinion between parents (divorce, adoption), etc.

The legal authorisation involves, as in adults, the patient's right to know and understand what is happening and the obligation of doctors to present the required, expected and necessary information that leads to an "educated decision" on their condition.

The ability to understand is extremely diverse both in caregivers and children and should be carefully evaluated by the doctor in order to opt for a more rational and convincing information, depending on many different factors: the intellectual and educational level, temper, trust, suspicion or fear, any previous experience but also influences of other physicians or qualified persons, relationships between parents and their own children; the latter proving to be confident or reluctant and sometimes fearing more the investigation than the disease. Depending on these factors and many others it has to be decided the level of information

concerning the rates of morbidity / mortality of the suggested procedure – too many details can frighten the subjects leading to refusal of the investigation / treatment, too little may expose the doctor at charges of an incorrect way of getting the acceptance. The specialist should opt for a "rational" informing with an exposure of significantly frequent or severe risks in order to convince a rational patient. On the other hand presenting only the major elements of risk may displease those who want an exhaustive presentation of them.

As a basic principle of the medical ethics, the informed consent has been established in the Universal Declaration on Bioethics and Human Rights at the 30th session of the General Conference of UNESCO of the 19th of October 2005. Article 6 of this declaration presents the consent based on the principle of autonomy in the spirit of the fundamental human rights (the right to life and freedom) and equally on the respect of the human dignity (UNESCO, 2005; Martin, 2011). Medicine, which presumes immediate interventions on the healthy or sick individual, directly affects these fundamental values regulated by the law, and consequently, their application involves the responsibility of both the physician or the system proposing them, and the patient or the community that accepts them. This responsibility also presumes the acceptance of the negative consequences of the medical procedure which involves at its turn the capacity to make decisions. Therefore, the procurement of the informed consent must rely on sufficient and accurate information.

When providing information, it must be considered the patient's level of understanding, his/her cultural values and the degree of tolerance regarding the knowledge of the truth (Adnan et al., 2006). That is why the language used, the manner of expression, the contents of information must be adapted to the level of understanding and perception of the patient, as the legal connotation of the consent must never be forgotten. We underline that, in legal terms, the informed consent is an at-will agreement, either express or tacit, given by a person with discernment, which is not vitiated by fraud and represents a concordance between the intimate will and the one declared of the patient.

Communication and obtainment of the child's consent is often influenced by the presence or absence of relatives or other persons responsible for him. Special conditions are encountered in children with impaired intellectual development or psychiatric disorders, orphans, deprived of legal representatives or institutionalized, alcohol or drugs consumers, members of religious sects as well as in emergency situations where there is practically no time to obtain the consent of relatives or in cases of impaired status of small patients in which an early endoscopy can contribute to the diagnosis and management of these conditions. It can also be mentioned the declination by the child of the initial consent, in which case it has to be requested from the parents or even through legal mandate in rare situations when public interests exceed the rights of the patient (Shield and Baum, 1994; Anderson, 1993; Miller et al, 2004).

Of course, these aspects get a special connotation in pediatrics, as it usually involves children, who are persons without discernment, or with limited capacity of understanding medical information. That is why the informed consent appears as a responsibility of the next-of-kin, parents or legal guardians. Article 4 of the Chart of Hospitalized Children issued by UNESCO, provides the child's right to be informed, of course, in a manner adapted to his/her age, provided that this informing is done by the physician, the caregivers and parents (Molina et al., 2011). There is even a regulation, in the fourth article in the Hospitalized Child Carta of UNESCO, which stipulates the right of the child to be informed, of course in an accessible manner depending on his age (Wail and Raynal, 2000). This attitude is firstly justified under psychological aspect because the anxiety caused by silence and secrecy can induce a state of unease to the child and can even worsen the disease, and, secondly, it must be considered that nowadays the perception and intelligence of a child is superior to the previous generations, starting even since the childhood.

Informed consent is a legal part of the observation sheet, it must be signed and dated and its content necessarily includes

- a complete diagnosis and prognosis of the disease that requires an endoscopy;
- type and description of the procedure including conscious sedation / anesthesia;
- the indications and the benefits of the procedure;
- the physical discomfort and complications: severity, incidence, risk;
- the alternative possibilities, the results and their reasonable risks;
- the prognosis in case of refusal (Zuckerman et al., 2007; Sinner and Johnson, 2002; Ladas, 2006).

