GRIGORE T. POPA UNIVERSITY OF
MEDICINE AND PHARMACY IASI

# HABILITATION THESIS

## OPENING DOORS IN GASTROENTEROLOGY

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**2022**

*"We keep moving forward, opening new doors, and doing new things, because we're curious and curiosity keeps leading us down new paths."*

*Walt Disney*

# CONTENTS

# ABBREVIATION LIST

**AFM** - Atomic Force Microscopy
**ARE** - AU-rich elements
**ASGE** – American Society of Gastrointestinal Endoscopy
**BMI** – body mass index
**BP** – blood pressure
**CaK Wt%** - calcium concentration in the hair
**CAP** - controlled attenuation parameter
**CAT** - catalase
**CD** - Crohn disease
**CDAI** - Crohn's Disease Activity Index
**CDC** - Centers for Disease Control and Prevention
**CM** - contrast media
**CNADTCU** - National Council for Attestation of University Degrees, Diplomas and Certificates
**CRE** - carbapenem-resistant enterobacteriaceae
**CRP** - C-reactive protein
**CSPH** - clinically significant portal hypertension
**CuK Wt%** - copper concentration in the hair
**CXR** - chest X-ray
**DSC** - Differential Scanning Calorimetry
**EDX** - Energy dispersive X-ray spectroscopy
**ERCP** - endoscopic retrograde cholangiopancreatography
**ESGE** - European Society of Gastrointestinal Endoscopy
**ESKD** - end-stage kidney disease
**ESPGHAN**- European Society for Paediatric Gastroenterology Hepatology and Nutrition
**ESR** - erythrocyte sedimentation rate
**ESS** - Epworth Sleepiness Scale
**FDA** - Food and Drug Administration
**FeK Wt%** - iron concentration in the hair
**FT-IT** - Fourier Transform Infrared Spectroscopy
**GPx** - glutathione peroxidase
**H2O2** - hydrogen peroxide
**HADS** - Hospital Anxiety and Depression Scale
**HDL** - high-level disinfection
**HOCM** - *High osmolality contrast media*
**HR** – heart rate
**IBD** - inflammatory bowel diseases
**IBS** – irritable bowel syndrome
**IGH**- Institute of Gastroenterology and Hepatology
**LOCM** - Low osmolality contrast media

**LSM** – liver stiffness measurement
**MDA** – malondialdehyde
**MDRO** - multidrug-resistant organisms
**MFIS** - Modified Fatigue Impact Scale
**MgK Wt%** - magnesium concentration in the hair
**MnK Wt%** - manganese concentration in the hair
**MRCP** - Magnetic resonance cholangiopancreatography
**NAC** - N-acetylcysteine
**NAFLD** – Non-alcoholic fatty liver disease
**NASH** - non-alcoholic steatohepatitis
**NLR** - neutrophil-to-lymphocyte ratio.
**NSAID** - Non-steroidal anti-inflammatory drugs
**OT/OTR** - oxytocin/oxytocin-receptors
**PAMPs** - pathogen-associated molecular patterns
**PAW** - Plasma- activated water
**PEP** - post-ERCP pancreatitis
**PON1** - peroxiredoxins and paraoxonase
**PPAR-γ** - peroxisome proliferator-activated receptor-γ
**PROMIS** - Patient-Reported Outcomes Measurement Information System
**PRRs** - pattern recognition receptors
**PHT** - portal hypertension severity
**PSQI** - Pittsburgh Sleep Quality Index
**RCCC** - Romanian Club for Crohn's Disease and Ulcerative Colitis
**REM** - rapid eye movement
**RNS** - reactive nitrogen species
**ROS** -reactive oxygen species
**SeK Wt%** - selenium concentration in the hair
**SEM** - scanning electron microscope
**SEM** - Scanning Electron Microscopy
**SES-CD** - Simple endoscopic score for Crohn's disease
**SK Wt%** - sulphur concentration in the hair
**SOD** - superoxide dismutase
**SRGH** - Romanian Society of Gastroenterology and Hepatology
**TBARS** - thiobarbituric acid reactive substances
**TGA** - Thermogravimetric Analysis
**TNF** - tumor necrosis factor
**UC** - ulcerative colitis
**UEFIDISCI** - Executive Unit for Financing Higher Education, Research, Development and Innovation
**ULN**- upper limit of normal
**WBC** - white blood cells
**WOS** – Web of Science

# REZUMAT

Teza de abilitare este structurată pe trei secțiuni, conform recomandărilor Consiliului Național de Atestare a Titlurilor, Diplomelor Certificatelor Universitare (CNADTCU). Lucrarea reprezintă o sinteză a activității mele profesionale, didactice și de cercetare științifică desfășurată în perioada 2009 -2021.

**Prima secțiune** a tezei de abilitare cuprinde o *sinteză* a traiectoriei mele academice, științifice și profesionale, urmată de *3 capitole* care detaliază direcțiile de cercetare abordate în perioada post-doctorală.

În introducere am trecut în revistă activitatea mea clinică, în relație cu pacientul, activitatea cu studenții și cu medicii rezidenți, activitatea administrativă desfășurată în cadrul comunității academice și a societăților profesionale, proiectele educaționale și de cercetare la care am participat și activitatea mea științifică ilustrată de principalele publicații la care mi-am adus contribuția.

*Activitatea profesională*. Sunt medic primar gastroenterolog și dețin un număr de 3 atestate și competențe, respectiv endoscopie digestivă diagnostică, endoscopie digestivă terapeutică, ecografie generală. Obiectivele mele profesionale sunt în strânsă legătură cu direcțiile de dezvoltare în activitatea de învățământ postuniversitar, enumerate în continuare.

*Activitatea didactică*. În prezent sunt conferențiar universitar la disciplina de Semiologie medicală și Gastroenterologie din cadrul Facultății de Medicină – UMF ″Gr T Popa″ Iași, poziție didactică pe care am ocupat-o prin concurs, după ce am parcurs toate etapele carierei didactice, trecând de la poziția de preparator universitar, la cea de asistent universitar, șef de lucrări, până la poziția actuală.

Pe tot parcursul carierei didactice am fost implicată în *activități administrative*, inițial la nivelul disciplinei și departamentului, ulterior, din 2016 am fost numită prodecan al facultății de Medicină.

*Activitatea științifică*. Realizările mele raportate la activitatea științifică și de cercetare de până în prezent se concretizează în autor sau coautor al unui număr de 5 manuale pentru studenți, autor a 5 capitole în două tratate medicale și 42 de capitole în 18 cărți de specialitate, 33 articole publicate în extenso în reviste indexate ISI cu factor de impact (15 autor principal și 18 co-autor), 10 de articole publicate în reviste ISI Proceedings, 28 de articole publicate în reviste indexate BDI și 28 de articole publicate in extenso în alte reviste recunoscute CNCSIS.

De asemenea menționez peste 100 de rezumate publicate în suplimente ale unor reviste indexate ISI și respectiv în volumele unor manifestări științifice cu ISBN precum și peste 50 de lucrări comunicate. Am susținut numeroase comunicări orale și postere la manifestări naționale și internaționale.

Ca o recunoaștere a activității științifice desfășurate până în acest moment menționez faptul că sunt autor a șase articole premiate de UEFIDISCI.

În același sens subliniez că am un număr de peste 345 citări, ceea ce a determinat un indice Hirsh de 11 (WOS), cu un factor de impact cumulat (pentru articolele în care am calitate de autor principal) de 36.269.

În a doua parte a primei secțiuni a tezei de abilitare am detaliat contribuțiile mele în direcțiile de cercetare abordate de la momentul obținerii titlului de doctor în medicină și până în prezent.

***Temele de cercetare abordate*** sunt organizate în trei mari capitole, raportate la preocupările științifice inițiale sau care au apărut ulterior: bolile inflamatorii intestinale,

gastroenterologia de tranziție de la patologia digestivă la vârstă pediatrică la patologia digestivă a adultului și endoscopia intervențională (CPRE).

Fiecare dintre aceste direcții de cercetare este prezentată în teza de abilitare structurat, după cum urmează: date teoretice – stadiul actual al cunoașterii în domeniu, rezultate obținute în urma cercetărilor proprii și vizibilitatea rezultatelor obținute (publicații în reviste de specialitate, citări, premii obținute).

În primul capitol al celei de-a doua secțiuni sunt prezentați o serie de factori epigenetici implicați în fiziopatologia bolilor inflamatorii intestinale cum ar fi – stresul oxidativ, o serie de microelemente, factori psiho-comportamentali.

Al doilea capitol al acestei secțiuni parte face referire la gastroenterologia de tranziție de la patologia digestivă la vârstă pediatrică la patologia digestivă a adultului. În această direcție am participat la cercetări care au urmărit preponderent aspecte din domeniul endoscopiei digestive și al tulburărilor funcționale la copil și adolescent.

Ultima direcție de cercetare detaliată în prezenta teză de abilitare este reprezentată de endoscopia digestivă terapeutică, domeniu de care m-am apropiat în ultimii ani atât prin prisma cercetărilor desfășurate cât și a publicațiilor. Sunt detaliate aspecte legate de colangio-pancreatografia endoscopică retrogradă (CPER) și a complicațiilor care pot surveni ca urmare a acestei manopere intervenționale iar cercetările au fost îndreptate în special în identificarea unor factori care pot determina aceste complicații și, implicit, a analizei unor măsuri de profilaxie care ar putea fi implementate în practică.

**Secțiunea a doua** a tezei de abilitare prezintă strategiile pe baza cărora îmi propun să îmi dezvolt cele trei domenii profesionale importante: activitatea academică, activitatea medicală și activitatea de cercetare științifică.

În ceea ce privește cercetarea științifică, proiectele mele pentru cercetările pe care le am în vedere pe viitor, derivă în mare parte din subiectele de cercetare care sunt enumerate și discutate în prima secțiune (studiul altor factori implicați în etiopatogenia bolilor inflamatorii intestinale, studiul afecțiunilor digestive comparativ la adolescent/adult tânăr vs vârstnic), dar îmi propun și alte direcții de cercetare: evaluarea non-invazivă a fibrozei în bolile hepatice cronice, rolul microbiotei în patologia digestivă (sindromul de intestin iritabil, steatoza hepatică), studiul patologiei bilio-pancreatice, importanța factorilor psihosociali în apariția patologiei funcționale digestive și modul în care rezultatele acestor cercetări ar putea fi implementate în practica clinică.

Un obiectiv important pentru mine îl reprezintă formarea unor nuclee de cercetare precum și dezvoltarea unei colaborări cu echipe de cercetare deja constituite. Tot cu acest scop, susțin interdisciplinaritatea în cercetare și intenționez să-mi extind colaborarea cu cercetători din alte domenii clinice (psihiatrie, pediatrie, chirurgie, imagistică medicală) și preclinice (farmacologie, fiziologie, fiziopatologie).

**Secțiunea a treia** și ultima include un număr de 500 referințe bibliografice relevante pentru subiectele discutate și care au fost utilizate pentru această teză și pentru articolele incluse.

# ABSTRACT OF THE THESIS

The habilitation thesis is structured in three sections, according to the recommendations of the National Council for Attesting University Degrees, Diplomas and Certificates. The thesis constitutes a synthesis of my professional activity, teaching career and scientific research carried out in the time period between 2009 and 2021.

**The first section** of the habilitation thesis comprises a *summary* of my academic, scientific and professional background, followed by *3 chapters* detailing the research directions undertaken during my post-doctoral period.

In the introduction I reviewed my clinical activity with patients, medical students, medical interns and resident doctors, the administrative work carried out within the academic community and professional societies, the educational and research projects in which I participated, and my scientific activity illustrated by the main publications to which I contributed.

*Professional activity*. I am a consultant gastroenterologist and hold 3 certifications and competencies, namely diagnostic digestive endoscopy, therapeutic digestive endoscopy, general ultrasonography. My professional objectives are closely related to the directions of development in postgraduate education, mentioned below.

*Teaching activity*. Currently I am an associate professor in the Medical Semiology and Gastroenterology department of the University of Medicine and Pharmacy – UMF 'Gr T Popa' Iași, a teaching position which I obtained by examination, after having traversed all academic ranks of the university teaching career starting with the position of teaching assistant followed by those of junior lecturer, senior lecturer, assistant professor and up to the current position. Throughout my teaching career, I have been involved in *administrative activities*, initially at the level of the discipline and department, and subsequently, since 2016, I have been appointed vice-dean of the Faculty of Medicine.

*Scientific activity*. My achievements related to the scientific and research activity up to the present are substantiated in having authored or co-authored 5 student teaching textbooks, authored 5 chapters in two medical treatises and 42 chapters in 18 specialty textbooks, 33 articles published in extenso in ISI indexed journals with impact factor (15 principal author and 18 co-author), 10 articles published in ISI Proceedings journals, 28 articles published in BDI indexed journals and 28 articles published in extenso in other CNCSIS recognized journals.

I should also mention over 100 abstracts published in supplements of ISI-indexed journals and in the volumes of scientific events with ISBN and over 50 research communications. I have given numerous oral communications and presented posters at national and international conferences.

As a recognition of the scientific activity which I have carried out so far, I would like to mention that I have authored six articles distinguished with the UEFIDISCI award.

In this regard, I would also like to underscore that I have achieved a number of over 345 citations which determined a Hirsh index of 11 (WOS), with a cumulative impact factor (for the articles in which I am the principal author) of 36.269.

In the second part of the first section of the habilitation thesis, I have detailed my contributions to the research areas of interest addressed in the time span stretching between my obtaining the MD (medical doctor) degree up to the present.

***The research topics addressed*** are organized in three main chapters, related to the initial or subsequent scientific concerns: inflammatory bowel diseases, transitional gastroenterology from pediatric to adult digestive pathology and interventional endoscopy (ERCP).
Each of these research areas is presented in the habilitation thesis structured as follows: theoretical data - the motivation for the choice of research topic, the current state of knowledge in the field, the results obtained from my own research and the visibility of the results obtained (publications in peer-reviewed journals, citations, awards obtained).
The first chapter of the second section presents a number of epigenetic factors involved in the pathophysiology of inflammatory bowel diseases such as oxidative stress, a series of microelements, psycho-behavioral factors).
The second chapter of this section refers to transitional gastroenterology from pediatric to adult digestive pathology. For this purpose, I have participated in research that has predominantly focused on aspects of digestive endoscopy and functional disorders in the child and adolescent.
The last line of research detailed in the habilitation thesis is represented by therapeutic digestive endoscopy, a field which I have approached in recent years both through the research carried out as well as publications. Aspects related to endoscopic retrograde cholangiopancreatography (ERCP) and the complications that can occur as a result of this interventional maneuver are detailed and research activities have been directed in particular toward identifying some of the factors which may determine these complications and, implicitly, the analysis of certain prophylactic measures that might be implemented in practice.

**Section II** of the habilitation thesis presents the strategies upon which I propose to develop my three important professional areas: academic activity, medical activity and scientific research activity.
As far as scientific research is concerned, the research projects that I am considering for the future derive for the most part from the research topics that are listed and discussed in the first section (the study of other factors involved in the etiopathogenesis of inflammatory bowel diseases, the comparative study of digestive disorders in the adolescent / young adult vs the elderly), but I am also considering other research areas: non-invasive assessment of liver fibrosis via modern methods in chronic liver diseases , the role of microbiota in digestive pathology (irritable bowel syndrome, hepatic steatosis), the study of bilio-pancreatic pathology, the significance of psychosocial factors in the development of functional digestive pathology and the mode in which the results of this research could be implemented in clinical practice.
An important goal for me is the formation of research centers as well as developing a collaboration with already established research teams. Also for this purpose, I support the interdisciplinarity in research and I intend to extend my collaboration with researchers from other clinical (psychiatry, pediatrics, surgery, medical imaging) and preclinical (pharmacology, physiology, pathophysiology) fields.

**The third** and final section includes a number of 500 bibliographical references relevant to the topics discussed and which have been used for this thesis and for the articles included.

# SECTION I

# PROFESSIONAL, SCIENTIFIC AND ACADEMIC ACHIEVEMENTS OVER THE POSTDOCTORAL PERIOD

## A. OVERVIEW OF THE ACHIEVEMENTS TO DATE

### BIOGRAPHICAL DATA

I was born on March 17, 1968, in Suceava. In 1986 I graduated from Ștefan cel Mare High School in Suceava – the Mathematics – Physics profile. Subsequently, upon passing the admission exam, I began attending the Department of General Medicine within the 'Gr T Popa' University of Medicine and Pharmacy Iasi, which I graduated in 1993.

### 1. PROFESSIONAL (MEDICAL) ACTIVITY

Upon completion of my undergraduate studies, I completed the internship year (1993-1994), which was compulsory at that time, and passed the national residency exam in 1994 ranking 173 and chose Gastroenterology as a specialty. In 2000, I became a gastroenterology specialist and since 2005 I have served as an attending gastroenterologist.

The clinical activity which I have performed served as a strong foundation for my continuing education as well as the interactive practical training of medical students.

Throughout the period following medical university graduation to the present, I have provided healthcare services (emergency, outpatient and inpatient) covering the entire spectrum of gastroenterologic pathology in a tertiary referral center for Moldova.

I have served for over 15,000 on-call hours, initially on the on-call line providing admission and monitoring of patients with digestive pathology presenting in the emergency department and requiring hospitalization, and subsequently on the endoscopy on-call line. I performed thousands of paraclinical investigations (abdominal ultrasonography, both diagnostic and therapeutic upper and lower digestive endoscopies, etc.).

Through my interaction with resident physicians both in daily patient care activities and on-call, I have contributed to the professional training of at least 30 gastroenterology fellows.

With regard to my professional development, I have attended specialization courses and obtained professional certifications and professional competencies. In 1998, during my residency, according to the legislation at that time, I attended the general ultrasonography training course organized by the 'Gr T Popa' University of Medicine and Pharmacy in Iasi, passing the ultrasonography competency exam in June 2001. (Competency Certificate Series A No. 0821).

During the period between 2007-2008, I attended the training course in diagnostic digestive endoscopy ('Gr T Popa' University of Medicine and Pharmacy, Institute of Gastroenterology and Hepatology, Iasi - under the guidance of MD PhD Carol Stanciu) and received the competency in diagnostic digestive endoscopy upon passing the national exams organized by the Ministry of Health in 2009. (Competency Certificate Series C No. 023152). I continued my training in the field of digestive endoscopy by attending the therapeutic endoscopy training course organized by 'Gr T Popa' University of Medicine and Pharmacy, Institute of Gastroenterology and Hepatology, Iasi – also under the guidance of Academician, Professor Carol Stanciu M.D. In 2019, I obtained a certificate of competency in therapeutic digestive

endoscopy, upon passing the national exams organized by the Ministry of Health. (Competency Certificate Series C No. 046603).

**Courses and competencies for the continuing professional development:**

- General Ultrasonography Course, 1998
- General Ultrasonography Competency, 2001
- Diagnostic Digestive Endoscopy Course, 2007-2008
- Diagnostic Digestive Endoscopy Competency, 2009
- Therapeutic Digestive Endoscopy Course, 2019
- Therapeutic Digestive Endoscopy Competency, 2019
- Gastroenterology Advanced Postgraduate Course, 2003, Amsterdam, Netherlands
- Regional Specialist Experience Exchange Meeting on Current Hepatitis Therapy, 2006, Medulin, Croatia
- Advanced Training in Elastography Technique Hitachi – Craiova, 2007
- Contrast Enhanced Ultrasonography Course 2009, Bucharest
- Advanced Endoscopic Technique Workshop, September 11-14, 2014
- Postgraduate Chronic Disease Management Course, 2014 CRONEX (Certificate of Graduation Series CHX No 020)
- Hemospray Hemostasis Device Utilization Course, 2015, Iasi

**Academic Affiliations**

- Romanian Society of Gastroenterology and Hepatology (since 2016 elected member of the Board of Directors)
- Romanian Society of Digestive Endoscopy
- Society of Physicians and Naturalists, Iași Branch
- Romanian Club of Crohn's Disease and Ulcerative Colitis
- Romanian Society of Neurogastroenterology

## 2. TEACHING ACTIVITY

In 2000, I obtained, by exam, the position of teaching assistant in the discipline of Medical Semiology - Gastroenterology and, in 2002, I ascended, again by exam, to the teaching rank of junior lecturer. In 2009 I was promoted, also by exam, to the teaching rank of senior lecturer in the discipline of Medical Semiology - Gastroenterology and, in February 2019, I rose to the teaching rank of associate professor in the same discipline.

As mentioned above, since 2000 I have served as lecturer in the discipline Medical Semiology - Gastroenterology. Throughout this period, I oversaw the practical coursework activities in medical semiology with third year students, gastroenterology rounds with fifth year medical students of the University of Medicine and Pharmacy G. T. Popa, Iaşi, specialized care internships in gastroenterology with students of the General Nursing Program offered by the same university. Since 2009, having been promoted to the teaching rank of senior lecturer, I was responsible for all teaching activities in the *Medical Semiology* course for third year, general medicine students, *Gastroenterology courses* for fifth year, general medicine students, and the course of *Specialized Care in Gastroenterology* for fourth year, General Nursing students.

Alongside the coursework with general medicine students and general medicine nursing students, my teaching activity also involved the coordination of resident doctors (gastroenterologists and related specialties) both by teaching specialty courses and by providing collaboration and guidance with medical assistance provided to patients in emergency situations (on-call) as well as supervising and coordinating specific explorations in the gastroenterology specialty.

Apart from the teaching and coordination of medical rounds for teaching purposes, I have developed and published three textbooks of gastroenterology and a specialized care guide for

general nursing students and have contributed (as chapter author) to the publication of 5 other guidebooks for students and young doctors.
The process of training and professional development of medical students and rezident doctors involves participation in a series of extra-curricular activities. To this end, I have guided both medical students and resident doctors in oral communications / clinical case presentations during student sessions and have coordinated a large number of students in preparing their undergraduate thesis during their final undergraduate year.
On a different note, I have contributed in each exam session with the development of exam topics (multiple choice questions) for students of all grade levels who came under the scope of my teaching activity.
In recognition of my well grounded medical training and didactic qualities, in each of the last 11 years, I have been chosen as a member of the committee responsible for the preparation of topics for the medical graduate examination and have been nominated 6 times to be part of the professional committee for the preparation of topics for the national residency exam.
Moreover, in recognition of my professional accomplishments, on the occasion of the 125th anniversary of the foundation of 'Gr T Popa' University of Medicine and Pharmacy Iaşi, I was awarded the 'C. Negoiţă' prize for teaching skills.
For my continuing professional development in this direction, I attended a series of **courses for the development of teaching skills:**

- Psychopedagogy Course, 2003, Department for the training of teaching staff, 'Alexandru Ioan Cuza' University of Iaşi.
- Business Presentations Program Course - September 2010 Cheile Grădiștei
- Advanced Business Presentations Program Course - 2011 Sibiu
- Facilitation Course: Improving Expression and Communication Skills - 2006 Sinaia

**3. RESEARCH ACTIVITY**

My research activity began in 2003 with the admission to doctoral studies on the topic '*The Influence of cholecystectomy on biliary reflux in patients with gallstones',* under the coordination of MD PhD Carol Stanciu. The results of the clinical research carried out in the field of the doctoral dissertation research topic led to the publication of 3 articles in extenso in ISI and BDI journals as well as in oral presentations and scientific poster communications at national and international events.
To date, **my scientific and research** activity includes: author and co-author of 5 chapters in medical treatises, 41 chapters in 17 specialty textbooks, 33 articles (15 main author and 18 co-author) published in extenso in ISI indexed journals with impact factor, 10 articles published in ISI Proceedings journals, 28 articles published in BDI indexed journals and 28 articles published in extenso in other CNCSIS recognized journals.
I should also mention over 100 abstracts published in supplements of ISI-indexed journals and in the publications of scientific events with ISBN as well as more than 50 communicated papers. In the period 2009-2021, I actively participated by giving numerous oral communications and presenting scientific posters at various national and international conferences and I was a moderator at more than 20 round tables.
In recognition of my scientific activity carried out so far, I should mention that I am the author of six articles awarded by UEFISCDI and three other works awarded by SRGH at national symposiums or congresses.
In the same regard, I emphasize that the scientific articles have attracted more than 345 citations in Clarivate Analytics Web of Science Core Collection and over 500 citations in Google Scholar, which have generated a Hirsch index of 11 according to Clarivate Analytics, respectively 13 in Google Scholar (Table I)

**Table I.** International scientific visibility

| | |
|---|---|
| NUMBER OF PUBLICATIONS IN CLARIVATE ANALYTICS DATABASE | 67 |
| CUMULATIVE IMPACT FACTOR (main author) | 36.269 |
| TOTAL NUMBER OF CITATIONS WITHOUT SELF-CITATIONS (CLARIVATE ANALYTICS)<br>MEAN CITATION NUMBER/PAPER PUBLISHED | 345<br>5.07 |
| HIRSCH INDEX (CLARIVATE ANALYTICS)<br>HIRSCH INDEX (GOOGLE SCHOLAR) | 11<br>13 |
| ORCID: **https://orcid.org/0000-0002-1394-8648** | |

My scholarly endeavors have also been directed toward research/educational projects and I have been actively involved in national and international projects obtained through competition.

I am also part of the management committee in two international grants and a member of the research team in one international grant and in 5 other national grants.

My clinical research has also included participation in international and national multicenter clinical trials in the field of digestive pathology. I have been the investigator in 7 multicenter clinical trials, studies in which I was involved on the basis of a contract obtained through feasibility competitions. I believe that participation in these studies has been beneficial for the continuining development of my research aptitudes, constituting a model of project design and subsequently adhering to a project and clinical protocol.

Throughout my professional endeavors, I have been actively involved in editorial work: I was the Editorial Secretary at the Journal for Continuing Medical Education - a scientific journal recognized by CNCSIS, as well as a *member of the editorial board* of the newsletter of the Romanian Society of Gastroenterology and Hepatology – SRGH.

**Development / Structural / Educational / Collaborative Networking Grants**

***a. International***

- *local coordinator*

1. Substitute member in COST project: CA17112 – Prospective European drug-induced liver injury network- 2018
2. Project Leader - Partner UMF Gr T Popa Iași - Erasmus Plus, contract no 2018-1-CZ01-KA203-048197 – Problem based and team-based learning strategies in the education of biomedical and natural sciences – TELSON

- *team member*

3. Team Member in the ERASMUS PLUS project contract 2018-1-RO01-KA203-0494121 – Case based learning and virtual cases to foster critical thinking skills of students (CLEVER)
4. Team Member in the ERASMUS PLUS project: HOPE -2018-1-RO01-KA202-049189: Strategic partnership project – field of professional training: Promoters of advanced oncogenetics open online training and multimedia raise awareness on multidisciplinary assessment of patients and their families at risk of hereditary or familial cancer
5. Team Member in the COST Action CA18122 project: European Cholangiocarcinoma Network
6. Team Member in the project - UEG Activity Grant - Translating FMT applications related to Clostridium difficile infection into local clinical practice Grant coordinators: Prof. Anca Trifan MD. PhD, Georgiana-Emmanuela Gîlcă-Blanariu MD. PhD. Partner societies:

British Society of Gastroenterology, Hungarian Society of Gastroenterology, European Helicobacter and Microbiota Study Group

***b. National***

*Member of the management/coordination team of national research/educational projects*

- Student Activity Coordinator in the project: Equity and Social Inclusion in UMF Iași – acronym EquitatIS - within the FDI Program 2018 – May 10, 2018 - December 15, 2018. Project Director Prof. Ionela Lacramioara Șerban MD PhD
- Regional expert in medical competency development - VHB N-E region in the project 'Training program for medical staff in Romania in the management of patients chronically infected with hepatitis B and C viruses – HEPATER'; POCU/91/4/8/107931 contract; project co-financed by the European Social Fund through the Human Capital Operational Program 2014-2020, implemented by the Fundeni Clinical Institute in Bucharest;

*Team Member in national research/educational projects*

- Teaching Assistant – in the project: Equity and Social Inclusion in UMF Iași – EIS 2.0 - within the FDI Program 2017 – September 1, 2017 – October 31, 2017 Project Director Prof Ionela Lacramioara Șerban MD PhD
- Senior researcher in the project: Nanoparticle-enabled Terahertz molecular imaging as advanced early-stage diagnostic of gastric neoplasia - PN III-PED - project director Prof Vasile Drug MD PhD
- Expert in competency development in VH screening family doctors (SE+NE region)' within the LIVE (RO)1 project - Training of medical staff in Romania for population screening of chronic infections with hepatitis viruses B/C/D MySMIS code 2014: 120640 POCU project code/308/4/9/120640
- Supervisor for Prevention Center 1 – partner of Sf Spiridon Hospital Iași within the LIVE project (RO)2 - Regional integrated program project for prevention, early detection (screening), diagnosis and treatment referral of patients with chronic hepatitis virus B/C/D infections'
- Teaching Assistant – in the project Professional counseling for students in medicine and integrated program of practice in the field of general and dental medicine. POSDRU/160/1.2/S/139881

***c. Direct contribution research studies:***

- Investigator in: Hemorrhagic esophageal varices, phase 2 trial (Vapreotide) 2004
- Investigator in clinical trial: Otilonium bromide in irritable bowel syndrome (OBIS), study code – MeFi/04/OBR-IBS/001 2007
- Investigator in: GS-US-174-0121 study: Hepatitis B- resistance to Lamivudinum 2009
- Investigator in CDEB 025A2301: A randomized, double-blind, placebo-controlled trial of the efficacy and safety of DEB025/Alisporivir in combination with peg-IFNα2a and ribavirin in hepatitis C genotype 1 treatment-naïve patients 2011-2013
- Investigator in CDEB025A2312 Multi-center long-term follow-up study for assessing the durability of sustained virological response in patients with chronic hepatitis C treated with alisporivir 2014-2015
- FIBRODICT Multicenter study: Non-invasive evaluation of fibrosis in chronic hepatitis C 2007-2008
- PROPHESYS - Multicenter study: Evaluation of response to antiviral therapy in chronic hepatitis C 2007-2008

**4. ACHIEVEMENTS IN THE ACADEMIC COMMUNITY**

From the very beginning of my teaching career, I have been involved in organizational and administrative activities, initially at the level of the discipline and subsequently at the level of

the department and the university. I am currently serving as the vice-dean of the University of Medicine.
Throughout my career, I have been the Secretary of Department III, Medical Specialties 1, since 2016 I have been elected member of the Board of the Medical Department I, a member of the Academic Studies Committee of the Senate and a member of the Curriculum Office and since 2020 I have been an elected member of the Teacher's Council of the University of Medicine.
Also from the beginning of my teaching career, I have participated in the organization and supervision of the admission exams and the Bachelor's Degree at Gr T Popa UMF Iaşi and in the last 8 sessions I have been the coordinator of the medical graduate examination at Gr T Popa UMF Iași and in the last 4 years I have also been the president of the admission committee for students with tuition in foreign currency.
All these administrative activities undertaken in the academic community require personal sacrifice, time commitment and social skills. Unquantifiable in the personal file assessment, they bring not only multiple responsibilities but also several advantages, in the first-place visibility in the academic environment, while representing at the same time a framework for the affirmation and practice of organizational skills and aptitudes.

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# B. SCIENTIFIC ACHIEVEMENTS

My habilitation thesis is entitled "Opening doors in gastroenterology" and reflects the study and research activities from over 20 years of my career.
The title was chosen in accordance with the research areas that addressed topics located at the border between specialties and between research and clinical activity.
This research has been done in collaboration with researchers in other medical or non-medical fields.
This habilitation thesis represents the synthesis of the main three directions of my postdoctoral scientific research:

- inflammatory bowel diseases,
- transitional medicine between childhood and adulthood
- interventional endoscopy (endoscopic retrograde cholangiopancreatography).

The articles I have published during this period as main author or in which I have participated as a co-author address a broader field, but the directions detailed below are the ones that have brought me the greatest satisfaction with the resulting publications, the visibility of the scientific journals and the number of citations.
Also, for each direction included in this thesis, the publications on the aforementioned topics were much more numerous but I chose to present only the original papers in which I actually participated as a researcher, coordinator or collaborator of the research team. Review-type publications (some of which we published in journals with an impact factor of more than 3, at the invitation of the editorial staff), as well as clinical cases are mentioned in the list of papers without being detailed. They demonstrate the interest of the research teams I was part of for the chosen topic.
This section in which we presented the scientific achievements consists of 3 chapters. Each chapter corresponds to a research direction. For each research direction, we structured the studies on subdirectories according to the scientific objectives we had.
My scientific achievements are presented in accordance with the *state of the* art at national and international level. The personal contribution and the resulting publications in ISI / PubMed journals are also highlighted.
All chapters start with the context and rationale of the research topic, continue with the scientific achievements in the field of research and detail the relevant publications.
Each chapter concludes with a summary of the results obtained on the research topics and scientific visibility of the publications in the field of research.

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# Chapter 1.
# BETWEEN RESEARCH AND CLINICAL PRACTICE IN INFLAMMATORY BOWEL DISEASES

## 1. 1. CONTEXT AND RATIONALE OF THE RESEARCH TOPIC

During my postdoctoral period, the first line of research I tackled in chronological order was related to inflammatory bowel diseases. This line is the most consistent and has brought me the greatest scientific satisfaction.

Inflammatory bowel diseases (IBD) have been an area of interest to me since 2009 and continue to be among my research interests today. The focus on this pathology has been fueled on the one hand by the increasing incidence and prevalence and on the other hand by the complex etiopathogenesis, which is still incompletely elucidated, despite significant progress in the understanding and management of inflammatory bowel diseases in recent decades.

As mentioned, in Romania, inflammatory bowel diseases have increasing incidence and prevalence, but still lower than in other European countries (Toader et al., 2006; Malczyk et al., 2009; Baumgart et al., 2011; Gheorghe et al., 2014; Goldis et al., 2019, Windsor, Kaplan, 2019). This positioning '*in time and space*' is an opportunity to conduct research with the aim of better understanding and management of this pathology, but also to explain this epidemiological pattern.

The fact that the evolution of IBD is unpredictable, that there is no medication with a curative aim and no elements to predict the response to various types of therapies makes it imperative to further study the pathophysiology of this category of diseases.

The main forms of IBD, CD (Crohn disease) and UC (ulcerative colitis) are inflammatory diseases with relapsing-remitting pattern, with complex etiopathogenesis. An important role in the onset and progression of these diseases is attributed to the interaction between the genetic and environmental factors. (Loddo, Romano, 2015). Since the genetic background involved in the etiopathogenesis of IBD is complex and difficult to pinpoint, the reseach focus has shifted to the environmental factors involved, all the more so as the external factors are, at least theoretically, modifiable.

In this context, we considered it appropriate to study some elements belonging to the main etiopathogenic links in IBD, with a potential role in modulating the inflammatory process.

I started the research in this field together with a team of gastroenterologists in collaboration with a group of researchers from the Faculty of Biology of A.I. Cuza University of Iași. Subsequently, the team has expanded and become multidisciplinary. On this research path, we collaborated with psychiatrists, psychologists, surgeons, pediatricians and researchers from the Institute of Macromolecular Chemistry "Petru Poni"in Iași.

The research I have been involved in over the last 10 years has tried to establish the role of endogenous elements in the emergence and progression of IBD. In distinct periods, from 2010 to the present, in chronological order, we have studied the involvement of oxidative stress (Achiței et al., 2013), then of psychological factors and sleep disorders (Gîlcă Blanariu et al., 2020) in modulating the evolution of IBD patients. Recently I conducted a study on the deficiency of some microelements and potential associations with the disease activity (Gîlcă Blanariu et al., 2021).

The research results in this area have been published in specialized journals, most of them ISI listed, with good international visibility (impact factor above 2).

## 1.2. SCIENTIFIC ACHIEVEMENT IN THE FIELD OF RESEARCH

The following are the research carried out in this field and the results published in the main original articles to which I contributed as main author or co-author.
I have divided the research I have conducted on inflammatory bowel diseases in two main subdivisions: 1. *contributions to the study of epidemiological and clinical-biological aspects* (analysis of epidemiological, clinical, biological and endoscopic particularities in North-Eastern Romania) and 2. *endogenous factors with a role in modulating the evolution of inflammatory bowel diseases* (the role of oxidative stress, psychological disorders associated with inflammatory bowel diseases, micronutrient deficiencies as well as the predictive value of some clinical-biological markers for assessing the evolution of IBD).

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### 1.2.1. Study of epidemiological and clinical-biological aspects in IBD

IBD predominantly affects the young, professionally active population, with an increasing incidence in the pediatric population. As a result, this pathology has a major negative impact on the quality of life, psychological status and professional efficiency, leading to social-economic costs beyond those directly required for the disease management (GBD, 2020).
On the other hand, prevalence remains high in the geriatric population, raising new challenges in the management of IBD, in the context of comorbidities that are commonly present in this age group. Patients with IBD in the over 65 age group represent the growing subpopulation in particular in Western European and North American countries. (Stepaniuk et al., 2015).
Less than a decade ago, in 2013, when our team conducted the observational study on the clinical, biological and epidemiological aspects of inflammatory bowel diseases in North-Eastern Romania (Achiței et al., 2013), the incidence of IBD in Western countries was reported to be increasing, with much higher incidences in Northern Europe (24.3/100,000 for ulcerative colitis - UC and 19.6/100,000 for Crohn's disease - CD) (Burisch et al., 2013), in Canada (19.2/100,000 for UC and 20.2/100,000 for CD) and in Australia (17.4/100,000 for UC and 29.3/100,000 for CD).
Subsequently, it was found that globally, the epidemiological evolution is distinct, depending on the degree of industrialization. Cohort studies in Asia, Africa and South America have consistently reported an increase in IBD incidence over the last decade, with regional variations that may be partially justified in the context of differences in risk factors, accelerated urbanization, limited access to health care, and differences in data recording (Ng et al., 2017).
In conclusion, incidence was found to be on a persistent upward trend in newly industrialized regions such as Africa, Asia and South America, while incidence remained stable or even showed a downward trend in North America and many Western European countries.
In addition to the stabilization of incidence, a number of epidemiological particularities have been observed in Western countries: increasing number of cases with onset at young age, especially in the pediatric population but with low mortality. This pattern lead to a longer disease course, resulting in new challenges, as patients experienced an increased variety of therapeutic agents (Windsor, Kaplan, 2019).
In terms of age at onset, the epidemiological peak of onset of CD and UC is in the 20-30 years and 30-40 years age groups, respectively. Although a widespread concept is that of a bimodal distribution of IBD incidence, with a second peak in the interval of 60-70 years, this

distribution is confirmed in less than one-third of epidemiological studies (Molodecky et al., 2012; Ruel et al., 2014). Overall, 5-25% of IBD cases are diagnosed during childhood or adolescence, while only 10-15% of IBD patients will be diagnosed at >60 years of age (Kelsen, Baldassano, 2008; Ruel et al., 2014).

Regarding the evolution of inflammatory bowel diseases in recent years, a relatively recent study, published 5 years after the publication of our study, which followed the evolution of CD cases in Europe, identified a course of the disease without significant differences between Western and Eastern countries, regarding the need for hospitalizations, the rate of surgery, the occurrence of strictures or fistulas (Burisch et al., 2018).

Therefore, the epidemiology of IBD is a complex and dynamic process with increasing prevalence, along with increasing incidence in areas previously known to have low incidence.

Situation in Romania

A first report of the situation at national level was in 2003 through a multicenter prospective study (18 centers) conducted under the aegis of the Romanian Society of Digestive Endoscopy, aiming at assessing the incidence and prevalence of IBD in the adult population over a one-year period (June 2002-June 2003). In this study, an incidence of 0.97/100,000 people/year was reported for UC, 0.50/100,000 people/year for CD, with a prevalence of 2.42/100,000 people/year for UC and 1.51/100,000 people/year for CD, these data placing Romania at that time among the European countries with the lowest incidence and prevalence. Regarding the distribution by gender, the M: F ratio was 1.31:1 in the case of CD, and 1.27:1 in the case of UC, respectively, and there were no significant differences in this respect. Regarding the incidence peaks, these were the 20-39 years age group for CD, respectively a relatively uniform incidence for UC in the 20-59 years age group. There was also a predominance of extraintestinal manifestations (14.8%) and a course burdened by the need for surgery (12.9%) in patients with CD compared to those with UC (5.2%, respectively 0.6%) (Gheorghe et al., 2004).

As regards the north-eastern region of the country, the first data were reported in 2008, in a study that aimed to record and follow up patients with IBD over a 10-year period, the incidence of IBD in north-eastern Romania being 1.89/100,000 inhabitants, with large variations between UC (1.54/100,000 inhabitants) and CD (0.35/100,000 inhabitants) (Toader E, 2008).

The most recent study focused on IBD epidemiology in Romania, published in 2019, including updated data included in the national IBD Prospect register reports a number of 2,724 patients, of whom for 2,248 complete data were reported. A slight predominance of UC with was reported 1,263 cases, whereas for CD 935 cases were reported, and for IBD unclassified - 500. Investigators have identified a trend of increasing number of IBD cases recorded in our country, but this could actually be due to better access to health services and, implicitly, an improvement in the diagnosis of this category of patients. From a phenotypic point of view, for UC, left-sided colitis cases predominated (50.5%), while for CD colonic (37.8%) and ileocolonic (37.6%) were the most common, a predominance of non-stenotic non-penetrating forms was also found, in contrast to previous reports (Burisch et al., 2013; Goldis et al., 2019). The same study reports a higher prevalence of UC in the north-eastern region of the country, respectively for CD in the south-eastern and south-eastern regions, outlining a north-south geographical gradient regarding the IBD subtype.