The physician should be aware of subjective and objective obstacles of obtaining this document – a difficult moment both for minor and for family; he must exploit his interpersonal communication skills and the spirit of compassion. Discussions (sometimes repeated) with the child and relatives involve-especially when the procedure includes a therapeutic part – an objective assessment of the intellectual, educational, and social level of the interlocutors.

We must take into account the fact that small infants and toddlers, sometimes older children and even teenagers can't describe the causes, nature and character of their suffering so that the discussion should be appropriate to the age of the subject.

The dialogue will be held in a special room providing an intimate atmosphere of trust and collaboration, using a friendly, encouraging and attractive language with simple and comprehensive terms, providing information and "common sense" arguments according to the capacity of the interlocutors to understand and to the opportunity to ask questions and get answers. Further efforts are needed to reduce natural reluctance and anxiety of children and parents. An appropriate amount of information must be presented in order to explain the steps and stages of the procedure and the interventional maneuvers: diet, bowel preparation, venous puncture for anesthesia, duration etc. Communication of disagreements, risks and potential complications are "keystone" in obtaining the informed consent, this requiring patience, tact and positive approach and a highlight of the doctor's experience and success rate. On contrary, the benefits of endoscopic exploration will be exposed.

The discussion will always respect the autonomy of decision, the dignity of the patient and will provide insurances about data confidentiality. Finally, understanding of the presented data should be checked, providing a reasonable time of reflection and decision both from parents and child. We don't have to forget to take the opinion for the eventual use of data obtained from endoscopic procedures in research purposes (Committee of Bioethics, 1995; Alderson, 1993; Kon, 2006). Same coordinates should be considered when the physician is confronted with resistance or persistent refusal of the parents or of the (non) "emancipated" child which should be respected both ethically and legally.

Therefore, if by the age of 4 a child fears the medical procedure and is looking for refuge and protection from parents, between the ages of 4 and 8, the curiosity and desire of socializing make the child accept and even take part to some medical maneuvers and procedures, attitudes which are also stimulated by the communication with those surrounding him/her, so that after the age of 8 he/she would actively participate to the performance of some procedures and application of certain therapeutic schemes, even with the rigors entailed by the disease. Sometimes it is impressive the compliance to treatment of a child suffering from a chronic illness, either diabetes, bronchial asthma, innate disability or neoplastic disease, regardless it is a painful procedure, a diet restriction or a long-term medication therapy (Buta and Buta, 2008).

In this context, it is a mistake to underestimate the child's capacity to understand, accept and even cooperate for the success of the treatment, especially the school age child, if he/she is explained in his/her terms the suffering, its causes and the possible medical remedies. Starting from these facts, the Convention of the U.N. from New York in 1991, regarding the

child rights, expressly provides that "the states guarantee the child who is capable of discernment the right to freely express his/her opinion on all the problems regarding him/her, his/her opinion being taken into consideration depending on the age and the degree of maturity."

The decision of a minor may not be rejected by any parental authority and can even be named an "informed refusal." The situation becomes more complicated when this option comes immediately before the beginning or during the endoscopy; this can lead to stop of the procedure and to a new counselling. Sometimes a good doctor or parents can encourage and persuade in a reasonable time the subject to accept the continuation of the investigation. Sometimes the presence of a psychologist is useful. At lower ages (under 7-10 yrs) parents and caregivers have the entire legal responsibility of refusal or cessation of the procedure.

This is because the capacity of consent also presumes the capacity of understanding the implications of a decision and equally the capacity of rationally assessing the consequences of the decision made (Cojocaru and Cojocaru, 2011). If by the age of 7-8 the child, even if he/she understands, prefers to "do as mummy and daddy say", while after the age of 9 he/she manifests the tendency of getting involved in a responsible manner, and starting with the age of 14-16 years his/her agreement and attitude must be considered (Wail and Raynal, 2000). When the consent cannot be guaranteed by parents or legal guardians, the society has the possibility to offer the agreement through its institutions, except for the emergency situations when the physician is authorized to make the most appropriate decision with the purpose of preserving life.