Consequently, the epidemiological evolution of IBD in Romania tends to blur the differences compared to Western countries, a phenomenon that can be explained on the one hand in the context of adopting a Western lifestyle and on the other hand by a better addressability to the specialist doctor for diagnosis and management (Toader et al., 2006; Brusnic et al., 2012; Gheorghe et al., 2014; Goldis et al., 2019; Windsor et al., 2019).

**Personal contributions**

The few studies published for Romania until 2010 showed at that time a lower incidence than the neighboring regions, an older average age and the predominance of mild and moderate forms of disease (Gheorghe et al., 2003; Toader et al., 2006; Toader 2008).
However, the data were slightly contradictory and there were methodological difficulties. Information from smaller local studies and clinical practice suggested that these characteristics had changed over the years and therefore required a new approach. Thus, at that time, I considered useful to analyze patients with IBD in the record of the reference center in the region of Moldova (Table 1.I).

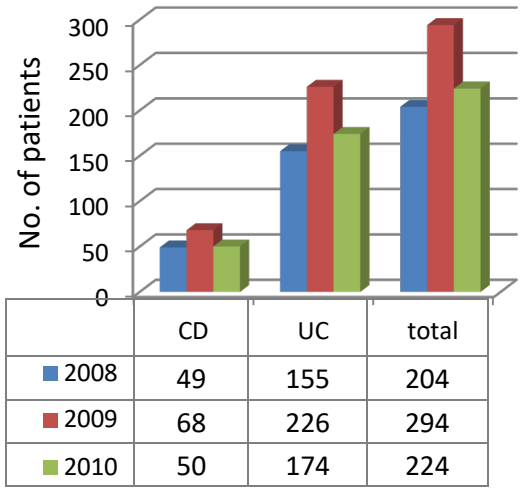
**Table 1.I.** Publications in the field of study of the epidemiological and clinical-biological aspects in IBD:

| |
|---|
| CLINICAL, BIOLOGICAL AND EPIDEMIOLOGICAL ASPECTS OF INFLAMMATORY BOWEL DISEASES IN NORTH-EAST ROMANIA.<br>Achitei Dorin, Gologan Elena, **Ştefănescu Gabriela**, Balan Gheorghe.<br>o *Rev Med-Chir Soc Med Nat,* vol.117, no2 /2013. |
| DYSPLASIA IN INFLAMMATORY BOWEL DISEASE: PERSPECTIVE FROM A TERTIARY CENTRE IN NORTHWESTERN ROMANIA.<br>Popa Iolanda Valentina, Drug Vasile Liviu, Popa Raluca, Savin Alexandra, Cardoneanu Anca, Bărboi Oana, **Ştefănescu Gabriela.**<br>o *Filodiritto Proceedings XXXVI National Congress of Gastroenterology, Hepatology and Digestive Endoscopy Cluj-Napoca,* 8-11 June 2016; p 346-350. |

Based from the aforementioned issues, in 2010 I participated with a research team from the Institute of Gastroenterology and Hepatology in Iași (the only tertiary gastroenterology center in North-Eastern Romania) in a retrospective study that aimed to analyze the epidemiological, clinical, biological and endoscopic characteristics, highlighting in particular the peculiarities in relation to other geographical areas and with older studies in Romania (Achiței et al., 2013).
The objectives of this study were to obtain an overall epidemiological overview by retrospective analysis of IBD cases hospitalized in the period 2008-2010 in IGH and to perform a descriptive analysis of cases from a clinical, biological and endoscopic point of view.

**Patients and method.** The study was designed as a retrospective analysis of cases diagnosed with inflammatory bowel disease, hospitalized at the Institute of Gastroenterology and Hepatology within 'St. Spiridon' Hospital from Iași in the period 1 January 2008 to 31 December 2010. The cases were selected electronically, entering keywords and diagnostic codes for the search.
I have included in the analysis demographic, clinical, biological and endoscopic data from the observation charts of all patients with inflammatory bowel diseases admitted to IGH during the mentioned period. The clinical and demographic data, the biological profile (inflammatory parameters ESR, CRP, Fbg, hematological, serum iron, etc.) and endoscopic parameters were registered for each patient. Also noted: for ulcerative colitis the Mayo clinical score and Mayo endoscopic score (Lewis et al.,2008) and for Crohn's disease the Montreal criteria (Satsangi et al., 2006) the Harvey Bradshaw index (Harvey, Bradshaw,1980) respectively the simple endoscopic score (SES-CD) (Daperno et al.,2004)
***Results:*** The total number of one-day and continuous hospitalizations with the diagnosis of inflammatory bowel disease in the period 2008-2010 was 722 (multiple hospitalizations for one patient were excluded). Compared to the total number of admissions in the same period, hospitalizations for IBD accounted for 2.27%. Ulcerative colitis was predominant in the study group, accounting for 76.87% of all cases (Figure 1.1)
The mean age of hospitalized patients was 45.7 ± 15 years, almost half of the subjects (46.79%) aged between 25 and 45 years. The gender distribution was sensitively equal across the whole

group of patients, with a slight predominance of male in the CD group and female in the UC group, without differences exceeding the threshold of statistical significance.
In terms of disease duration, the majority of hospitalized cases were new cases or with a disease course under 2 years (47.5% of all cases) (Fig. 1.2).




| | CD | UC | total |
|---|---|---|---|
| 2008 | 49 | 155 | 204 |
| 2009 | 68 | 226 | 294 |
| 2010 | 50 | 174 | 224 |

**Fig. 1.1.** Number of patients by year and type of disease

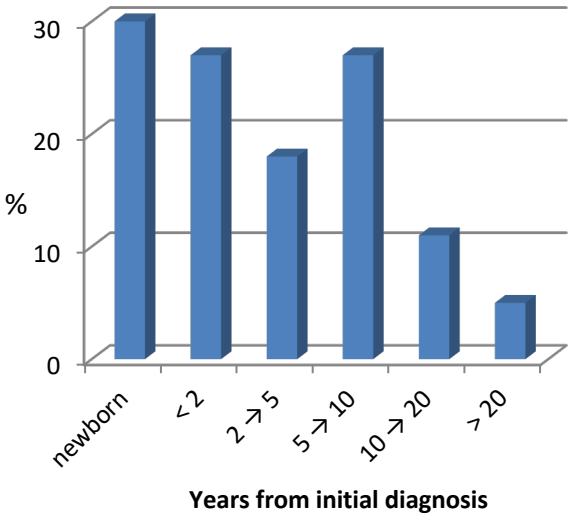



**Fig. 1.2.** Duration of the disease at evaluation

By calculating the age at diagnosis, we found in our group a maximum incidence in the age interval 35-45 years (35%) followed by the interval 25-35 years (27%). When analyzing according to disease subtype, the mean age of onset was 37.7 ± 13 years for Crohn's disease and, respectively, 42.8 ± 14 years for ulcerative colitis.
In terms of disease location, we found that in the subgroup of patients with UC, about half of the cases were left-sided colitis (endoscopic lesions present up to the splenic angle - 46.3%), followed by pancolonic forms (29.3%) and proctitis (24.4%).
In Crohn's disease, colonic involvement was documented in more than 50% of cases, isolated ileal location was found in only 14.3% of cases. Approximately 10% of patients had lesions in the upper digestive tract and approximately 25% had perianal lesions. Most cases of Crohn's disease had a non-stenotic, non-penetrating phenotype of 42%, 16.1% were stenotic forms.
27% of ulcerative colitis cases were severe, 33% moderate, and 37% mild. Whereas for Crohn's disease there were 10.7% severe, 50% moderate, 17.8% mild and 21.4% patients in remission.
35.25% of patients had at least one extraintestinal manifestation. The most common extraintestinal manifestations were ankylosing spondylitis and other seronegative spondyloarthropathies (13.6%) followed by cholestasis syndrome and ocular manifestations (uveitis, episcleritis).
Colonoscopy evaluation was performed in 72.8% of patients. More than half (52.38%) of patients with UC had an endoscopic Mayo score of 2 and 26.19% had a score of 3. In the Crohn's disease subgroup, according to the simple endoscopic score, most patients had mild or moderate forms, less than 10% being severe forms: 28.5% score<3, 23.8% score 4-10, 38.09% score 11-19 and 9.52% score >20.

**Discussion**
An estimate of IBD incidence in Iași County at that time can be made based on the data of this study: I considered that about half of the 240 annual cases of hospitalized IBD are from Iași

County (where IGH was the only reference center) and that at least 20% of the cases are incident (Gheorghe et al., 2003). Thus, by relating the 25 new annual cases in the county to a population of 772,000 inhabitants (according to the 2011 census), we obtain an incidence of IBD for Iași County at the time of the study, of 3,24 ‰. This value, although only estimated, is higher than the figures reported in the studies so far and highlights the trend of increasing incidence at that time.

Analyzing the results of the study, a first aspect to be mentioned is the ratio between the subtypes of the disease, namely the small number of patients with Crohn's disease (approximately 23%) compared to the cases of UC (77%). At that time (2008-2010), this proportion was particular to the NE region of Romania, the prevalence of Crohn's disease in other neighboring regions being at least 30% (Lacatos et al., 2011; Wiercinska-Drpalo et al., 2005). One possible explanation may be that CD was at that time underdiagnosed, especially the forms located in the small intestine as a result of technical and material difficulties.

In terms of age distribution of patients in the studied group, it was similar to that reported for Europe, with the disease predominating in younger adults (up to 45 years). Most cases included in the study were new or recently diagnosed cases.

The mean age of onset in the studied population was higher than in the high incidence areas in northern Europe, being around 40 years (Braegger et al., 2011). The age of onset was higher among patients with UC, which has been reported in most epidemiological studies. The high age at onset may be a characteristic of the region studied, but may also be related to a higher diagnostic delay.

Also, the mean age at the time of diagnosis of IBD was 39.7 years. This value is higher compared to studies in Northern Europe countries and the United States, where the *peak* incidence is below 35 years. (Braegger et al., 2011). However, referring to the data published for NE Romania by Toader et al. (mean age of onset 45 years), we can appreciate that there is a trend of decreasing age of onset of the disease or earlier diagnosis.(Toader,2008)

Our results showed that Crohn's disease onset is at significantly lower ages compared to UC. This is in line with most epidemiological studies in the country or abroad (Brusnic et al., 2012; Baumgart et al., 2011). However, this difference is larger in our study and can be a particularity of the area of Moldova.

Although we did not find statistically significant differences in prevalence by gender, we noticed a slight prevalence of Crohn's disease in men and of UC in women, as opposed to trends in other regions (Vatn, 2011).

According to the results obtained by us, at the level of 2011 there was no clear gender-related predisposition in the development of inflammatory bowel disease. However, if we analyze separately by subtype of disease, women had a slight predisposition for UC and men for CD, the overall ratio being roughly equal. Most studies found a higher prevalence of CD in women, even by 30%, and a slight prevalence of UC in men in areas with low incidence. (Baumgart et al., 2011) In our study the trend is reversed, the percentage of men with CD being higher than in the literature. This is also demonstrated in the multicenter study published by Gheorghe C et al., in 2014 where there is a clear ratio in favor of males for both conditions. It can be concluded that the slight male predominance, at least for CD, can be considered a regional particularity.

Analyzing the clinical-endoscopic features, in our study group the extraintestinal manifestations were reported in about one third of the subjects, the most common being the osteoarticular manifestations, consistent with the literature data (Vavricka et al., 2011).

Regarding the extension of endoscopically or imaging documented lesions, in our group for UC, left colitis predominated, and for Crohn's disease we observed a predominance of forms with isolated colonic involvement and ileocolic forms, isolated ileal location being very rare. This may have been a characteristic of CD in this region at the time, although insufficient

exploration of the small intestine may have contributed to the reduced proportion of pure ileal forms.
Despite the fact that the incidence and prevalence of Crohn's disease was lower than in neighboring regions (Gheorghe et al., 2003), it seems that there were more severe cases, 48% of cases being moderate or severe, and stenotic and/or penetrating complications being present in more than half of cases. This may be due to both the late diagnosis and the fact that the study was conducted in a tertiary Gastroenterology Center, where most severe cases with complications are referred.

**Conclusions**
The epidemiological characteristics and clinical-endoscopic forms of IBD manifestation are extremely variable between countries and even neighboring regions.
The main epidemiological characteristics of inflammatory bowel diseases in the NE region of Romania at that time were: the predominance of UC over Crohn's disease, the older age at onset for both entities and the higher tendency of CD to develop intestinal complications (stenosis, fistulae, perianal manifestations).
*Originality and applicability of the results in medical practice*
In 2010, at the time of publication of our study, few studies were available in the literature including patients with IBD in Romania and the data were contradictory, so our results contributed to outline the epidemiological and clinical-endoscopic characteristics of patients with inflammatory bowel diseases in the North-East region of Romania.
*Limitations of the study*
The limitations of our research lie in the retrospective nature, carried out over a relatively short period of time. Also, by including in the study only patients referred to a tertiary center, cases with mild forms of disease that are usually managed in the territory may have been underestimated.
*Future directions*
The epidemiology of inflammatory bowel disease has changed greatly over the last decade. For this reason, we consider that regular updates of the epidemiological data in Romania are necessary. This is now easier to achieve through the IBD-Prospect project. The regular publication of these data will provide a more accurate insight into the situation across the country and will highlight the regional specificities of IBD.

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## 1.2.2. Endogenous factors with a role in modulating the evolution of inflammatory bowel diseases

The second topic addressed in this line of research was the study of endogenous elements with a role in modulating the evolution of inflammatory bowel disease.
As previously mentioned, the pathogenesis of IBD is complex and far from being elucidated. The most plausible hypothesis is that genetic factors, gut microbiota, environmental factors and, above all, the innate and acquired abnormal immune response are involved. Interactions between these elements result in an imbalance of a fragile homeostasis between the host immunity and gut microbiota. (Guan, 2019**;** Kim, Cheon, 2017**;** Lloyd-Price et al., 2019; Alemany-Cosme et al., 2021)
Despite the fact that these hypotheses have been the focus of attention in translational research over the past two decades, no biomarkers have yet been identified to ensure the certain diagnosis of IBD and to predict the evolution in the medium and long term (Tian et al., 2017; Metwaly, Haller, 2019; Krzystek-Korpacka et al., 2020). The incidence and prevalence of IBD

in recent years is increasing, the time from the first symptoms to the final diagnosis of IBD has not been shortened in recent years, nor has the exponential progress made in understanding the pathogenesis of IBD changed the position of endoscopy, which, however invasive it may remain, is the primary method of diagnosis (Cantoro et al., 2017).
In this context, we considered it appropriate to study some elements belonging to the main etiopathogenic links in IBD, with a potential role in modulating the inflammatory process in IBD. The research I have conducted since 2010 has sought to establish the role of endogenous factors on the outcome of patients with inflammatory bowel disease. In chronological order, the three etiopathogenic links studied were: oxidative stress, psychological status (anxiety, depression, sleep disorders), and the study of micronutrient deficiencies (Table 1.II).

**Table 1.II.** Publications in the field of endogenous factors involved in IBD pathogenesis

| |
|---|
| DIFFERENT PROFILE OF PERIPHERAL ANTIOXIDANT ENZYMES AND LIPID PEROXIDATION IN ACTIVE AND NON-ACTIVE INFLAMMATORY BOWEL DISEASE PATIENTS<br>Achiței Dorin, Ciobică Alin, Bălan Gheorghe, Gologan Elena, Stanciu Carol, **Ștefănescu Gabriela**.<br>o *Digestive Diseases and Sciences* 2013, **IF – 2.550** |
| SLEEP IMPAIRMENT AND PSYCHOLOGICAL DISTRESS AMONG PATIENTS WITH INFLAMMATORY BOWEL DISEASE – BEYOND THE OBVIOUS<br>Gîlcă-Blanariu Georgiana Emmanuela.; **Ștefănescu* Gabriela**; Trifan Anca; Moscalu Mihaela; Dimofte Mihai Gabriel, Ștefănescu Cristinel, Drug Vasile Liviu, Afrăsânie Vlad Andrei, Ciocoiu Manuela.<br>o *J.Clin. Med* 2020, **IF- 4.242** |
| NEW INSIGHTS INTO THE ROLE OF TRACE ELEMENTS IN IBD<br>Gîlcă-Blanariu Georgiana-Emmanuela, Diaconescu Smaranda, Ciocoiu Manuela, **Ștefănescu Gabriela**,<br>o *BioMed Research International* 2018, **IF – 2.197** |
| FUNGAL DYSBIOSIS IN INFLAMMATORY BOWEL DISEASE- WHERE ARE WE?.<br>Gîlcă Georgiana-Emmanuela, **Ștefănescu Gabriela*,** Ciocoiu Manuela<br>o Neurogastro 2017 - Meeting Of The Romanian Society Of Neurogastroenterology With Rome IV Regional Central East European Meeting, Proceedings |
| HAIR EDX ANALYSIS—A PROMISING TOOL FOR MICRONUTRIENT STATUS EVALUATION OF PATIENTS WITH IBD?<br>Gîlcă-Blanariu Georgiana-Emmanuela ,Coroabă Adina, Ciocoiu Manuela, Trifan Anca, Dimofte Mihai Gabriel, Diaconescu Smaranda, Afrăsânie Vlad Andrei, Bălan G.Gheorghe, Pinteală Tudor.; **Ștefănescu, Gabriela.**<br>o *Nutrients* 2021, 13, 2572. **IF – 5.719** |
| EVALUATING PREDICTIVE FACTORS FOR DISEASE ACTIVITY AMONG PATIENTS WITH INFLAMMATORY BOWEL DISEASE<br>Gîlcă-Blanariu Georgiana-Emmanuela, **Ștefănescu Gabriela** *, Afrăsânie Vlad Andrei, Gologan Elena, Mitrică Dana Elena, Timofte Oana, Bălan Gh. Gheorghe, Olteanu Andrei Vasile, Ciocoiu Manuela.<br>o *Med. Surg. J. – Rev. Med. Chir*., 2020. |

We considered that this research is of interest and that the results could have clinical applicability for developing strategies for personalized approach to the patient with inflammatory bowel disease and for identifying new therapeutic resources.

### *1.2.2.1 Study of the role of oxidative stress in IBD*

Oxidative stress, defined as an imbalance between prooxidants and antioxidants, is closely associated with the inflammatory response and has been shown to be involved in the pathogenesis of many metabolic, inflammatory, or tumor disorders (Halliwell, Gutteridge , 2007).

Relatively recently, it has been suggested that oxidative stress may also be involved in the pathogenesis of IBD, as several genetic risk loci relevant to oxidative stress have been identified associated with IBD. Moreover, it is proven that in patients with IBD oxidative stress is an important trigger of neoplastic transformation (Tian et al, 2017).
Activated immune cells of the intestinal mucosa release many reactive oxygen and nitrogen species (ROS and RNS, respectively), such as superoxide, hydrogen peroxide and hydroxyl radicals (Khor et al., 2011). These are highly reactive and unstable molecules and can cause tissue damage, especially if the antioxidant defense system is low or ineffective. (Kruidenier et al., 2015). Recently it has been noted that ROS can lead to the accumulation of inflammatory cells, being not only a consequence but a trigger of inflammation (Hendrickson et al., 2002) However, there is not yet sufficient evidence for this theory.
In order for cellular homeostasis to be maintained, prooxidant activity must be counterbalanced by antioxidants.
The first line of defense comprises antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), peroxiredoxins and paraoxonase (PON1), which mainly contribute to preventing the formation of free radicals and neutralize those already formed. This defense mechanism also includes chelating proteins (transferrin, ceruloplasmin, albumin), which are responsible for sequestering free iron and copper and preventing them from participating in the Fenton reaction.
The second line of defense neutralizes free radicals by donating electrons: glutathione (GSH), uric acid, cysteine, bilirubin, carotenoids and vitamins A, E and C (Ighodaro, Akinloye, 2018**;** Mirończuk-Chodakowska et al., 2018).
The third and fourth line of defense focus on removing prooxidant damage at the molecular and cellular level, respectively.
Increased markers of oxidative stress (oxygen free radicals and lipid peroxides) as well as decreased antioxidant defenses have been demonstrated to play an important role in the inflammatory process in IBD patients (Lih-Brody et al., 1996; Koutroubakis et al., 2004; Kruidenier et al., 2003), but there are also studies in which no changes have been reported (Durak et al., 2000; Tu¨zu¨n et al., 2002).
In general, changes in oxidative stress parameters were observed in patients with active disease compared to control groups, while no changes in oxidative stress markers were identified in patients in clinical and biological remission compared to control subjects (Maor et al., 2008; Beltra´n et al., 2010).
In conclusion, the number of studies assessing oxidative stress in different stages of IBD is relatively small and the results were conflicting (Krzystek-Korpack et al., 2020; Balmus et al.,2016).

**Personal contributions**

Considering the above mentioned background, the main objective of our study was to assess the serum-specific activity of two antioxidant enzymes – superoxide dismutase (SOD) and glutathione peroxidase (GPX) and a lipid peroxidation marker, malondialdehyde (MDA), in the serum of patients with active / inactive inflammatory bowel disease compared to a control group. We investigated the existence of possible differences between different categories of patients and how the results could be related to the existing etiopathogenic theories.
Superoxide dismutase is an essential antioxidant enzyme that detoxifies superoxide anions ($O2^{-}$) resulting from the activity of activated neutrophils and macrophages, converting them to hydrogen peroxide (hydrogen peroxide – H2O2) (Bild et al., 2012). Glutathione peroxidase acts further in the extracellular environment converting H2O2 to O2 and H2O (Kruidenier , 2015; Pietarinen-Runtti et al., 2000). Together with catalase, a cell active enzyme, SOD and GPX are the main markers of antioxidant defense (Hata et al.,1997). MDA, a highly reactive

thiobarbituric acid degradation product, results from peroxidation of polyunsaturated fatty acids and arachidonic acid metabolism and has been used extensively as a marker of lipid peroxidation (Beltrán B et al., 2010; Alzoghaibi et al., 2007)

**Patients and study method**

We conducted a prospective study on a group consisting of 41 patients with confirmed diagnosis of IBD (27 with UC and 14 with Crohn's disease) consecutively referred to the Institute of Gastroenterology and Hepatology Iași between October 2010 and April 2011. They were divided according to disease activity into two study groups: those with active disease (21 subjects) and those in remission (20 subjects). Remission was defined as a CDAI (Crohn's Disease Activity Index) score less than 150 (for Crohn's disease) or a total Mayo score less than 3 (for UC) (Freeman, 2008; Lewis et al., 2008). The control group consisted of 18 healthy subjects (students, hospital staff) matched to the study group in terms of age, gender and body mass index. Knowing the influence of smoking on oxidative stress, the percentage of smokers was also taken into account when selecting controls (Hritcu et al., 2009). Ongoing medication was not stopped before the tests were performed. Subjects with other decompensated chronic conditions and those receiving antioxidant medication were excluded.

Disease activity was evaluated using CDAI for Crohn's disease and Mayo clinical score for UC. Blood samples were collected during the morning, on fasting status. The serum was then centrifuged and then transferred to Ependorf flasks which were stored at -40°C until retrieved for analysis.

The actual determination of specific oxidative stress markers was carried out by the team of researchers from the Faculty of Biology of A. I. Cuza University of Iași with whom I collaborated in this study.

- *Determining the activity of superoxide dismutase (SOD)*

Serum SOD activity was measured by the percent inhibition rate of enzyme reaction with the substrate WST-1 (a *water-soluble tetrazolium dye*) and xanthine oxidase using a specialized kit (SOD Assay-FLUKA, 19160) according to the manufacturer's recommendations.

The percentage of inhibition was expressed as units of SOD activity.

- *Determining the activity of glutathione peroxidase (GPX)*

The glutathione peroxidase (GPX) activity was measured using the GPX cellular activity assay kit CGP-1 (Sigma).

- *Determining the serum concentration of malondialdehyde (MDA)*

Malondialdehyde levels were determined by thiobarbituric acid reactive substances (TBARS) assay.(Padurariu et al., 2010)

*Data Analysis*

The results for antioxidant enzymes activity and MDA level were analyzed using one-way ANOVA. All results were expressed as mean ± SEM. Post hoc analyses were performed using Tukey's honest significance test in order to compare active and non-active IBD groups. Crohn's disease and ulcerative colitis patients were analyzed separately within the active and inactive groups also using one-way ANOVA. Pearson's correlation coefficient was used to evaluate the connection between the oxidative stress parameters and CRP.

The study was conducted in accordance with the provisions of the Declaration of Helsinki and was approved by the local Ethics Committee. All subjects received and signed the Informed Consent.

**Results**

The analysis of covariance showed that, with respect to age, gender, smoking status, and BMI, there were no significant differences between IBD patients and healthy control subjects. There

was also no significant difference between patients with active disease and those in remission concerning disease subtype, disease duration, or type of treatment (Table 1.III).

**Table 1.III.** Demographic and clinical data in the control, remission and active disease groups
[a] Each value represents mean SD; [b] Analysis of covariance

| | Control ($n$ = 18) | Remission ($n$ = 20) | Active disease ($n$ = 21) | $F$[b] | $P$ value[b] |
|---|---|---|---|---|---|
| Age[a] | 36.39 ± 13.7 | 35.5 ± 14.6 | 34.71 ± Il | 0.08 | 0.924 |
| Gender (male/female) | 8/10 | 11/9 | 13/8 | 1.1 | 0.263 |
| Active smokers | 33.3 % | 30 % | 33.3 % | 0.55 | 0.577 |
| BMI[a] | 24.2 ± 3.9 | 23.4 ± 3.7 | 21.7 ± 4.8 | 2.11 | 0.069 |
| Time from initial | | | | | |
| Diagnosis (years)[a] | — | 4.75 ± 5.53 | 3.47 ± 4.33 | 0.68 | 0.415 |
| Crohn's disease (%) | — | 30 % | 38.1 % | 1.3 | 0.077 |
| Ulcerative colitis (%) | — | 70 % | 61.9 % | 1.3 | 0.08 |
| Medication | | | | | |
| 5-ASA | — | 75 % | 61.9 % | 2.3 | 0.057 |
| Corticosteroids | — | 20 % | 33.3 % | 2.19 | 0.06 |
| Azathioprine | — | 15 % | 4.7 % | 2.03 | 0.095 |
| Biological agents | — | 15 % | 9.5 % | 1.7 | 0.112 |

Regarding the specific activity of SOD, we observed a significant decrease in the group of patients with IBD in remission (F(1.36)= 5, $P$ = 0.03), compared to the control group (Fig. 1.3). Patients with active disease had higher levels of SOD than controls, but this difference was not statistically significant. (F(1.37) = 1, $P$ = 0.27). The *post-hoc* analysis revealed significant differences between the groups with IBD in remission and in active phase ($P$ = 0.004).

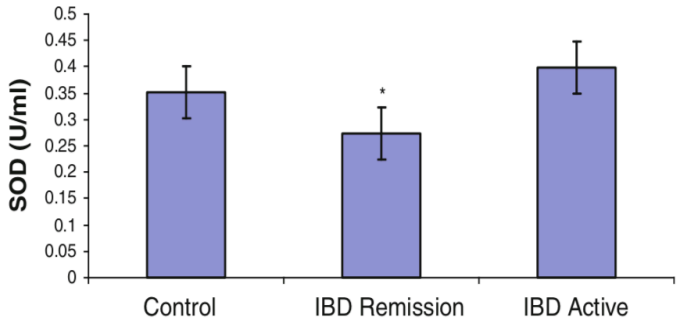



**Fig. 1.3.** Superoxide dismutase specific activity in the serum of control, IBD remission, and IBD active subjects. The values are mean± SEM (n=18 in control, 20 in IBD remission and 21 active group)

Analyzing the subgroups with Crohn's disease and UC, we found that the differences were more pronounced in those with Crohn's disease, with a significant decrease in SOD activity in patients in remission compared to controls (F(1.22) = 8.03, $P$ = 0.009) and higher values of SOD in active disease compared to subjects with UC ($P$ = 0.36). However, the statistical analysis did not reveal significant differences between the subgroups with CD or UC in remission ($P$ = 0.07) or in active phase ($P$ = 0.35).

The values obtained when evaluating the other antioxidant enzyme studied (GPX) had a similar distribution. We found a significant decrease in enzyme activity in patients in remission compared to the control group (F(1.36) = 7, $P$ = 0.01). As in the case of SOD, the group with active IBD had a higher value of GPX compared to the controls, without meeting the criteria of statistical significance (F(1.37) = 2, $P$ = 0.23) (Fig. 1.4.). The *post-hoc* analysis also revealed

significant differences between patients in the group in remission compared to those with active disease ($P = 0.002$).
Subgroups consisting of patients with UC and CD showed a very similar behavior in terms of specific GPX activity, with significant decrease in value in remission and a consistent but still statistically insignificant increase in active phase. At *post-hoc* analysis, differences between groups with active and inactive disease were maintained for each subgroup ($P = 0.03$ for CD and $P = 0.01$ for UC).
In the case of lipid peroxidation quantified by the MDA concentration, the statistical analysis showed a significant increase in the MDA value in the group with active IBD compared to the controls ($F(1.37) = 5$, $P = 0.04$). However, no significant differences from controls were observed in patients in remission. ($F(1.36) = 1$, $P = 0.3$) (Fig. 1. 5). Unlike the other two determinations, the *post-hoc* analysis did not reveal significant differences between patients in remission and those in active phase ($P = 0.408$). In the subgroups with CD and UHC there were no statistically significant differences between the two conditions in the groups of remission ($P = 0.53$) and active phase ($P = 0.93$).

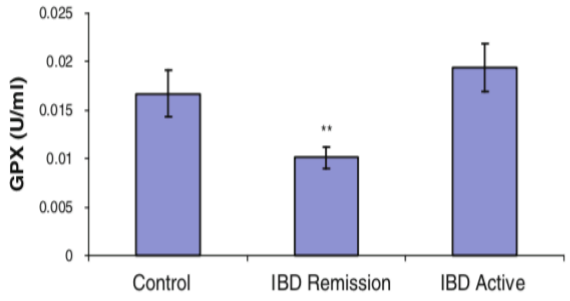



**Fig.1.4.** Glutatione peroxidase specific activity in the serum of control. IBD remission, and IBD active subjects. The values are mean ±SEM (n=18 in control, 20 in IBD remission and 21 IBD active group)**p=0.01.

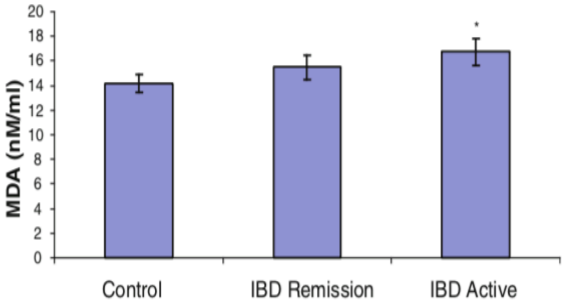



**Fig.1.5**. Malondialdehyde concentration in the serum of control. IBD remission, and IBD active subjects. The values are mean ±SEM (n=18 in control, 20 in IBD remission and 21 IBD active group)*p=0.04.

The detailed results of the enzymes measured in the study groups expressed as the mean ± SEM and the value of *P* are illustrated in Table 1.IV
By analyzing the correlation between the 3 enzymes analyzed and the serum CRP value, we observed in the case of GPX a weak and insignificant positive correlation ($n = 41$, $r = 0.185$, $P = 0.245$). In contrast, SOD levels had a moderate positive correlation with CRP values, reaching statistical significance 58, $P = 0.021$). Similarly, MDA value correlated positively with the systemic inflammation quantified by the PCR value ($n = 41$, $r = 0.362$, $P = 0.020$).

**Discussion**

In patients with active intestinal inflammation, we showed increased values of peripheral antioxidant enzymes (GPX and SOD) with very similar dynamics. This reflects an intense production of oxygen free radicals as a result of mucosal injury. So far there have been conflicting data, with many studies showing similar results (Tu¨zu¨n et al., 2002, Maor et al., 2008; Beltra´n et al., 2010), but there are also authors who have observed no difference (Akman et al., 2012; Reimund et al., 2000).
On the contrary, patients in clinical and biological remission did not only have significantly lower levels of SOD and GPX compared to patients with active disease, but also showed a statistically relevant decrease in these antioxidant enzymes compared to the control group. The similar distribution of both markers supports this conclusion. There are few authors who have identified significantly low values of SOD and/or GPX in the serum of patients with IBD in

remission (Reimund et al., 2000; Geerling et al., 2000), most studies finding no difference (Maor et al., 2008; Beltra´n et al., 2010). However, there are a limited number of papers that have investigated the antioxidant enzyme system in different phases of IBD activity (Maor et al., 2008;), and the number of patients enrolled in most studies has been relatively small. GPX is a selenium-dependent enzyme, therefore low levels of plasma selenium found in IBD patients may explain our results (Geerling et al., 2000, Hinks et al., 1988, Gîlcă et al., 2021). Moreover, by examining the antioxidant defense in the colonic mucosa, many authors have shown significant decreases in the values of these enzymes (Lih-Brody et al., 1996; Dagli et al., 1997; Tsunada et al., 2003).

**Table 1.IV.** Specific activity of SOD, GPX and MDA in active and non-active disease compared with control subjects

| | SOD (U/ml) | *P* | GPX (U/ml) | *P* | MDA (nM/ml) | *P* |
|---|---|---|---|---|---|---|
| Control (*n* = 18) | 0.351 ± 0.028 | | 0.0167 ± 0. 0024 | | 14,183 ± 0.712 | |
| Non-active disease (*n* = 20) | 0.273 ± 0.026 | 0.036* | 0.0101 ± 0.0010 | 0.013* | 15.473 ± 0.992 | 0.302 |
| CD (*n* = 6) | 0.202 ± 0.025 | 0.009* | 0.(X)99 ± 0.002 | 0.134 | 14.517 ± 1.896 | 0.839 |
| UC (*n* = 14) | 0.304 ± 0.032 | 0.287 | 0.0101 ± 0.0013 | 0.035* | 15.883 ± 1.165 | 0.202 |
| Active disease (*n* = 21) | 0.399 ± 0.030 | 0.274 | 0.0194 ± 0.0025 | 0.232 | 16.720 ± 1.148 | 0.047* |
| CD (*n* = 8) | 0.436 ± 0.048 | 0.132 | 0.0183 ± 0.0026 | 0.682 | 16.760 ± 1.791 | 0.117 |
| UC (*n* = 13) | 0.376 ± 0.039 | 0.610 | 0.0200 ± 0.0038 | 0.444 | 16.695 ± 1.491 | 0.108 |

Separate values are given for CD and UC subgroups
Each value represents mean ± SEM
**P* value considered statistically significant if < 0.05

A possible explanation for the decrease in GPX and SOD in patients in remission may be the consumption of these antioxidant enzymes during the active phases of the disease. Another more tempting hypothesis is that patients suffering from IBD, even in remission, have a poor antioxidant defense. This depletion could be present even before the onset of the disease. Thus, low antioxidative capacity could be a risk factor for UC and Crohn's disease. Many patients in remission do not achieve mucosal healing and experience ongoing intestinal inflammation (Lichtenstein, Rutgeerts, 2010). Therefore, poor antioxidant defense may explain the continuity of cellular damage.

Patients with UC and Crohn's disease had similar levels of GPX activity. However, the specific SOD activity showed a wide variation between disease activity and remission, with a sharp increase in active phase and a marked decrease during remission. Given the systemic nature of Crohn's disease and the fact that SOD is the first enzyme to interfere with the antioxidant chain may partly explain the obtained results. However, the small number of patients with Crohn's disease could represent a limitation, thus the results should be interpreted with caution.

Along with antioxidant enzymes, lipid peroxidation, assessed by serum MDA levels, was significantly increased in patients with active disease. Given the high concentration of ROS as deducted from the increased values of SOD and GPX, tissue injury due to alteration of structural lipids may be a logical consequence, as demonstrated by other working groups (Tu¨zu¨n et al., 2002; Maor et al., 2008; Alzoghaibi et al., 2007). There are also studies that found no difference in peripheral MDA values during active disease compared to controls (Beltrán et al., 2010; Barbosa et al., 2003). We also demonstrated that patients in remission have higher levels of serum MDA, but the difference was not statistically significant. For none of the three markers determined did we find statistically significant differences between patients with UC and those with Crohn's disease. This may be a further argument for etiopathogenic mechanisms common to the two conditions.

As the number of evidence regarding the involvement of oxidative stress in IBD has increased, more interest has been focused on antioxidant treatment. In fact, the first effective drug used to

treat IBD (sulfasalazine) has been shown to have an antioxidant effect by scavenging free oxygen metabolites. Many antioxidants have been proposed as supplements in patients with inflammatory colitis (vitamins, unsaturated fatty acids, N-acetyl-L-cysteine). The results regarding the increase of the total antioxidant capacity were mostly positive (Geerling et al., 2000; Aghdassi et al., 2003).
The therapeutic applicability of SOD is limited by low cell permeability, short half-life, immunogenicity and high production costs. A derivative of superoxide dismutase (lecithinized) has been used with good results in the alleviation of colonic inflammation in animal models and in pilot studies.
Regarding the prevention of relapse, complications or positively influencing the course of the disease, no studies have been published so far demonstrating the effectiveness of antioxidants.

**Conclusions**
In this study we demonstrated increased levels of oxidative stress and lipid peroxidation in patients with active inflammatory bowel disease. Our results also suggest that IBD patients in remission have a reduced antioxidant defense compared to healthy individuals. This could be a consequence of the disease but can also be interpreted as a pre-existing condition. In the latter hypothesis, dysfunction of antioxidant capacity can be considered as a predisposing factor for IBD development and therefore may be an argument for the etiological theory of oxidative stress in IBD.
*Originality and applicability of the results in medical practice*
Most studies in the literature have been conducted on animal models, and of those conducted on patients very few are randomized trials (Suzuki et al., 2008). Our study was among the first conducted on groups of patients with IBD. We were able to demonstrate the presence of significant changes in the oxidative stress markers in the serum of patients with active inflammatory bowel disease compared to healthy subjects. We also demonstrated that patients with inactive disease showed a decreased antioxidant enzyme profile and increased lipid peroxidation. Evidence of the involvement of oxidative stress in the pathogenesis of these diseases is a premise for further studies on the role of antioxidants in the treatment of IBD.
*Limitations of the study*
Of course, there are several limitations of our study, which include the relatively small size of the groups that we used and the fact that the background treatment was not stopped when the study was conducted. Even if the inclusion of some smoking subjects did not statistically influence the results of the research, for rigorousness it is preferable to avoid enrolling active smokers in studies targeting oxidative stress. Future studies should consider all the factors that may alter oxidative stress
*Future directions*
In order to clarify the role of oxidative stress in the etiopathogenesis of IBD, case-control cohort studies are necessary to assess as many parameters as possible. In practice this is difficult to do, as evidenced by the fact that even today, 10 years after the development of our study, the published data on the significance of oxidative stress in inflammatory bowel disease are contradictory (Krzystek-Korpacka et al., 2020;)
In other words, in the light of the results of the study, randomized clinical trials to establish the efficacy of antioxidant medication in the treatment of IBD would be warranted.

### *1.2.2.2. Implications of psychological impairment and sleep disorders in patients with inflammatory bowel disease*

In the early 90s, during the early period of the research aimed at identifying links between psychiatric impairment and UC, a systematic review of the literature, on 138 clinical studies published up to that time, concluded that there was no association between psychiatric

pathology and UC and that most of the studies demonstrating the opposite were in fact methodologically flawed (North et al., 1990).
Subsequent, prospective, case-control studies in a large number of patients have shown an increased prevalence of depressive and anxiety disorders in IBD patients (Walker et al., 2008). A relatively recent systematic review of the literature, which included 171 studies, and a number of 158,371 patients with IBD, reported a prevalence of anxiety disorder of 20.5%, respectively of depression of 15.2%, these comorbidities reaching even higher percentages in patients during disease flare (Neuendorf et al. 2016). Risk factors for the development of psychopathological comorbidities were also investigated, including an aggressive IBD phenotype, the presence of an active disease and female gender. (Gracie et al., 2019).
On the other hand, a number of studies have reported the presence of quantitative and qualitative sleep disorders in patients with IBD. These include reduced sleep duration, which has been highlighted as a risk factor for triggering UC (Ananthakrishnan et al., 2013) or daytime sleepiness and fatigue. These are common manifestations of chronic inflammatory diseases such as IBD and may influence their progression. A study under the aegis of the American Society of Crohn's Disease and Ulcerative Colitis, which included 3,100 patients with IBD, reported that patients with CD and sleep disorders had a double risk for active disease at 6 months, an association that was not maintained in patients with UC. The same study revealed associations between the presence of sleep disorders and disease activity, corticosteroid use, but also the presence of depression (Ananthakrishnan et al., 2013).
Many of the studies conducted in patients with IBD, concurrently assessed psychopathological comorbidities and sleep quality, and the multivariate modelling of the data included in a systematic review of the literature including data from 1,444 studies, revealed that both anxiety and disease activity are predictors of impaired sleep quality in these patients (Brooks et al., 2016).
Assumptions regarding the role of psychopathological disorders and sleep disturbance on the inflammatory process
The close connection between sleep and the immune system has been demonstrated. A proper circadian rhythm is fundamental to regulating the immune system. Sleep influences cell-mediated immunity, highlighting the connection between its alteration and various proinflammatory mediators, such as IL-1 and tumour necrosis factor- (TNF-α), which influences IBD activity (Qazi, Farraye, 2019).
Sleep deprivation has also been shown to contribute to systemic inflammation. This is reflected by changes in the level of pro-inflammatory cytokines such as IL-1β, IL-6. Increases in IL-6 and IL-1β, respectively, during sleep deprivation has been shown in both animal model and clinical studies. Prolonged sleep deprivation for 64 hours has been associated with increased NK cell and monocyte activity (Frey et al., 2007; Haack et al., 2007). Another study showed that re-initiation of sleep after a period of deprivation contributes to a decrease in the levels of monocytes, NK cells and lymphocytes, and is associated with an increase in IL-2 production. (Ranjbaran et al., 2007)
There is a complex interaction between environmental factors, including diet, stress, altered microbiota, and the immune system. These factors are recognized by the immune system as pathogen-associated molecular patterns (PAMPs), which trigger the activation of some pattern recognition receptors (PRRs). This activation of PRRs leads to pro-inflammatory response, by production of pro-inflammatory cytokines, which also play a role in sleep modulation. These include interleukin-1 (IL-1) and tumor necrosis factor (TNF), which promote the prolongation of the non-REM phase of sleep at the expense of the REM phase.
Regarding IBD, one of the first studies to assess sleep in this group of patients was carried out by Zimmerman et al. He conducted a case-control study in male patients with IBD and irritable

bowel syndrome, respectively, and assessed the quality of sleep using the questionnaire method. The study showed a higher prevalence of sleep disorders in IBD patients and identified the presence of diarrhea among predictors of sleep disorders (Zimmerman, 2003). Another study evaluating sleep disorders in the pediatric IBD population identified an important percentage (54%) of sleep disorders, with morning fatigue and sleep fragmentation most commonly reported. (Nachmias et al., 2006).