2.8.3. Features of the informed consent in the pediatric gastroenterology

It must be emphasized from the beginning the fact that, from the point of view of bioethics, there are major distinctions and particular shades in approaching different specialties and subspecialties within Pediatrics. In this context, gastroenterology has a special place especially due to the technical possibilities of which it disposes nowadays, from sequential and interventional endoscopic explorations to transplants. Besides the purely medical side in the diagnostic approach of the digestive pathology, extremely beneficial nowadays, the technical possibilities also involve risks through their invasive character which must be carefully assessed and offered as information to the patient (Ament, 1997). Therefore, it is often necessary that each of these technical possibilities should rely on a separate ethical approach, even though, overall, the basic ethical principles are found in each of them.

A special place in the pediatric gastroenterology is taken by the endoscopic approach of digestive pathology, with its two sides: diagnostic endoscopy and therapeutic or interventional endoscopy, a new technical possibility that has quickly evolved in the last 40 years. We remind the fact that the first digestive endoscopy was performed in 1970 in Los Angeles on a newly born. The technical development of flexible endoscopes has allowed the extension of endoscopic investigations, so that nowadays pediatric gastroenterology can no longer be practiced without endoscopic exploration, whose main advantage is that it allows the visualization of the lesion, the in-situ biopsy and even some immediate interventions (Burlea and Lupu, 2009). Besides the huge benefits brought in the therapeutic exploration and approach of the alimentary duct, it must not be overlooked the fact that the procedure itself, even if it is minimum invasive, still induces a state of discomfort to the patient, related both to the pre-anesthesia or anesthesia and to the maneuvers itself required by this procedure. Relatives must be informed on these aspects (Friedlander et al., 2009).

It is unanimously accepted the fact that presently the contribution of endoscopy plays a decisive role in the diagnosis of some diseases, formerly mentioned as hypothetic only, among which: the gastroesophageal reflux disease, unexplainable repeated vomiting, gastrointestinal hemorrhage dysphagia or odynophagia, ingestion of caustic substances or foreign bodies, the

presence of polyps, thoracic or abdominal pains having an uncertain cause, chronic infections or the presence of chronic inflammatory diseases of the gastrointestinal tract, etc. (Burlea et al., 2005). It must be considered the fact that, besides the diagnostic aspect whose precision is incontestable, as it visualizes the lesion in real time, the digestive endoscopy also offers immediate therapeutic solutions, such as the endoscopic hemostasis of esogastroduodenal non-varicose acute bleedings, the extraction of foreign bodies, the sclerotherapy of esophageal varicose, the dilatation of esophageal stenosis, endoscopic polypectomy, percutaneous endoscopic gastrostomy etc. (Burlea et al., 1999).

Although the endoscopy is generally considered a hypo aggressive procedure, it still presumes a series of risks which must be presented to the patient. Therefore, starting from vomiting produced by premedication or the discomfort related to the insufflation with air during the endoscopic maneuver, until the unwilling but possible complications, such as: the inflammation of the neck, retropharyngeal hematoma, detachment or loss of teeth, laryngeal trauma, esophageal and laryngeal perforations, although very rare, can occur. Of course, the percentage of complications can be higher in interventional endoscopy. Knowing the limits of endoscopy is compulsory to a physician because this prevents him from approaching assimilated situations, as contraindications such as: cardiovascular instability, breathing dysfunctions, the affectation of the cervical spine, the deterioration of the neurological status, suspicion of intestinal perforation, hemorrhagic diathesis, massive hemorrhage (Burlea et al., 2003).

From an ethical point of view, the four basic principles governing the physician-patient relationship must also be observed in the pediatric gastroenterology. Therefore, the autonomy in the sense of respecting the option of the patient, the non-mischief – which corresponds to the ancient principle "primum non nocere", the principle of the benefit for the patient, in the sense that the procedure must contribute to the amelioration and healing of the disease, and finally, the spirit of justice and equity, in the sense of non-discriminatory use of resources which are often limited and represent a major dilemma for the physician when he knows that the economic reasons can influence his/her medical decision are aspects that must be taken into consideration (Bailey et al., 1996; Vicol, 2011).