The relationship between psychopathological comorbidity and the degree of inflammation has a complex pathophysiological underpinning, partly related to the impairment of the gut-brain axis, which involves a complex interaction between neuroendocrine pathways, the autonomic nervous system and the gastrointestinal tract.

The increased risk of developing psychopathological symptoms in patients with IBD, especially during disease flares, is well known. However, the presence of these symptoms during remission has been less studied. In turn, psychopathological comorbidity has a series of negative effects on patient outcomes, by reducing adherence to treatment and by requesting repeated medical evaluations and paraclinical assessments (Gracie et al., 2019).

The psychological status can influence the physiological functions of the digestive tract by generating a stress response, which determines the activation of the hypothalamic-pituitary-adrenal axis, resulting in increased intestinal permeability. The sequence of these events has been demonstrated in animal model (Santos et al., 1999).

The increased activity of the sympathetic autonomic nervous system, observed in people under stress and associated with increased secretion of catecholamines, may exert proinflammatory effect on the digestive tract, following the stimulation of mast cells and macrophages, mediated by proinflammatory cytokines (Farhadi et al., 2005). This inflammation-sustaining cortege may indirectly facilitate the action of the microbiota on the nervous system by creating the microbiota-gut-brain axis (Gracie et al., 2019).

An abnormal activation of the enteric immune system is part of the etiopathogenesis of visceral hypersensitivity , which is due in part to its overexposure to the lipopolysaccharide envelope of intraluminal bacteria, possibly in the context of increased intestinal permeability (Gracie et al., 2019). On the other hand, studies conducted on the murine model have shown that psychological well-being is correlated with a specific gut microbiota composition (Emge, et al., 2016). The relationship between psychological status and microbiota is vagally mediated by the inhibitory role of cholinergic mediation on proinflammatory cytokines (Matteoli, Boeckxstaens, 2013). This type of parasympathetic reflex is reduced under stress. A reduced vagal tone is associated with reduced levels of salivary cortisol (Pellissier et al., 2014). From this premise, pilot studies have been conducted investigating the role of vagal stimulation in achieving remission in patients with CD (Bonaz et al., 2016).

The visceral hypersensitivity is clearly demonstrated in patients with IBD and it seems that, besides the autonomic nervous system, the sensitive neural pathways have an important role. In this regard, a meta-analysis including 13 observational studies in patients with IBD revealed the presence of irritable bowel-like symptoms in 1/3 of patients with UC and in almost 50% of patients with CD. These symptoms are underpinned by visceral hypersensitivity as a pathophysiological substrate. (Halpin, Ford, 2012).

In other words, one aspect that makes it difficult to establish the relationship between inflammation and psychopathological manifestations is the therapeutic intervention addressing IBD. It is known that medication, and in particular corticosteroid therapy, can influence the psychological status independent of the disease itself.

However, a study conducted in patients who did not take corticosteroids and who did not have surgery related to IBD showed the presence of depression with a higher prevalence than in the control group (Addolorato et al., 1997). However, another study conducted in patients with

IBD could not demonstrate the predictive value of depression on the risk of relapse in patients with UC (Langhorst et al., 2013).

In summary, it is believed that between altered psychological status and gut inflammation, there is a two-way relationship (Gracie et al., 2018) and, moreover, it seems that the altered psychological status, sleep disturbance and inflammation cause reciprocal exacerbations. In this context, the study of psychopathological disorders alongside sleep assessment are important aspects for understanding the etiopathogenic mechanisms involved. (Keefer et al., 2006)

*Psychological and sleep assessment tools in patients with inflammatory bowel disease*

Both for assessing psychological status and sleep, the most widely used tool in clinical trials is the questionnaire. Validated questionnaires are available to assess sleep quality, fatigue, daytime sleepiness, of which the best known are the Pittsburgh Sleep Quality Index (PSQI), the Patient-Reported Outcomes Measurement Information System (PROMIS), the Modified Fatigue Impact Scale (MFIS) and the Epworth Sleepiness Scale (ESS).

PSQI is most frequently used to assess the quality of sleep, both in various research settings (Ali et al., 2013) and in clinical practice, as it reflects the quality of sleep by assessing seven components by evoking various types of events in the past month. The PSQI questionnaire includes a total of nineteen items, it assesses the seven components of sleep quality: the quality of sleep subjectively assessed by the patient, the use of sleep medication, the latency until sleep, the duration of sleep, the habitual efficiency of sleep, the degree of daytime dysfunction resulting from improper sleep. Patient responses, for each of the seven components assessed resulted in scores between 0 - representing no impairment of that component - and 3 - representing severe impairment of that component. As a consequence, the score obtained by summing the components ranges between 0 and 21. A score value greater than or equal to 5 corresponds to an altered quality of sleep, with diagnostic sensitivity of 89.6% and a specificity of 86.5% Ali et al., 2013; Buysse et al., 1989; Ibáñez et al., 2018)

To assess psychological status, there are also numerous questionnaires, some of which have as an advantage the ease of application, others the possibility of simultaneously assessing the depression and anxiety that frequently coexist. A commonly questionnaire used in studies evaluating IBD patients is the Hospital Anxiety and Depression Scale (HADS) questionnaire, which includes 7 questions for the assessment of anxiety and 7 for the assessment of depression, and which can identify borderline disorders and the presence of anxiety and/or depression. This questionnaire based on patient reporting of symptoms has been validated for use in patients with IBD (Yamamoto-Furusho et al., 2018). To assess anxiety and depression, respectively, using this questionnaire, the maximum score is 21 for each component. The higher the score obtained, the greater the severity of the symptoms; the threshold for clinical diagnostic significance of anxiety and depression is 11, while a score between 8 and 10 suggests minor impairment. (Zigmond, Snaith, 1983,)

The place of psychological and sleep assessment in the management of patients with inflammatory bowel disease

Although it is known that stress, anxiety and depression contribute significantly to altering the clinical and biological status of patients with IBD (Sajadinejadz, et al., 2012), the connection between stress and the evolution of IBD is still uncertain. Recent studies following this connection have highlighted a potential causal link between psychological dysfunction and the onset and exacerbation of subsequent IBD activity (Melinder, et al., 2017; Gracie et al., 2018).

Impaired sleep quality is associated with significant reduced quality of life, (Knowles et al., 2018) with sleep disturbance reported not only in patients in flare-ups, but also in patients in remission (Ali, et al., 2013; Keefer et al., 2006; Gîlcă-Blanariu et al., 2020).

Although in patients with IBD and depression the reduced interest in usual activities could lead to decreased adherence to treatment, which could lead to disease flares, it is difficult to estimate

the individual impact of each psychopathological factor (Nigro et al., 2001). The influence of anxiety/depression on reduced adherence to treatment has been reported by Zelikovsky, who identified a 12% decrease in adherence in patients with anxiety/depression symptoms. (Zelikovsky, Schast, 2008)

A systematic review, conducted by Brooks A.J. et al. aimed to assess the risk factors and the impact of psychological comorbidity in young patients with IBD. Of the 1,444 studies identified, only 30 met the quality criteria for inclusion. Following the analysis carried out, the following risk factors for psychological comorbidity were identified: low social-economic status, parental stress, older age at diagnosis, but also strictly disease-dependent elements (disease activity, use of corticosteroids). (Brooks et al., 2016). Taking into account all these elements, the question arises on how the assessment of sleep can be integrated into the management of patients with IBD, so that there is a benefit regarding the evolution of the disease and the improvement of the quality of life. Screening for psychopathological and sleep disorders could be included as a non-invasive element to reflect subclinical inflammation (Kinnucan et al., 2013; Ali et al., 2013; Ananthakrishnan et al, 2013).

This approach is essential if we relate to current treat-to-target or patient reported outcome strategies.

From the data currently available, it is not certain how best to approach the investigation/management of psychological comorbidity and the alteration of sleep quality in patients with IBD, but a multidisciplinary team is certainly necessary, including the gastroenterologist specialized in IBD, the psychologist, the psychiatrist and possibly the somnology specialist.

**Personal contributions**

Based on these premises, and in line with our interest in the study of the mechanisms involved in the etiopathogenesis of inflammatory bowel diseases, we considered that it is of interest to further investigate the relationship between psychopathological comorbidity, sleep disorders and the course of inflammatory syndrome.

Prior to our study published in 2020, no data related to the psychological status and quality of sleep in IBD patients have been reported in Romania.

In this context, we conducted a study in which we followed the psychopathological disorders and sleep changes in a group of patients with IBD (Gîlcă et al., 2020)

The primary objective of this study was to characterize sleep disorders in IBD patients using the Pittsburgh Sleep Quality Index (PSQI) questionnaire.

The secondary objectives of this study were to identify possible correlations between sleep disturbance and inflammatory markers, and between sleep disorders and altered psychological status (depression, anxiety) in patients with IBD. We also aimed to analyze the potential correlations between sleep disorders, disease activity and other relevant parameters for the evolution of IBD patients (nutritional status, CRP/albumin, neutrophil-to-lymphocyte ratio =NLR) as well as the relationship between the medication administered and the presence of sleep disorders, respectively, the presence of psychopathological disorders. Among secondary objectives, in the prospect of significant correlations, establishing– cut-off values for components of PSQI and HADS scoring with predictive value for disease activity was included.

**Patients and method**

We conducted a case case-control study, carried out in the Institute of Gastroenterology and Hepatology within St. Spiridon County Clinical Emergency Hospital, Iasi, in which we recruited consecutive patients with IBD for a period of 6 months (March 2019 and September

2019). In the control group we included healthy subjects, evaluated under a screening program for CRC, carried out at the Regional Institute of Oncology, Iași.
We investigated the hypothesis according to which, for adult patients with IBD, there is a significant alteration in the quality of sleep in comparison with healthy subjects.
*Patient selection*
The inclusion criteria were: patients aged 18-70 years, able to sign an informed consent, diagnosed with IBD on clinical, biological, endoscopic and histologically confirmed criteria.
The exclusion criteria consisted of situations (comorbidities/medication) that may interfere with the quality of sleep: evolving pregnancy, sleep apnea, BMI > 35, the presence of an acute infection, associated neoplasia or history of neoplasia, associated severe cardiopulmonary pathology, BMI > 35, associated psychiatric or thyroid pathology, patients working in night shifts, patients with psychotropic medication or dietary supplements used for sedative purposes, myorelaxants, thyroid hormones, amphetamines, etc. (Kryger et al., 2010)
*Parameters assessed*
Demographic characteristics, clinical aspects (presence and type of extraintestinal manifestations), current and previous medication, possible surgery due to inflammatory bowel disease were recorded in the database. Disease activity was assessed by Mayo scores for UC and CDAI for CD.
The biological parameters analyzed were: markers of systemic inflammation (C-reactive protein, fibrinogen, ESR), fecal calprotectin, CRP/albumin ratio, neutrophils/lymphocyte ratio (NLR).
The assessment of sleep was done using the Pittsburgh Sleep Quality Index (PSQI) questionnaire, and the psychological status using the Hospital Anxiety and Depression Scale (HADS) questionnaire. In the study we conducted, we considered pathological any PSQI score $\geq 5$ and any HADS score $\geq 8$, respectively (compatible with the presence of a depressive or anxious disposition).
The sleep disturbance profile in IBD patients was assessed by comparison with a control group consisting of healthy volunteers recruited from among patients who presented for colonoscopy screening, without any digestive complaints and no lesions during the colonoscopy assessment.
The two questionnaires (PSQI and HADS) were applied by the same interviewer to all subjects included in both the study group and the control group to ensure a correct and uniform application as there is no validated Romanian version.
*Statistical Analysis*
The statistical analysis of data was performed using SPSS version 24 (IBM Corporation, North Castle Drive, Armonk, NY, USA). Continuous variables were reported as mean values and standard deviation, or as median with 25th–75th percentiles. The comparisons between the analyzed groups were performed using Student's t-test, the Mann–Whitney U Test or the Kruskal–Wallis test for continuous variables, depending on the homogeneity of the data series, based on Levene's test. The comparison among the groups was based on the results of McNemar, Yates or Pearson chi-square tests. The univariate correlation analysis was completed based on the results of Spearman Rank Order Correlations tests. The multivariate analysis of prognostic factors for PSQI values was achieved using an ordinal regression model. The prediction power of some variables was assessed, based on the ROC curve, by evaluating the area under the ROC curve (AUC). The calculated significance level (p-value) within the applied tests was considered significant for $p < 0.05$.
*Ethical considerations*
The study protocol and all procedures included therein were in accordance with the recommendations of the Ethics Committee, the 1964 Declaration of Helsinki and its subsequent regulations. The ethical opinion was obtained from all the institutions involved, namely Grigore

T Popa University of Medicine and Pharmacy, Iași, the Regional Institute of Oncology and Sf Spiridon County Clinical Emergency Hospital Iași. The informed consent was obtained from all the participants included in the study.

## Results

*Descriptive statistics*

After calculating the optimal batch size, 110 patients (54 F and 56 M) with a mean age of 41.5 years were included in the study group, and 66 healthy subjects with a mean age of 44.3 years were included in the control group. There were no statistically significant differences between patients in the study group and those in the control group in terms of age, gender and background. The demographic data are shown in Table 1.V .

**Table 1.V.** Descriptive statistics for the sociodemographic characteristicsin the study and control group.

| Characteristics | Study Group | | Control Group $n = 66$ | Statistic Test | $p$-Value |
|---|---|---|---|---|---|
| | **Ulcerative Colitis $n = 76$** | **Crohn's Disease $n = 34$** | | | |
| Age, year (mean ± SD) | 45.1 ± 14.9 | | 44.3 ± 16.1 | 1.6957 † | 0.4283 |
| | 46.2 ± 15.6 | 42.6 ± 13.5 | | | |
| Gender (M/F), *n* (%) | 36/40 | 20/14 | 26/40 | 3.4368 ‡ | 0.1793 |
| | (47.4%/52.6%) | (58.8%/41.2%) | (39.4%/60.6%) | | |
| Environment (U/R), *n* (%) | 58/18 | 26/8 | 42/24 | 3.28561 ‡ | 0.1934 |
| | (76.3%/23.7%) | (76.5%/23.5%) | (63.6%/36.4%) | | |

† Kruskal–Wallis test; ‡ Pearson chi-square test; marked effects are significant at $p < 0.05$.

In both study subgroups (CD respectively UC) there were both patients in remission and patients with active disease (UC -57.9% active forms; CD - 29.41% active forms). The percentages were calculated based on Mayo disease activity scores and CDAI, respectively. There were statistically significant differences in the activity of the disease ($p = 0.0201$), with a higher percentage of patients with active disease in the group of patients with UC.

Biochemical parameters were assessed only for patients in the study group. With respect to the disease type, significant differences between CD and UC were only observed for hemoglobin level and for the presence of extraintestinal manifestations.

There were also statistically significant differences between the group of patients with UC compared to the group with CD and for fecal calprotectin values. Fecal calprotectin values were significantly higher in the UC subgroup than in CD subgroup. These aspects reflect more severe forms of the disease in the group with UC, which is consistent with the percentages reflecting the activity of the disease. (Table 1. VI).

By analyzing the extraintestinal manifestations, the most commonly identified was peripheral arthritis, present in 18.42% of patients with UC, respectively in 5.88% of patients with CD. The second most common extraintestinal manifestation was sacroiliitis, present in 7.89% of patients with UC, without being found among patients with CD studied. Joint manifestations could be responsible for changes in sleep through pain potential.

*Assessment of sleep quality and psychological status*

Following the application of the PSQI questionnaire, we found an alteration in the quality of sleep in patients with IBD, compared to the control group ($H = 31.3107$, $p < 0.001$). In the study group (IBD) the median PSQI score was 8 with interquartile range (5; 10) and in the control group the median PSQI score was 4 (3; 7). When assessing PSQI by disease subtype (CD vs UC), there were no significant differences ($p = 0.1913$) (Figure 1. 6).

**Table 1. VI.** Descriptive statistics for biochemical parameters in the study group.

| Disease Activity | Study Group | | Statistic Test | *p*-Value |
|---|---|---|---|---|
| | Ulcerative Colitis *n* = 76 | Crohn's Disease *n* = 34 | | |
| Active disease, *n* (%) | 44 (57.9%) | 10 (29.4%) | 7.625 ‡ | 0.0201 * |
| Mayo or CDAI score, median (Q25; Q75) | 3 (1; 7) | 129 (43; 200) | - | - |
| Hemoglobin, (mean ± SD) | 12.87 ± 2.33 | | | |
| | 12.55 ± 2.42 | 13.63 ± 1.95 | 4.9888 ♯ | 0.0276* |
| Seric iron level, (mean ± SD) | 56 ± 34.51 | 64.25 ± 43.26 | 1.1026 ♯ | 0.2961 |
| Albumin, (mean ± SD) | 3.97 ± 0.58 | 4.05 ± 0.47 | 0.3582 ♯ | 0.5509 |
| Leucocytes, (mean ± SD) | 8358 ± 2887 | 8644 ± 3280 | 0.2112 ♯ | 0.6468 |
| PMN/Ly, median (Q25; Q75) | 2.65 (1.72; 3.80) | 2.75 (2.26; 3.86) | 0.9055 § | 0.3651 |
| Platelets, median (Q25; Q75) | 314657 (287775; 341540) | 325529 (293124; 357934) | 0.2267 § | 0.6349 |
| ESR 1h, (median (Q25;Q75) | 7.8 (5.9;9.8) | 6.4 (4.9;7.9) | 1.0525 § | 0.3083 |
| CRP, (median (Q25;Q75) | 2.2 (1.22; 3.19) | 3.25 (0.85; 5.64) | 0.4269 § | 0.6694 |
| CRP/Albumin, (mean ± SD) | 0.83 ± 2.32 | 1.13 ± 2.11 | 0.3322 ♯ | 0.5657 |
| Fibrinogen, median (Q25;Q75) | 417 (402; 432) | 439 (408; 470) | 2.1885 ♯ | 0.1424 |
| Fecal calprotectin, median (Q25;Q75) | 384.6 (293.9; 475.3) | 269.5 (265.2; 272.5) | 1.1856 § | 0.0237 * |
| Peripheric arthritis, *n* (%) | 14 (18.4%) | 2 (5.9%) | 3.1473 ‡ | 0.0345 * |
| Sacroiliitis, *n* (%) | 6 (7.9%) | - | 4.5902 ‡ | 0.0321 * |
| Pyoderma gangrenosum, *n* (%) | 1 (1.3%) | - | - | |
| Uveitis, *n* (%) | 2 (2.6%) | - | - | |
| Total EIM (% among disease subtype), *n* (%) | 21 (27.6%) | 2 (5.9%) | 17.6101 ‡ | 0.0001 * |
| Autoimmune thyroiditis, *n* (%) | 6 (7.9%) | 2 (5.9%) | 0.1463 ‡ | 0.7072 |
| Arterial hypertension, *n* (%) | 16 (21.1%) | 4 (11.7%) | 0.8094 ‡ | 0.3683 |
| Diabetes mellitus, *n* (%) | 10 (13.2%) | 6 (17.6%) | 0.3808 ‡ | 0.5371 |
| Biliary lithiasis, *n* (%) | 2 (2.6%) | 2 (5.9%) | 0.7084 ‡ | 0.4179 |

♯ t-student test; § Mann–Whitney U Test; ‡ Yates, (*) marked effects are significant at $p < 0.05$.

We also identified higher values of HADS among patients with IBD, both at the Anxiety Subscore (HADS-A) and at the Depression Subscore (HADS-D). The differences between the IBD group and the control group reached the threshold of statistical significance ($p<0.01$) for both subscores (Table 1. VII). In the group with IBD the median and interquartile range for the HADS-D score were 8 (5;11) and 7(5;10) for the HADS-A (Table 1. VII).

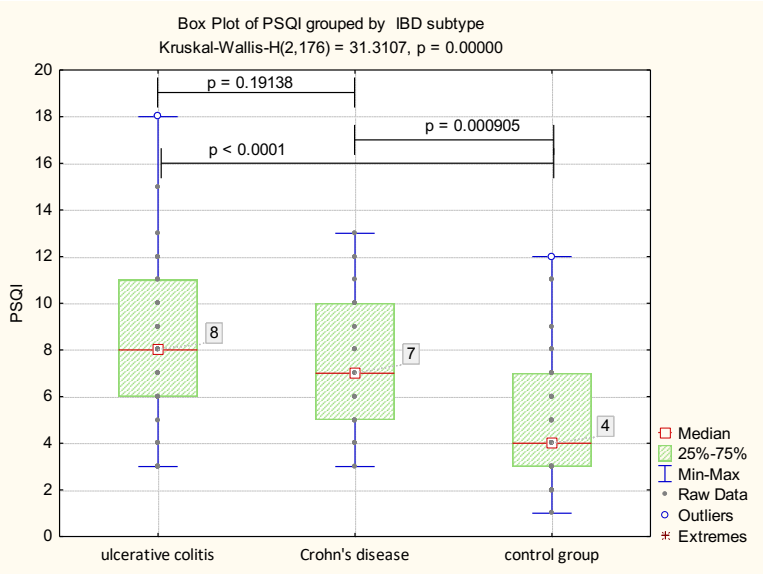



**Fig. 1.6.** Evaluation of global Pittsburgh Sleep Quality Index (PSQI) scores among the studied group

**Table 1.VII.** Descriptive statistics regarding sleep impairment and psychological distress in the study and control group.

| Characteristics | Study Group | | Control Group | Statistic Test | *p*-Value |
|---|---|---|---|---|---|
| | Ulcerative Colitis *n* = 76 | Crohn's Disease *n* = 34 | | | |
| **PSQI,** median (Q25;Q75) | 8 (5; 10) | | 4(3; 7) | 31.3107 ^ | <0.0001* |
| | 8 (6; 11) | 7(5; 10) | | | |
| **HADS-A,** median (Q25;Q75) | 7 (5; 10) | | 4 (2; 6) | 38.605 ^ | <0.0001* |
| | 8 (5; 10) | 7(6; 10) | | | |
| **HADS-D,** median (Q25;Q75) | 8 (5; 11) | | 5 (4; 7) | 10.1394 ^ | 0.0063 * |
| | 8 (5; 11) | 8(4; 10) | | | |

^ Kruskal–Wallis test, (*) marked effects are significant at $p < 0.05$.

*Influence of disease activity on sleep quality*

According to the results obtained, the activity of the disease significantly influenced the quality of sleep. Patients with active IBD in flare-ups had significantly higher PSQI scores (median -9) than patients in remission (median-7) (p<0.001). It is worth noting that, although patients in remission had significantly lower scores than those with active forms of disease, the PSQI score had values above normal (Figure 1.7). Subjects in the control group had normal values at PSQI score (median – 4).

We further studied the correlation between disease activity scores (Mayo for UC and CDAI for CD, respectively) and PSQI score. We identified a statistically significant correlation between PSQI score values and Mayo score (Spearman correlation coefficient = 0.411, p=0.0001), while the correlation was not statistically significant between PSQI score and CDAI score (Spearman correlation coefficient =0.004, p= 0.982) (Table 1.VIII ).

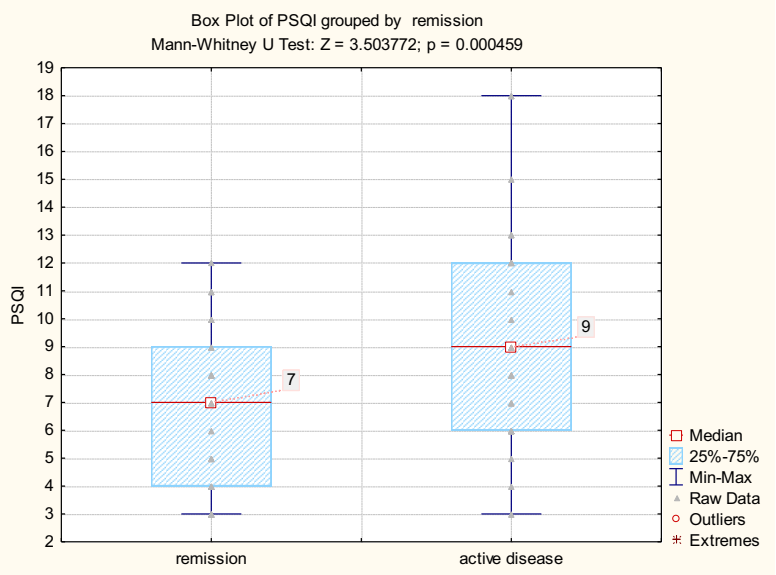



**Fig. 1.7.** The differences in PSQI value between remission and disease activity.

**Table 1.VIII.** The correlation between sleep quality and disease activity.

| PSQI Score vs. | Correlation Coefficient | *p*-Value |
|---|---|---|
| **Mayo** | 0.411212 | 0.0001 * |
| **CDAI** | 0.0040 | 0.982 |

Spearman Rank Order Correlations, (*) marked effects are significant at $p < 0.05$.

*Assessment of the link between sleep alteration and a range of clinical and biological parameters*

We aimed to analyze whether other parameters correlate with the PSQI score. We included in the analysis the age, presence and type of extra-intestinal manifestations, biological markers (hemoglobin, CRP, fibrinogen, fecal calprotectin, albumin). We identified statistically significant correlations between PSQI score and extraintestinal manifestations (p=0.0172), NLR, CRP/albumin ratio and inflammation markers: CRP (p=0.0013), fibrinogen (p=0.0029) and fecal calprotectin (p=0.036) (Table I.IX).

Age and presence of anemia (reflected by hemoglobin level) did not correlate with altered quality of sleep. In contrast, in the study group, we identified statistically significant correlations between PSQI score values and the presence of extraintestinal manifestations, markers of inflammation (CRP, fibrinogen, fecal calprotectin), NLR and CRP/albumin ratio. (Table 1. IX)

**Table 1.IX.** Correlations between PSQI score and clinical and biological parameters.

| PSQI Score vs. | Correlation Coefficient | *p*-Value |
|---|---|---|
| Age | 0.0399 | 0.6790 |
| Extraintestinal manifestations | 0.2754 | 0.0172 * |
| Hemoglobin | 0.0719 | 0.460 |
| Fecal calprotectin | 0.4772 | 0.03608 * |
| CRP | 0.2183 | 0.0013 * |
| Fb | 0.2073 | 0.0029 * |
| NLR | 0.2101 | 0.0280 * |
| CRP/Albumin | 0.3226 | 0.0010 * |

Spearman Rank Order Correlations, marked effects are significant at $p < 0.05$.

*Evaluation of the cut-off value for the parameters that correlated with the PSQI score*

For parameters that correlated with the PSQI score (CRP, NLR and CRP/albumin), we used the analysis of the ROC curve in order to determine cut-off values that have predictive potential for sleep disorders (PSQI score ≥5) in patients with IBD.

Following these analyses, we obtained for NLR the cut-off value 3.4 (AUC = 0.6612; 95% CI: 0.5519-0.7705, $p = 0.0197$), for the CRP/albumin ratio - cut-off value of 0.367 (AUC = 0.6806, 95% CI: 0.5576-0.8036; $p = 0.01038$) and for CRP - cut-off value of 1.13.

The comparative analysis of AUC for CRP, NLR and CRP/albumin ratio demonstrated a comparable predictive power of sleep disorders for the 3 parameters evaluated, with a slightly better value for CRP/albumin ratio (Figure 1.8).

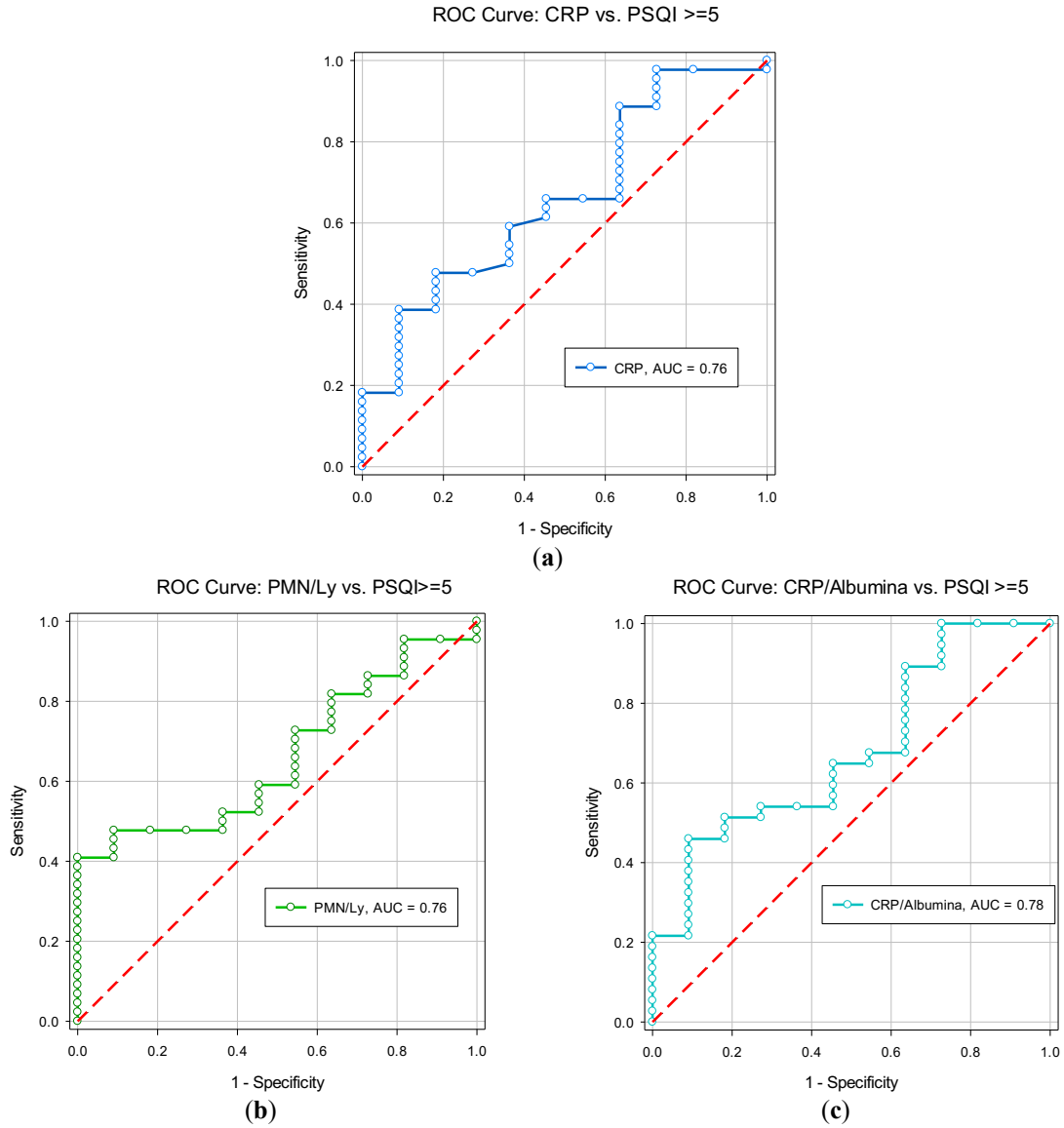



**Fig. 1.8.** ROC curves evaluating the prediction power of (**a**) CRP, (**b**) PMN/Ly ratio, (**c**) CRP/albumin ratio on PSQI values.

*Assessment of the link between altered quality of sleep and altered psychological status*

Another objective of the study was to assess the potential correlations between psychopathological changes (anxiety/depression) and sleep disorders. We identified a moderate, statistically significant correlation between the PSQI score and the HADS-A and HADS-D components in the HADS score (Table 1. X).

**Table 1.X.** Correlation between PSQI and Hospital Anxiety and Depression Scale (HADS) score in the inflammatory bowel disease (IBD) group. Univariate analysis.

| PSQI Score (PSQI Score ≥5) vs. | Correlation Coefficient | *p* -Value |
|---|---|---|
| HADS depression | 0.4157 | 0.00001* |
| HADS anxiety | 0.4062 | 0.00001* |

Spearman Rank Order Correlations, (*) marked effects are significant at $p < 0.05$

It should be pointed out that in the group of patients with IBD and high PSQI score (PSQI ≥5), we identified an important percentage of patients (69.1%) who, although they had sleep disorders, were not receiving psychotropic therapy.

*Highlighting the particular aspects related to components of the PSQI score*

We comparatively analyzed the values of each PSQI component subscore in the study group, by type of illness - UC and CD - and in the control group.

Statistical analysis using comparison tests appropriate to the type of data and their distribution showed that all components of the PSQI score contributed significantly to the alteration of sleep in patients with IBD compared to the control group, except for component 6 - use of sleep medication, which did not differ between the study group and the control group (p= 0.9004) (Table 1. XI ).

**Table 1.XI.** Evaluating sleep impairment through each PSQI component in the study and control group.

| | Study Group | | Control Group (*n* = 66) | Statistic Test | *p*–Value |
|---|---|---|---|---|---|
| | UC (*n* = 76) | CROHN (*n* = 34) | | | |
| PSQI—median (Q25;Q75) | 8 (5; 10) | 8(6; 10) | 4(3; 7) | 4(3; 7)^ | <0.0001* |
| Comp 1—subjective sleep quality, *n* (%) | 0/1/2/3 12/32/20/12 (13.2%/31.6%/ 39.5%/15.8%) | 0/1/2/3 0/26/6/2 (0%/76.5%/ 17.7%/5.9%) | 0/1/2/3 34/26/4/2 (51.5%/39.4%/ 6.1%/3.1%) | 36.4388 ‡ | <0.0001* |
| Comp 2—sleep latency, n (%) | 12/32/20/12 (15.8%/42.1%/ 26.3%/15.8%) | 10/6/16/2 (29.4%/17.7%/ 47.1%/5.9%) | 18/28/14/6 (27.3%/42.4%/ 21.2%/9.1%) | 15.7472 ‡ | 0.0151* |
| Comp 3—sleep duration, n (%) | 8/48/14/6 (10.5%/63.2%/ 18.4%/7.9%) | 6/22/2/4 (17.7%/64.7%/ 5.9%/11.8%) | 26/30/10/0 (39.4%/45.5%/ 15.2%/0%) | 14.2552 ‡ | 0.0269* |
| Comp 4—habitual sleep efficiency, n (%) | 20/32/10/14 (26.3%/42.2%/ 13.2%/18.4%) | 14/16/2/2 (41.2%/47.1%/ 5.9%/5.9%) | 52/10/4/0 (78.8%/15.2%/ 6.1%/0%) | 26.0609 ‡ | 0.0002* |
| Comp 5—sleep disturbances, n (%) | 4/60/12/0 (5.3%/78.9%/15.8%) | 4/28/2/0 (11.8%/82.4%/5.9%) | 12/42/12/0 (18.2%/63.6%/ 18.2%) | 9.8583 ‡ | 0.0401* |
| Wake up for urine emission (yes), *n* (%) | 72 (94.7%) | 30 (88.2%) | 50 (75.8%) | 11.1233 ‡ | 0.0038* |
| Wake up due to diarrhea (yes), *n* (%) | 24 (31.6%) | 8 (23.5%) | 0 (0%) | 24.489 ‡ | <0.001* |
| Wake up due to nightmares (yes), *n* (%) | 26 (34.2%) | 12 (35.3%) | 18 (27.3%) | 1.0184 ‡ | 0.5974 |
| Wake up due to pain (yes), *n* (%): | 34 (44.7%) | 12 (35.3%) | 16 (24.2%) | 6.5024 ‡ | 0.0365* |
| Abdominal pain, *n* (%) | 16 (21.05%) | 6 (17.65%) | 2 (3.03%) | -0.5518 ‡ | 0.0019* |
| Joint pain, *n* (%) | 16 (21.05%) | 6 (17.65%) | 12 (18.18%) | -0.0720 ‡ | 0.6579 |
| Headache, *n* (%) | 2 (2.63%) | 0 (0%) | 2 (3.03%) | 0.0485 ‡ | 0.0915* |
| Comp 6—use of sleep medication | 66/6/2/2 (86.8%/7.9%/ 2.6%/2.6%) | 28/4/0/2 (82.4%/11.7%/ 0%/5.9%) | 56/6/2/2 (84.9%/9.1%/ 3%/3%) | 2.1993 ‡ | 0.9004 |
| Comp 7—daytime dysfunction | 12/24/28/12 (15.8%/31.6%/ 36.8%/15.8%) | 0/18/8/8 (0%/52.9%/ 23.5%/23.5%) | 18/30/16/2 (27.3%/45.5%/ 24.2%/3.1%) | 24.1853 ‡ | 0.0004* |

^ Kruskal–Wallis test; ‡ Yates or Pearson chi-square test; (*) marked effects are significant at $p < 0.05$.

*Influence of medication on sleep quality*

In order to have a comprehensive picture of the potential factors involved in sleep disorders in patients with IBD, we have also studied the influence that IBD-specific therapy has on the PSQI score. The highest PSQI scores were recorded among patients receiving corticosteroid therapy with a median PSQI score of 11, with PSQI values recorded for this subgroup of patients being statistically significantly higher ($p<0.05$) than the score obtained in all other subgroups of patients, (Figure 1.9).

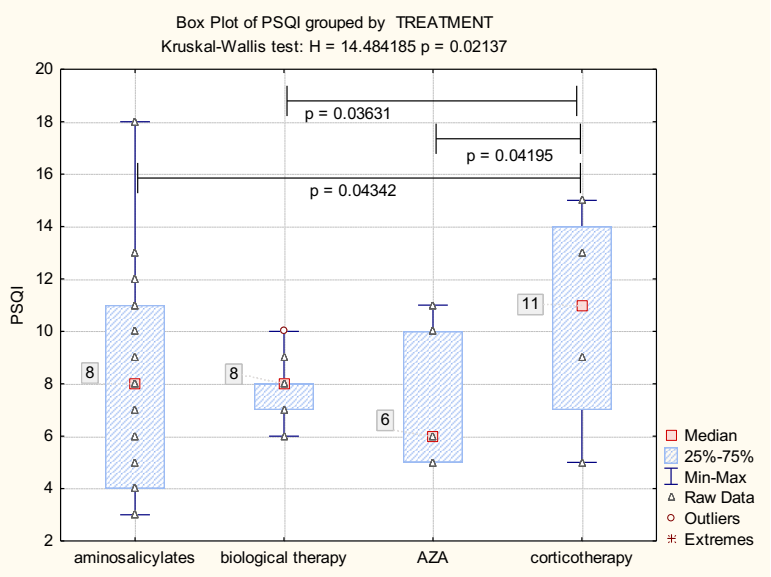



**Fig. 1.9.** The values of PSQI score among various treatment options.

## Discussion

The research presented, aimed at assessing sleep disorders in patients with IBD is, to our knowledge, one of the first studies that followed the presence and characterization of sleep disorders in patients with IBD. At the same time, we aimed to identify quantifiable parameters that would allow the implementation of criteria to recommend the screening of sleep disorders and psychopathological disorders

A first element revealed by our study is that the alteration in the quality of sleep is frequently present in IBD patients, compared to healthy subjects, a result similar to those of other studies recently reported in the literature. Specifically, we have identified the presence of sleep disorders in over two thirds of patients with IBD (70.9%), a percentage comparable to that reported in other studies. Compared to the control group (consisting of healthy subjects), we have identified significantly increased values of the PSQI score in patients with IBD, but without significant differences between the forms of IBD (CD and UC), aspect consistent with other reports in the literature (Sobolewska-Wlodarczy et al., 2018; Marinelli et al., 2020; Hood et al., 2018). At the same time, we also identified significant correlations between the PSQI score and all the inflammatory markers assessed (CRP, fibrinogen, fecal calprotectin). Our results are similar to those reported by Marinelli et al. (Marinelli et al., 2020)

The PSQI score was significantly higher in patients with active disease - median PSQI 9 (6;12) than in patients in remission, but it is worth noting that in patients with IBD in remission, the median PSQI score was above the upper limit of normal of 7 (4;9).

PSQI values in the group of patients with UC were increased proportionally to the Mayo activity score, so that increased PSQI values were found predominantly during disease flare.

In the group of CD patients studied, no statistically significant correlation of PSQI score values with CDAI score was identified, the presence of sleep disorders in this category of patients

being independent of disease activity. This result may reflect a distinct pathophysiology of sleep disorders between the two subtypes of the disease, a hypothesis also supported by a study that found that sleep disturbance increases the risk of flare-ups in patients with CD, but not in those with UC (Ananthakrishnan et al., 2013).