Consequently, one of the serious ethical problems of the physician is the answer to the questions: what is fair, what is the good and what is the correct way to be followed in solving medical problems. This way must follow the achievement of the maximum benefit to and minimum suffering of the patient and the reasonable use of the available resources (Buta and Buta, 2008).

2.8.4 Some considerations regarding the informed consent in pediatrics

As in any other medical specialty, the pediatrics also involves ethical considerations regarding the medical procedure which must be taken into consideration, even if it is about assigned responsibility:

- the informed consent must be obtained for all procedures, sometimes even for parts of them, such as: anesthesia, interventional endoscopy, superior or inferior endoscopy,
- the patient must be informed on the risks of the procedure,
- the therapeutic options, risks and benefits must be discussed,
- the economic restrictions must not influence the quality of the medical procedure,
- endoscopists must have the necessary competence for performing procedures,
- the quality standards must be complied with (Mac Sween, 1997).

Although the informed consent has evolved along the time from an ethical concept to a legal obligation, as it is nowadays practiced, it is based on the same ethical principles, even if it is about the pediatric age. Therefore, informing the child about his/her disease and the possible therapeutic remedies must be done even when his/her responsibility is limited or

assigned. This information can have positive effects, as it answers to some specific needs existing even at the pediatric age, such as: the need to respect him/her as a human being, as a consequence to treat him/her as a subject and not as an object of the medical procedure, the need to know the reality that he/she must fight and bear, without humiliation and without shattering his/her trust, the need to actively and autonomously participate in the recovery, the need to have responses to his/her questions, regardless of the type of disease of which he/she suffers.

Of course, the achievement of these goals is a relatively simple problem for those who work with children, as their experience helps them build a language and an attitude adequate to the child's psychology, otherwise being a problem of time and availability (Friedlander et al., 2011). These become necessary because, besides the medical aspect of the consent, rather human we would say, there is also its legal effect, somehow different. The difference consists in the fact that the legal aspect approves the act of will as an autonomous desired consent, fully aware of it and not inflicted (Mason and Mc Call, 1999).

We would also emphasize that although the humanitarian connotation goes through both aspects (medical and legal), there is a clear distinction between the medical humanism and the legal humanism, fact that makes the medical law oscillate between these two aspects. The medical humanism presumes the use of all the possibilities that can contribute to re-establishing the health condition of the patient, thus making the physician to assume the entire responsibility that he/she can afford. Therefore, from the point of view of medical humanism, the patient is the person who trusts himself/herself to the physician for the recovery of health, based on a relation of full confidence. Unfortunately, the medical humanism is not found in the legal humanism. In the legal humanism prevails the patient's autonomy that does not refer to the physician in order not to give up to his/her autonomy. Moreover, for justice, the person who must represent the interest of the patient is the patient himself/herself (in the case of the children, the parents or the next-to-kin) and not the physician (Buta and Buta, 2008; Mason and Mc Call, 1999).

Sometimes, this dilemma makes the physician hesitate when offering complete information to the patient, thus maintaining one more type of medical paternalism, because although the patient's interest is the one that determines the medical information, no one ever knows who the judge of this interest is particularly when undesired situations occur in the evolution of the disease or during the medical procedure (Miron et al., 2009).

2.8.5 Conclusions

The ethical principles of the medical procedure have assumed new valences in the conditions of the modern medicine that has become high-tech and interventional.

The informed consent becomes a compulsory instrument in pediatrics, with its features of communication, understanding and responsibility.

Accelerated progress in the last 3-4 decades of gastrointestinal endoscopy techniques, both diagnostic and therapeutic, associated with developing innovative endoscopic accessories and their introduction in pediatric practice contributed to a new subspecialty – Pediatric Gastroenterology. Diagnosis and identification of new aspects in child's digestive pathology also coincided with the development of new opportunities for effective nonsurgical treatment of various lesions. The invasive nature of these practices raised numerous and complex legal and ethical issues concerning their knowledge and acceptance by parents and minor patients in the informed consent. The large variety of clinical situations raise many dilemmas to medical staff between autonomy of the decision versus optimal approach in the interest of the small patient, divergence between parental consent and child's refusal / acceptance, difficulties of considering the child's opinion according to age group and "competence" and the optimal attitude in emergency situations, particular social context or "informed refusal "of the

minor/caregivers. In all cases the decision will be taken by the doctor in the patient's best interest and in respect of the rules of ethics and legality.

Endoscopy presumes a special ethical approach, having in regard its invasive character which, besides the actual benefits in the diagnostic and therapeutic approach, also presumes risks.

In modern medicine, communication and information are essential even in the relation to a patient of pediatric age, and from the ethical perspective of this relation, one must take into consideration the differences between the medical humanism – common to those who practice medicine and focused on the patient and his/her suffering, and the legal humanism – that is grounded on the person's autonomy and freedom.

SECTION II. Further Academic, Professional and Scientific Development

Academic Perspectives

I come from a university with tradition, "Grigore T. Popa" University of Medicine and Pharmacy, which has a lot to offer to the society and it is a leading brand of Romanian medicine and pharmacy, through its past and present. The accumulated capital must be used and enhanced, especially in its areas of performance such as research and practical, formative activity for the generations that address us. We have taken on a mission and a set of objectives, but they cannot be developed on a sustainable basis without the adoption of fundamental values that I must honor and promote in all my decisions:

- humanism – our university provides medical-pharmaceutical education, and the object of our activity is the human being and his health, whether he is the student in the process of development and training, or whether he is the patient in search of health. Their interest will have to remain paramount and be promoted in all its dimensions;
- diversity – we are part of cultures which, although often they are different, they have lived together for centuries and have influenced each other. Bringing them together in a form of constructive interculturality can and should induce a synergistic effect and should become the engine of progress of the university. Our common goal must be the performance and promotion of the new that can only come from the promotion of unity in diversity, a reality of the contemporary world that we must properly understand;
- freedom of thought and expression - apart from being a right, it is also a proof of spiritual, cultural and professional maturity, as they are values inextricably linked to tolerance and mutual respect. From this freedom can be born constructive opinions and ideas that applied in practice to fuel progress and communion in the educational instructional process. This right will be guaranteed when it is expressed honestly, applied correctly promoting the spirit of teamwork, without affecting in any way the dignity of the other;
- value recognition – as the university's most important resource, each member of it, teacher or student, mentor or disciple, will benefit from the merit recognition and the identification of the appropriate form of professional development;
- solidarity – we belong to a community joined by us, which has adopted us and each of us is responsible to defend its interests and reputation. Team spirit must be redefined, as well as collective interest, in perfect balance with the personal one. I will always advocate that our values be placed in a fair relationship with common values, contributing to the general progress of society.

My intention is to promote quality education, with an emphasis on its practical and applied side. I will support the creation of a simulation center for students and residents to allow the development of their practical skills. In this direction, I will promote the use of modern technical means in the process of training and formation of students, residents and trainees for postgraduate improvement. I will also promote an in-depth curricular reform to ensure a modern, attractive and efficient education, without overlapping, that meets the demands of the labor market and, where possible, to allow for the deepening of the residency theme.

I would like to develop competence and skill-based education, apply theoretical knowledge to practical work, streamline practical activities and clinical internships so than, at

graduation, each graduate may have a demonstrable package of minimal procedures that they have performed and are able to replicate. I will consider updating students' handbooks at short intervals (4-5 years) and whenever necessary, in accordance with the weight of each handbook in the curricula, and improving the accessibility of students to bibliographic and other information resources of the University. I am also planning to publish a handbook of pediatrics for general medicine assistants' students, which should be both a useful tool in their preparation and an update of knowledge in the field.

I will try to streamline the study formations for a judicious use of the existing resources, and to increase the interaction between teacher and student, in an active and effective mentoring. I will implement student assessment procedures taking into consideration the specifics of the discipline but aiming at maximizing the objectivity and the efficiency of these assessments. I will create a framework for identifying individual student skills that will support their career orientation towards specific general medicine and dentistry fields. I will also create the necessary framework so that they can participate in scientific research (student scientific circles, scientific events, scientific performance grants). I plan to organize residency training into an institutionally coordinated structure, identified in the teaching standard, where appropriate, aiming at increasing the residents' training quality.