Also arguing that the pathophysiology of sleep disorders differs depending on the form of disease is the fact that the analysis of the items in the component of the PSQI score in patients with IBD has identified significant differences both compared to the control group and between disease subgroups. From the analysis on subcomponents of sleep disorder, we have identified that, in patients with IBD, an important contribution is the subjective perception and presence of nocturnal awakenings, while in the control group, subjects are dissatisfied with the habitual efficiency of sleep. In patients with UC in remission, nocturnal awakenings and reduced sleep duration make a major contribution. All these aspects support the contribution of psychological factors to the presence of sleep disorders in IBD, even independently of disease activity.

Another aspect found was that in IBD sleep disorders were correlated with the presence of extraintestinal manifestations, in particular with the joint manifestations, present in 5.88% of patients with CD and in 26.31% of patients with UC. This result could be attributed to the nocturnal joint pain component, occurring in the context of inflammatory arthropathies, which could cause frequent awakenings in this subgroup of patients. However, analyzing the specific reasons for nocturnal awakenings reported by patients in the IBD group, we did not identify statistically significant differences between the frequency of nocturnal awakenings due to arthralgia compared to the control group.

The relationship between fatigue and sleep quality, reflected by daytime dysfunction, is known. Since one cause of fatigue is anemia and, because in IBD anemia is frequently diagnosed, we followed up on whether anemia correlates with sleep disorders but did not identify statistically significant correlations. These results are consistent with results of other studies (Marinelli et al., 2020).

When considering the quality of sleep, an important aspect in the case of chronic diseases is related to treatment. Sleep disorders are frequently reported as adverse effects of medication. In this context, we analyzed the relationship between sleep disorders and medication specific to the digestive disorder.

In our group we found that the administration of corticotherapy was associated with sleep alteration. The PSQI values recorded in this subgroup were significantly higher than in patients receiving any other type of medication ($p<0.05$). For no other therapeutic class (aminosalicylates, azathioprine, biological therapy) there were no significant increases in PSQI scores compared to the control group. (Sofia et al., 2018; Lee et al., 2018).

This result is explainable, since corticosteroid treatment is intended for patients with moderately/severely active forms and symptoms of the disease (nocturnal diarrhea, abdominal pain) definitely contribute to sleep alteration. In addition, as previously mentioned, a systemic inflammatory syndrome has been shown to contribute to sleep alteration. In addition to the increased disease activity present in this subgroup of patients, sleep disorders may also be related to side effects of corticosteroids. This aspect is an additional argument for avoiding corticotherapy in the control of disease flares, by choosing strategies in which corticotherapy should be used for as short a period as possible. Among the therapeutic options in inducing remission without corticosteroidsis earlier initiation of biological therapy. Stevens et al. demonstrated that the use of anti-TNF therapy and vedolizumab could even lead to an improvement in sleep, depression and anxiety in patients with moderate / severe IBD, stressing that the effect is seen 6 weeks after initiation of treatment and is maintained up to 1 year (Stevens et al., 2017).

A particular issue identified in the study group was the low use of sleep medication (psychotropic medication, dietary supplements), which was found only among 13.16% of

patients with UC and 17.65% of patients with CD, despite a high prevalence of sleep disorders and psychopathological disorders. These findings are inconsistent with the results reported by other authors regarding the frequent use of sleeping pills in patients with IBD (Ranjbaran et al., 2007; Graff et al., 2006). In our study the use of this type of medication was not significantly different between the study group and the control group, although sleep disorders were significantly more common in the IBD group. Reduced use of medication for sleep disorders among patients with IBD is explainable both due to the lack of a routine assessment of the psychological status and quality of sleep among patients with IBD, and of a restraint of patients with IBD to report issues related to psychological impairment. The neglect by patients of psychological comorbidity may also be partly due to a fear of stigmatization, the more so as the age segment affected is in young adults, many of whom have difficulty adjusting to the burden of a chronic illness occurring at a young age, with an impact on the quality of life and productivity in the workplace.

Based on the fact that the prevalence of sleep disorders is increased in patients with IBD (Wilson et al., 2015; Sobolewska-Wlodarczyk et al., 2018; Marinelli et al., 2020) and taking into account the association between increased values of PSQI and values of markers reflecting systemic inflammation, we aimed to determine cut-off values with the potential to predict an impaired quality of sleep (PSQI ≥5) for CRP, NLR and CRP/albumin. By analyzing the ROC curve we obtained a cut-off value of 1.13 for CRP, of 3.4 for NLR and a cut-off value of 0.367 for the CRP/albumin ratio. For all 3 determined values, we calculated a high specificity (> 90%) of the determined cut-offs for these parameters.

**Conclusions**

Our study highlighted the impact of sleep disorders on the evolution of IBD patients, by identifying them both among patients with active disease and those in remission. Thus, we emphasized the opportunity to analyze the quality of sleep in these patients and to include it in the routine assessment of this category of patients.

It remains necessary to establish a therapeutic strategy dedicated to sleep disorder and psychological comorbiditytailored for IBD patients, including both sleep hygiene and cognitive-behavioral therapy and pharmacological therapy as proposed options. Further studies are needed to highlight the optimal method of assessing both psychological comorbidity and sleep disturbances, and targeting them therapeutically, so as to obtain the beneficial effect on disease activity. At the same time, it may be useful to assess these disorders as predictive factors for the subsequent occurrence of a disease flare and for an improved understanding of the intricate pathophysiology between psychological disorders such as anxiety and depression, sleep disorders and IBD activity.

Regarding the role of sleep disorders in IBD's etiopathogenesis, pharmacological therapy requires further studies, as does assessing the impact of this type of intervention on the subsequent activity of the disease. More research is also needed to assess whether sleep disturbance could be a prognostic factor for disease onset and to better understand the immunological background between sleep disturbance and IBD activity.

All these results justify the importance of integrating an assessment of psychological status in the monitoring plan of IBD patients. This is all the more important as the effective identification and management of associated psychological comorbidity can contribute to improving the control of IBD (Marinelli et al, 2020).-

*Originality and applicability of the results in medical practice*

Although these results require validation in subsequent studies, on larger groups of patients with IBD, these findings are promising, in terms of the potential for implementation in daily practice of assessing sleep disorders. Using a questionnaire such as PSQI is readily available in the clinical setting of assessing IBD patients and, could help identify those patients who require

screening in this regard. However, the usefulness of these parameters is limited for patients with systemic inflammatory syndrome, reflected by biological markers (such as CRP and fibrinogen), while for patients with IBD in remission, other solutions should be identified to screen for sleep disorders.

The integrative approach of patients with IBD should involve- in addition to treating inflammation and managing lifestyle issues- evaluation of stressors, which influence the quality of life and may impact the course of the disease.

The medical care of patients with IBD is predominantly provided in outpatient setting, where the time slot allocated for evaluation is limited. In this context, routine assessment of sleep disorders can be very difficult to implement (Qazi, Farraye, 2019). A management simplification could be achieved by introducing screening by questionnaires (e.g. PSQI) for patients with IBD who meet a certain biochemical profile. Patients could complete the questionnaire in the waiting room or it could be sent prior to the visit by email and returned completed at the time of the medical visit.

Such an approach can facilitate the management of patients with IBD both in terms of patient assessment and progress towards an integrative strategy.

Our study was the first of its kind conducted in Romania and among the first in Europe to assess both the sleep disorders and the psychological status of patients with IBD, compared to a control group consisting of healthy subjects. Also, at the time of starting our research, no other studies were reported to assess the predictive potential of CRP, NLR, CRP/albumin values for sleep disorders. Further studies confirming the usefulness of these parameters as viable benchmarks in investigating sleep disturbance among the comorbidities of IBD patients are needed.

*Limitations*

Our study has several limitations, which should be mentioned and which highlight the need for further research in this area. First, we conducted a study in a tertiary gastroenterology center, in which addressability is increased especially for more severe cases. However, patients in remission were also included in our study. Second, the use of a self-report, retrospective method to evaluate sleep quality, without an objective quantifiable method, could be a limitation. Nevertheless, studies investigating sleep disorders in patients with IBD, using both the PSQI questionnaire and polysomnography, have identified significant correlations between some of the parameters assessed in PSQI and polysomnography results, such as reported sleep duration and sleep efficiency (Keefer et al., 2006).

Third, the correlations between disease activity and PSQI were based on activity scores (Mayo and CDAI) and we did not include endoscopic assessments to evaluate disease severity (colonoscopy appearance and histology). However, we did include faecal calprotectin level in the assessment of disease activity, which is considered a good alternative for assessing disease activity.

*Future directions*

The use of objective measurements (such as polysomnography, actigraphy, electroencephalography) concurrently with sleep questionnaires to assess sleep quality and related daytime dysfunction could be a future direction to improve knowledge of sleep patterns in patients with IBD.

The use of psychotherapy, anxiolytics and antidepressants in IBD therapy should be established by large, randomized control trials. The impressive level of psycho-emotional manifestations and their perpetuation throughout the course of the disease seem to justify this at least in selected patients.

Well-designed prospective longitudinal studies clarifying the temporal link between psychological manifestations and inflammatory bowel diseases would also be useful.

### *1.2.2.3. The role of nutritional factors in the outcome of patients with IBD*

Malnutrition is another commonly identified issue in patients with IBD. Both protein-calorie malnutrition and micronutrient deficiency are caused by altered intake, impaired absorption, direct gastrointestinal loss or hypercatabolic state (Cho, Yang, 2018). Along with the poor absorption and loss in the intestine, one of the pathophysiological substrates of micronutrient deficiency in the inflammatory context is the inhibition of scaffold protein production in the liver, through the action of proinflammatory cytokines. It is proven that the inflammatory response generates the sequestration of microelements in the liver (Gîlcă-Blanariu et al., 2018).

Clinically, nutritional deficiencies are associated with the significant risk of an unfavorable outcome resulting in prolonged hospitalization (Nguyen et al., 2016), complicated perioperative course and higher mortality (Song et al., 2014).

While macronutrient deficiencies can be identified relatively easily in the baseline assessment, and can be subsequently corrected by diet and by supplementation, micronutrient deficiencies cannot be diagnosed and managed as easily.

The role and prevalence of micronutrient deficits is poorly assessed in routine practice. Apart from serum calcium, magnesium and iron levels, the remaining trace elements are not part of the usual assessement of IBD patients.

Micronutrients deficiency in IBD patients and implications in the inflammatory process

Given that the gut is an interface with luminal elements of external origin, immunological surveillance at this level is warranted, with a role in the adaptive immune response (Maloy, Powrie, 2011). Studies on animal models have shown that the chronic inflammatory process within IBD involves recruitment of neutrophils and macrophages, which contribute to oxidative stress, with the production of nitric oxide and superoxide in significant amounts (Zhu, Li, 2012). Other reactive oxygen species (xanthine oxidase, 5-lipoxygenase) are also known to be involved in the etiopathogenesis of IBD. Beyond the prooxidative enzymes, a depletion of elements with antioxidant role also occurs in IBD. Reduced antioxidant capacity is present not only during disease flares, but also during remission , which could suggest that decreased antioxidant capacity plays a role in triggering flares (Zhu, Li, 2012; Achitei et al., 2013).

Based on these aspects and taking into account the fact that a number of microelements such as selenium, zinc and copper are constituents of enzymes involved in oxidative stress, an important link in the inflammatory process, we will further focus on their analysis.

*Iron,* as mentioned, is one of the few trace elements routinely assessed. This is of utmost importance, as the role of iron deficiency and anemia in the evolution of IBD is well known.

The prevalence of anemia among IBD patients is estimated to be about 16% for patients with mild forms and as high as 68% for hospitalized patients. Although the pathogenesis of anemia in IBD patients is multifactorial, the main causes are inflammation, leading to intestinal blood loss and decreased iron absorption in the context of IBD (Vagianos et al., 2015). As a result, in addition to complete blood count, it is essential to assess iron deficiency, which is largely responsible for anemia in patients with IBD (Lopez et al., 2016). Moreover, iron deficiency can negatively influence the outcome of patients with IBD even in the absence of anemia. (Dignass et al., 2015).

*Zinc and Copper.* In the balance of oxidative status, zinc acts as coenzyme in key reactions of the immune response, having an antioxidant role. In activated macrophages, zinc inhibits iNOS activity by approximately 90%, preventing the production of reactive oxygen species and cellular dysfunction (Mohammadi et al., 2017). Among micronutrients, together with zinc, copper plays an important antioxidant role within copper-zinc superoxide dismutase, whose activity is documented to be reduced during IBD, leading to a reduction in free radical scavenging capacity; however, the causes of the decreased activity of this enzyme are questionable, and can be partly attributed to chronic inflammatory phenomena, but also to the zinc deficiency in this category of patients (Mohammadi et al., 2017; Seguí et al., 2004).

*Selenium.* Another micronutrient with potential to combat oxidative stress is selenium, especially due to its role in the composition of selenoproteins. Of these, the most studied for antioxidant potential is glutathione peroxidase (GPx) through its four isoforms, expressed in the gut (Papp et al., 2007). Selenoprotein P is another type of antioxidant selenoprotein, similar to GPx isoform 4 and responsible for transporting selenium to various tissues (Andoh et al., 2005). Selenoprotein S is expressed in the digestive tract in Paneth cells and intestinal macrophages, having as main roles the antioxidant action and the transport of selenium to other tissues, its activity being reduced in the context of IBD (Speckmann et al., 2014).
In this context, one of the research topics in the field of IBD addressed by our team focused on the analysis of micronutrients.

**Personal contributions**
Because inflammation may affect serum concentrations of micronutrients, serum levels may be of limited value in reflecting the state of trace elements in chronic inflammatory diseases such as IBD. On the other hand, the serum concentration of microelements such as selenium may be influenced by a number of external factors (e.g. administration of iodine or gadolinium-containing contrast agent). In addition, the determination of the serum concentration of a constituent reflects its level at a given point in time without being able to provide information on the long-term situation. (Cho, Yang, 2018).
The concentration of micronutrients in the skin appendages (nails or hairs) is less influenced by other biological parameters, such as those of inflammation, or by the influence of diet and also has the advantage of using a non-invasively collected sample. Moreover, compared to serum assessment, the determination of micronutrients in the appendages reflects their status over a longer period of time, while the serum concentration highlights the level of micronutrients strictly at the time of collection.
In this context, we aimed to determine the concentration of microelements in a biological product in which their concentration reflects as stable and faithful as possible a deficiency, respectively in the hair.
The study was initiated in collaboration with a research team from "Petru Poni" Institute of Macromolecular Chemistry in Iași.
Our goal was to investigate the concentration of some minerals (selenium, zinc, copper, iron, sulphur) in the hair in patients with IBD, compared to a control group and to investigate the possible correlations between the level of micronutrients and the parameters relevant to the activity of the disease.

**Patients and method**
We conducted an observational study on a group consisting of 42 patients diagnosed with IBD - ulcerative colitis (UC) (n = 25) and Crohn's disease (CD) (n = 17). The inclusion criteria for the study group were: confirmed diagnosis of IBD, age 18 to 70 years, and written consent of the patients. Disease activity was assessed by calculating the Crohn's disease activity index (CDAI) for patients with CD and Mayo score for UC. The control group consisted of healthy subjects (n = 37). Patients with associated infections, those using micronutrient supplements, those with a history of malignancies or metabolic disorders (obesity, dyslipidemia, thyroid dysfunction) were excluded. Also the use of therapeutic agents or hair cosmetics (medicinal shampoos that may interfere with the results of investigations) and restrictive diets in the last 6 months were exclusion criteria for both the study group and the control group.
For all subjects (in both the study group and the control group), the hair concentrations were determined for the following trace elements: iron (Fe%), magnesium (Mg%), calcium (Ca%), zinc (Zn%), copper (Cu%), manganese (Mn%), selenium (Se%) and sulphur (S%),
In addition, the serum albumin concentration, the inflammatory markers (C-reactive protein, complete blood count, with the calculation of the neutrophil-lymphocyte ratio -NLR) were

determined.
Colonoscopy was performed for all patients with IBD and entero-CT was added in cases where it was necessary to assess the extent of the disease.
*Microelement analysis of the hair.*
The determination of the concentration of microelements in the hair was carried out by the team of researchers from the "Petru Poni" Institute. Energy dispersive X-ray spectroscopy (EDX) was used for the analysis. The hair sample was taken from the occipital region and included a minimum of 10 strands for each subject. EDX analysis was performed at the root end of the hair after the samples were cleaned using the Hess procedure. The procedure consisted of placing the hairs in small pots with distilled water containing a drop of detergent and sonicating them for 5 minutes. The sample was then washed in distilled water, sonicated for 5 minutes in absolute acetone and allowed to dry (Hess et al.,1990, Coroaba et al., 2020).
After the cleaning process, the hair samples were examined with a Quanta 200 scanning electron microscope (SEM) at 30 kV in high vacuum mode. The EDX system mounted on Quanta 200 SEM was used to identify elements and perform quantitative analysis. Elemental analysis was performed on both the surface and cross-section of the hair specimens. No secondary treatment or processing was required.
*Statistical analysis.*
The statistical analysis was performed using SPSS v25.0 (SPSS, Inc, Chicago, IL). The comparisons between the analyzed groups were performed using Student's t-test, Kruskal-Wallis test or Chi-square test, depending on the homogeneity of the data series. For comparisons among 3 groups, the one-way ANOVA analysis was used. For evaluating various correlations of the studied parameters, Pearson correlation or Spearman correlation test was performed, depending on the distribution of data.
Ethical issues
The study protocol and all procedures included in the study were in accordance with ethical standards and the 1964 Declaration of Helsinki and subsequent amendments. The study was conducted after obtaining the ethical approval from the Ethics Committee of Grigore T Popa University of Medicine and Pharmacy, Iași (25.11.2018) and from 'St Spiridon' County Clinical Emergency Hospital Iași (No 45/04.09/2019).
The protocol used for the processing of biological samples subject to analysis by EDX, which were collected in accordance with the regulations of the European Directive EC No. 206, was approved by "Petru Poni" Scientific Council of the Institute of Macromolecular Chemistry. Upon inclusion in the study, informed consent was obtained from all patients included.

**Results**
37 patients with IBD (25 patients with ulcerative colitis and 12 with Crohn's disease) were included in the study and 31 healthy subjects were included in the control group. There were no statistically significant differences between patients in the study group and those in the control group in terms of demographic data, respectively age, gender and background.
The socio-demographic, clinical, biological, endoscopic and therapeutic indications for the studied subjects are shown in Table 1.XII.
*Analysis of the concentration of micronutrients in the hair*
We performed the comparative analysis of the concentration of chemical elements and in particular of the micronutrients between the study group (patients with IBD) and the control group.
Taking into account the possibility of a distinct pathophysiology of deficiencies in IBD patients, we performed the analysis of the concentration of microelements by subtype of disease and compared them to each other and to the control group. Analyzing the differences between patients with UC and those with CD, we identified concentrations with statistically significant differences by subtype of disease for manganese ($p=0.009$). There were no major

differences between carbon, nitrogen, oxygen, silicon, sulphur, zinc, calcium, magnesium, iron and selenium concentrations when analyzed by subtype of disease. Comparing the concentrations of the chemical elements between the study group and the control group, statistically significant differences between each subtype of disease (analyzed separately) and the control group were found for most of the elements analyzed.