Another objective is to broaden the offer of postgraduate courses, promote interdisciplinary courses, develop inter-institutional exchanges of students and teachers. I will insist on developing the ability to synthesize theoretical knowledge and clinical judgement with the practice of the specialty through personal initiative and the progressive assumption of responsibilities (participation of students in writing patient charts, in patient follow-up, recording their evolution and treatment, participation in on-call duty, both in our clinic, in the ER room, as well as in the other hospital clinics).

The final objective of the pediatric internship will be to harmonize the theoretical, practical and behavioral training with national and international standards for an optimal insertion of graduates in line with the demands of the labor market. Another objective is raising awareness among residents, their harmonious social integration and the collegial spirit at the workplace.

Continuous feedback will also be provided for residencies and specializations in order to optimize the implementation of these forms of education.

Professional perspectives

Practicing the profession remains a desideratum of becoming a professional in order to gain experience in the field and apply theoretical knowledge following the scientific approach to pediatric pathology. The future of the academic career is closely linked to the results of clinical trials, to disseminate their results. Clinical activity is based on the outcome of study, both personally and by consulting the literature in the field. The activity at the patient's bedside remains the keystone for gaining experience in the profession, a source of inspiration for scientific research and also an important source in the training of future practitioners. It is also the practical way of permanent confrontation between the novelty in science and the reality of hospital case studies.

Thus, as far as my own professional training is concerned, I will persevere through up-to-date theoretical documentation by browsing through literature studies and online information, subscriptions or purchase of internationally acknowledged books, all of which contribute to the continuous broadening of the knowledge horizon for an optimal clinical and therapeutic approach to pediatric patients.

Scientific perspectives

Medical publishing will continue at a rate of 5 - 6 articles per year at reputable ISI-indexed journals or in international databases. The documentation will represent a daily activity as a support for the scientific activity that I propose using the information sources in the field as well as the participations in the scientific meetings. I will continue my research in the areas in which I obtained results trying to find the appropriate and documented answers to the unanswered questions.

Therefore, further investigation of the relationship between gastroesophageal reflux disease and body mass index using nutritional parameters could shed light in this area, as obesity as a nutritional disease is on the rise. Also, sideropenia appears constantly in reflux patients and its presence would be the cause of the tendency towards anemia by altering erythrocyte values.

In respiratory pathology, the statistically significant correlation of gastroesophageal reflux disease was found to exists only with recurrent wheezing compared to the literature which considers asthma as more frequent.

The relationship with duodeno-gastric reflux could be an important research topic given that the alkalinity of duodenal reflux can neutralize esophageal acidity and consequently alter pH-metric results. A comparative study in which the presence or absence of duodeno-gastric reflux is investigated in conjunction with the determination of esophageal acidity could open new horizons in pediatric gastroenterology research.

To determine the distant prognosis of the disease, patients included in the database could be periodically re-evaluated following a standard protocol over a period of at least 10 years. This long-term periodic reassessment would provide greater certainty about the course of the disease, its relapses, the detection of complications and their evolution.

I am available to address topics referring to professional ethics, bioethics (I have published two articles on this subject, and I contributed to the composition of the volume "Medicine and Society"), pedagogy and general medical culture, the history of medicine by approaching and participating in the activities of well-represented societies in our university.

I will study the short- and long-term consequences of not vaccinating children and I will publish the results with the necessary comments considering the prophylactic importance of modern vaccinology.

As a conclusion, the scientific work should answer the questions and meet the needs of our clinical work. Hence the need to combine scientific research with practical activity which represents an inexhaustible source of research topics. Increasing the quality of life and the life expectancy are two daily issues in medicine and we must keep in mind that preventive medicine starts from childhood. In this context, the approach to prevention goes beyond the strict framework of infectious pathology and must include equally important areas of the child's life, including nutrition, movement, social life and education. Health problems related to nutrition and vaccination are very common in the pediatric population, with clear negative impact on their quality of life. Thus, intensive clinical research is a necessity in the field. I would like that clinical research in the areas mentioned above, including my research activity, to be included in a strategic planning, which would involve communication and inter-institutional collaboration, so I intend to promote it. At the same time, I intend to remain open to new developments and to any new approach that may arise in such a broad and comprehensive area of child health.

SECTION III. References

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