**Table 1.XII.** Patient characteristics.

| **Patient characteristics** | **Study group n = 37** | | **Control group n=31** | ***p*- value** |
|---|---|---|---|---|
| | **UC (n=25)** | **CD (n=12)** | | |
| Age, median (Q25;Q75) | 43.5 (30; 59.5) | | 32 (29; 42) | .05§ |
| | 46 (32.5; 65.5) | 33 (27.5; 44.5) | | |
| | | | | |
| Sex (M/F), n(%) | 19/18 (51.4/48.6) | | 16/15 (51.6/48.4) | .981 ŧ |
| | 13/12 (52/48) | 6/6 (50/50) | | |
| | | | | |
| Urban vs. rural area (U/R), n(%) | 29/8 (78.4/21.6) | | 28/3 (90.3/9.7) | .286 ŧ |
| | 20/5 (80/20) | 9/3 (75/25) | | |
| | | | | |
| BMI (average ± SD) | 21.97 ± 1.5 | | 23.08 ± 2.2 | .259# |
| | 22.61 ± 1.95 | 21.97 ± 1.5 | | |
| Disease activity score | Mayo score 3(1;7) | CDAI score 146.5(52.5;276.5) | | |
| Active disease, n (%) | 22 (59.5%) | | NA | NA |
| | 16 (64%*) | 6 (50%*) | NA | .65 ŧ |
| | | | | |
| Disease location* | Proctitis 6(24%)<br>Left-sided colitis 13 (52%)<br>Pancolitis 6(24%) | Ileum (L1) 2(16.6%)<br>Colonic (L2) 7 (58.3%)<br>Ileocolic (L3) 3 (25%) | NA | |
| **Biological parameters** | | | | |
| Hemoglobin, Median (Q25;Q75) | 13.2 (11.7; 14.3) | 14.6 (13.4-16.4) | 3.85# | **<0.001*** |
| | 13 (11.5; 14.2) / 13.7 (12.57; 14.57) | 14.6 (13.4-16.4) | 8.19 A | **0.01*** |
| Seric iron level median (Q25;Q75) | 51 (19; 82.5) | 100 (56-116) | 4.14# | **<0.001*** |
| | 47 (17.5; 81.5) / 100 (56-116) | 100 (56-116) | 8.93 A | **<0.001*** |
| CRP median (Q25;Q75) | 1.16 (0.2; 2,41) | 0.23 (0.21-0.32) | 10.778 § | **0.001*** |
| | 1.16 (0.13; 2.41) / 0.23 (0.21-0.32) | 0.23 (0.21-0.32) | 7.716 W | **0.001*** |
| Albumin (medie ± DS) | 4.08 ± 0.45 | 5.72 ± 0.63 | 1.43# | 0.158 |
| | 4.1 ± 0.44 / 4.1 ± 0.44 | 5.72 ± 0.63 | 1.007 A | 0.371 |
| Fibrinogen (medie ± DS) | 422.17 ± 59.32 | 301.94 ± 61.4 | - 8.084# | **<0.001*** |
| | 414.52 ± 61.24 / 414.52 ± 61.24 | 301.94 ± 61.4 | 33.254A | **<0.001*** |
| **Treatment** | | | | |
| Treatment followed* | aminosalycilates 18 (72%)<br>azathioprine 1 (4%)<br>biological therapy 4 (16%)<br>first diagnosis-no prior treatment 2 (8%) | azathioprine 6 (50%)<br>biological therapy 4 (33.3%)<br>first diagnosis-no prior treatment 2 (16.7%) | NA | |

*percentage according to disease subtype, § Kruskal-Wallis test, ŧ Chi-square test, #-ANOVA.

For the concentration of magnesium (MgK Wt%) in the hair, we identified statistically significantly lower values in the group of patients with CD compared to the control group ($p<0.001$). The MgK Wt% values were lower in the UC group than in the control group, they reached the threshold of statistical significance ($p=0.005$). There were no statistically significant differences between the CD and the UC groups on this element ($p=0.09$) (Figure 1.10).

The values of the sulphur concentration in the hair (SK Wt%) were statistically significantly higher in the groups of patients with IBD (both CD and UC) compared to the control group ($p<0.001$), but there were no statistically significant differences between the values of SK Wt% between the two subtypes of the disease (CD versus UC) ($p = 0.967$) (Figure 1.11).

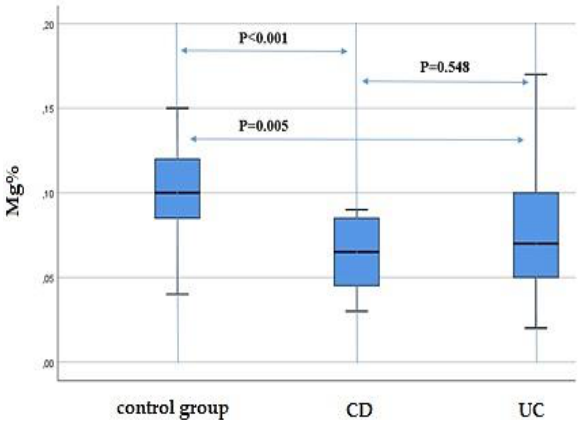



**Fig. 1.10** Evaluation of hair magnesium concentration (Mg%) between IBD subtypes (UC and CD) and control group

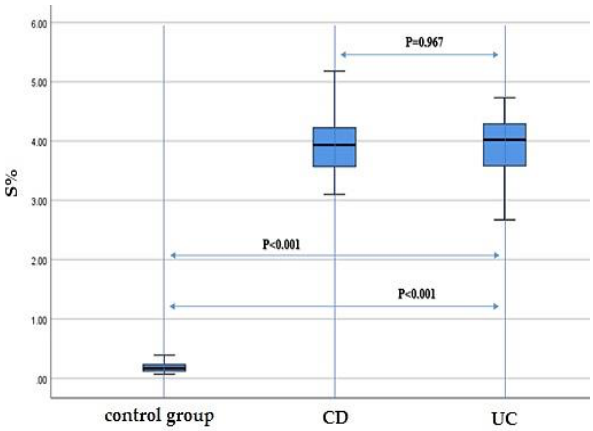



**Fig. 1.11.** Evaluation of hair sulfur concentration (S%) between IBD subtypes (UC and CD) and control group

By analyzing the concentration of calcium in the hair (CaK Wt%), we identified statistically significantly lower values in both the group of patients with UC and in the group of patients with CD compared to the control group ($p<0.001$), but without identifying statistically significant differences between the two subtypes of the disease ($p=0.185$) (Figure 1.12).
For manganese (MnK Wt%), the concentration in the hair had statistically significantly higher values in patients with UC, both compared to the control group ($p=0.003$) and compared to patients with CD ($p=0.009$). No statistically significant differences ($p=0.81$) were found between the MnK Wt% values in the group of patients with CD and the control group (Figure 1.13).

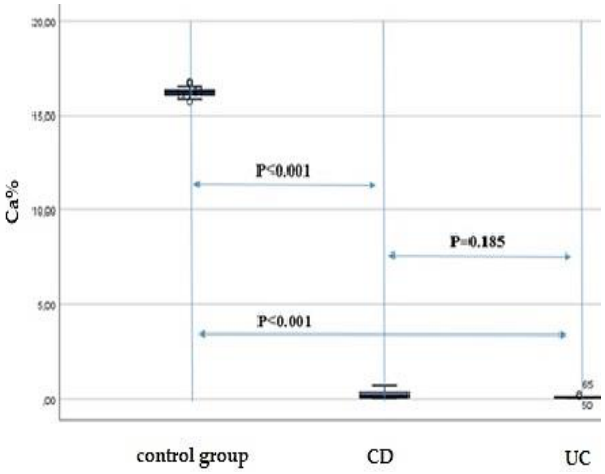



**Fig. 1.12.** Evaluation of hair calcium concentration (Ca%) between IBD subtypes (UC and CD) and control group.

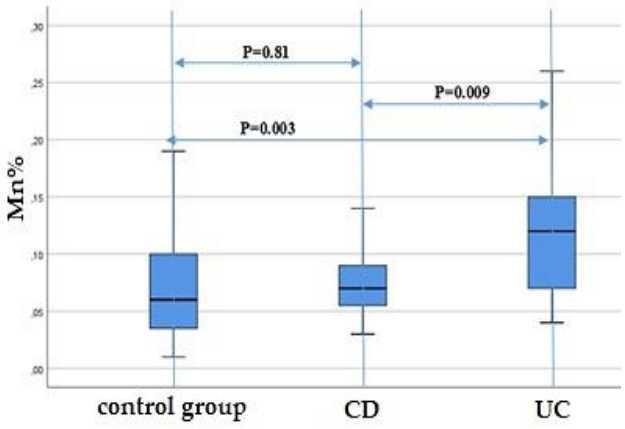



**Fig. 1.13.** Evaluation of hair manganese concentration (Mn%) between IBD subtypes (UC and CD) and control group.

By analyzing the iron concentration in the hair (FeK Wt%), we identified lower values of iron concentration in both subtypes of the disease (CD/UC) compared to the control group, both recording differences that reached the threshold of statistical significance ($p<0.001$). No

statistically significant differences in FeK Wt% (p= 0.716) were found between the CD and UC groups (Figure 1.14).
The value of copper in the hair (CuK Wt%) was statistically significantly lower in both the group of patients with CD (p=0.015) and in the group with UC compared to the control group, but without statistical significance (p=0.106) while its values were not significantly different between the two subtypes of patients with IBD (CD versus UC) (p=0.462) (Figure 1.15).

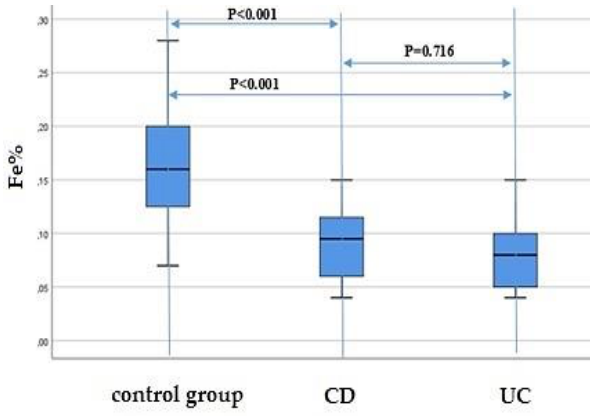



**Fig. 1.14.** Evaluation of hair iron concentration (Fe%) between IBD subtypes (UC and CD) and control group.

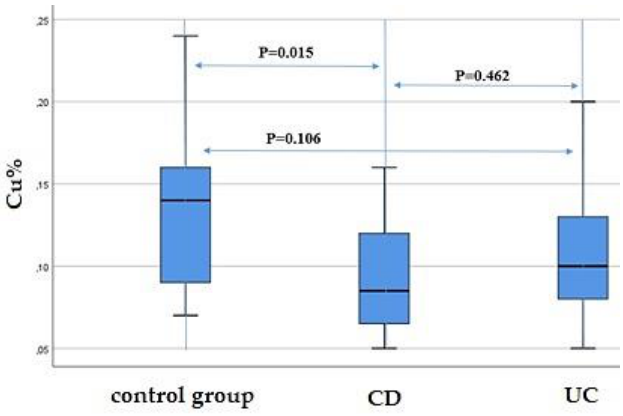



**Fig. 1.15.** Evaluation of hair copper concentration (Cu%) between IBD subtypes (UC and CD) and control group.

As far as zinc concentration in hair is concerned, there were no statistically significant differences between IBD patients and control group (p=0.697 and p=0.832), although a slightly lower median hair zinc concentration was identified for UC and CD patients compared to control group (Figure 1.16).
Hair selenium concentration was decreased both among UC and CD patients compared to the control group (p=0.002 and p<0.001, respectively). However, there was no statistically significant difference between selenium hair concentration between UC and CD patients (p=0.952) (Figure 1.17).

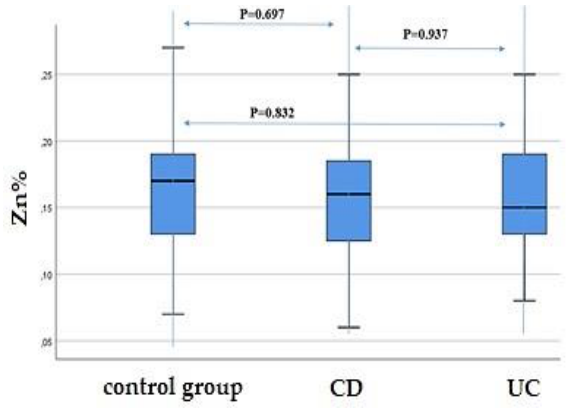



**Fig. 1.16** Evaluation of hair zinc concentration (Zn%) between IBD subtypes (UC and CD) and control group.

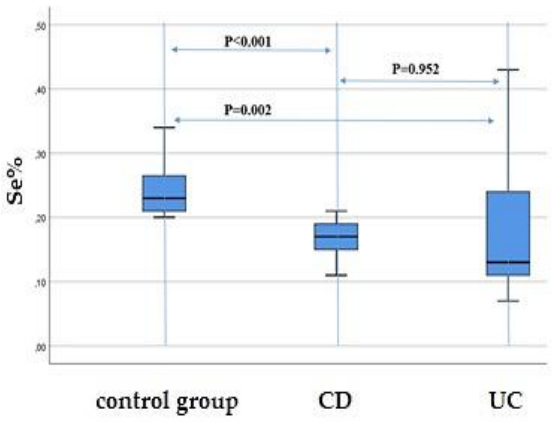



**Fig. 1.17.** Evaluation of hair selenium concentration (Se%) between IBD subtypes (UC and CD) and control group.

*Clinical and biological correlations between the activity of the disease and the concentration of microelements in the hair in patients with IBD*
We analyzed the correlations between the concentration of various minerals and microelements and the IBD activity, represented by Mayo and CDAI activity scores, respectively. We

identified a trend towards negative correlation, with statistically significant values between the value of the calcium concentration and the activity of the disease (p<0.001) Crohn's disease but not for ulcerative colitis. Also for Crohn's disease, we found a strong positive correlation between sulphur concentration and disease activity, without identifying correlations for ulcerative colitis. (Table 1. XIII).

**Table 1.XIII.** Evaluating the correlations between mineral and trace elements and disease activity.

| Evaluated element | CD disease activity | | UC disease activity | |
|---|---|---|---|---|
| | Correlation coefficient | *p*-value | Correlation coefficient | *p*-value |
| Mg%╥ | -.147 | .649 | .186 | .375 |
| S%^ | **.585** | **.046*** | .112 | .594 |
| Ca%╥ | **-.772** | **.003*** | -.058 | .782 |
| Mn%╥ | -.269 | .398 | .133 | .525 |
| Fet%╥ | .315 | .319 | .006 | .978 |
| Cu%╥ | -.024 | .940 | .052 | .804 |
| Zn%^ | .696 | .126 | .009 | .966 |
| Se%╥ | .269 | .398 | -.006 | .978 |

^Pearson correlation test, ╥ Spearman correlation test; *marked results are significant for p<0.05.

By analyzing the potential correlations between the concentration of the chemical elements in the hair and the value of the biological parameters reflecting the systemic inflammation, we did not identify statistically significant correlations (Table 1.XIV).

**Table 1.XIV.** Evaluating the correlation between hair micronutrient concentration and inflammatory markers- IBD patients.

| Evaluated element | CRP | | CRP/ALB | | NLR | | Fibrinogen | |
|---|---|---|---|---|---|---|---|---|
| | Correlation coefficient | *p*- value | Correlation coefficient | *p*- value | Correlation coefficient | *p*- value | Correlation coefficient | *p*- value |
| Mg%╥ | -.284 | .088 | -.257 | .125 | -.261 | .119 | -.288 | .094 |
| S% ^ | .135 | .425 | .145 | .391 | .171 | .311 | .206 | .236 |
| Ca%╥ | -.297 | .074 | -.301 | .07 | -.266 | .112 | -.119 | .497 |
| Mn%╥ | .185 | .274 | .161 | .342 | .112 | .511 | -.255 | .139 |
| Fe%╥ | -.133 | .432 | -.122 | .474 | -.003 | .987 | -.154 | .379 |
| Cu%╥ | -.110 | .516 | -.130 | .442 | .016 | .987 | -.279 | .105 |
| Zn% ^ | -.061 | .722 | -.06 | .725 | .063 | .713 | -.094 | .589 |
| Se%╥ | .144 | .396 | -.043 | .799 | .059 | .728 | .189 | .277 |

^Pearson correlation test, ╥ Spearman correlation test, CRP/ALB= CRP to albumin ratio, NLR= neutrophil-to-lymphocyte ratio.

When studying the correlation between each type of micronutrients and BMI, we did not find any statistically significant correlation between the hair concentration of any of the studied micronutrients and BMI, neither in the study group, nor in the control group (Table 1.XV).

**Table 1.XV.** Evaluating the correlation between type of micronutrients and BMI.

| Evaluated element | UC patients | | CD patients | | control group | |
|---|---|---|---|---|---|---|
| | Correlation coefficient | *p*-value | Correlation coefficient | *p*-value | Correlation coefficient | *p*-value |
| Mg%╥ | -0.002 | 0.993 | -0.099 | 0.759 | 0.143 | 0.442 |
| S%^ | -0.01 | 0.962 | 0.333 | 0.290 | -0.2 | 0.917 |
| Ca%╥ | 0.088 | 0.675 | -0.147 | 0.649 | -0.083 | 0.657 |
| Mn%╥ | 0.113 | 0.592 | 0.563 | 0.057 | -0.006 | 0.976 |
| Fe%╥ | 0.023 | 0.911 | -0.011 | 0.974 | -0.268 | 0.144 |
| Cu%╥ | 0.091 | 0.665 | 0.204 | 0.526 | 0.225 | 0.220 |
| Zn%^ | -0.087 | 0.678 | 0.409 | 0.187 | -0.198 | 0.285 |
| Se%╥ | -0.272 | 0.189 | 0.309 | 0.329 | 0.077 | 0.680 |

^Pearson correlation test, ╥ Spearman correlation test.

**Discussions**

The alteration of nutritional factors among IBD patients might be perceived at a first glance as related to macronutrient deficit, considering the importance of the altered protein status, especially hypoalbuminemia on IBD evolution and complications (Nguyen et al.,2008; Ghoneima et al., 2019]) Beyond protein malnutrition, micronutrient deficits, including trace elements, should also be considered, since this issue might affect up to half of the patients with IBD (Weisshof, Chermesh, 2019). Although this aspect might be of higher impact at the beginning of disease course, especially for patients with longer diagnostic delay, various nutritional deficiencies might appear, persist or worsen throughout the disease course, due to poor intake, impaired absorption (Cho, Yang, 2018)

The most frequently used method for evaluating trace elements is measuring serum concentration, considering it is an accessible method (Yakut et al., 2010; Hwang et al., 2012). Since there is prior evidence that inflammation may alter the serum micronutrient concentrations, the use of serum levels is limited in reflecting body nutrient status in chronic inflammatory diseases such as IBD (Galloway et al., 2000; Duncan et al., 2012; Cho, Yang, 2018).Considering these aspects, an alternative to be considered for evaluating trace elements status is measuring hair concentration. The approach of measurement of multiple elements in the scalp hair is being applied for several diseases (Wołowiec et al., 2013), ranging from diabetes (Hotta et al., 2018) to autoimmune disorders. There is evidence for the usefulness of trace element evaluation in patients with psoriasis (Seneczko, 2004) and alopecia areata (Coroaba et al., 2020). There have also been some isolated studies using hair samples for measuring trace elements concentration in patients in specific settings (Ogasawara et al., 2020). Taking into account the previously mentioned aspects, we opted for mineral and trace elements evaluation by measuring hair concentration using SEM and EDX.

One patient characteristic which might influence hair concentration of various elements is age. Among the IBD patients included in our study, the UC patients had a higher age (in average almost 10 years higher), compared to the control group and CD patients, although the statistical significance of this difference reached borderline significance. The absence of a significant age difference between CD patients and control group, together with registering significantly lower hair calcium concentration among CD patients compared to the control group might be an indicator of calcium reduction in young CD patients. In what concerns both calcium and magnesium concentrations, one study analyzing the age dependence of various elements concentrations highlighted higher calcium and magnesium concentrations in younger subjects (those under 25 years old). Consequently, the authors stated that there may be an age dependence of the concentration of these trace elements, but this is difficult to demonstrate regarding scalp hair concentration, if the number of samples collected per age group is not large enough (Dignass et al., 2015) As far as the concentration of other studied elements is concerned, the same study showed that sulfur, zinc and copper concentrations do not significantly vary with age (Dignass et al., 2015).

We identified lower hair concentrations for several minerals and trace elements between IBD patients and healthy controls, namely lower iron, magnesium, calcium, and selenium, with statistically significant differences between patients and control group. Our results are consistent with a previous study including pediatric IBD patients and measuring both serum and hair concentration of trace elements (Cho, Yang, 2018). In the study we performed, there were no statistically significant differences in the hair concentration of these four elements between patients with UC compared to CD patients. While the hair iron deficiency might not be of clinical significance, considering the extensive research already undergone related to the importance of righteous management of low serum iron

level and presence anemia in IBD patients, with practical guidelines established (Niepel et al., 2018; de Baaij et al., 2015), there is still a need for better understanding of other micronutrients' status and management. The magnesium deficit is of important clinical practice and requires prompt diagnosis and management, considering its pivotal role in cell signaling, genomic stability, DNA repair processes (Stritt et al., 2016; Workinger et al., 2018; Kruis, Phuong, 2016), but also due to its influence on other minerals such as the transport of calcium (Kruis, Phuong, 2016). Beyond the typical clinical picture of low magnesium levels, such as muscle cramps, arrhythmia, impaired tissue repair, the importance of magnesium deficit is also reflected in accentuating depression and enhancing fatigue among IBD patients (Christakos et al., 2011). Therefore, magnesium supplementation could contribute to improving IBD evolution for patients with deficit of this mineral, but further evidence is needed in order to generate specific dosing, time of supplementation and optimum monitoring of magnesium status in IBD patients.

Another mineral which was deficient among the studied IBD group was calcium, which was also negatively correlated with disease activity for CD patients. These findings suggest the presence of a calcium deficit in CD patients, especially during disease flare. Calcium deficiency is pathophysiologically well-founded, considering that calcium is mainly absorbed in the ileum (Ghishan, Kiela, 2010) and that almost 40% of the CD patients included in our study did have ileal involvement. This underlines the importance of monitoring calcium status in IBD patients, especially among CD patients and particularly during disease flare, when the presence of ileal inflammation impairs calcium absorption, which will mainly take place through transcelullar active transport (Huybers et al., 2008) in this setting. Beyond the impairment of calcium absorption and the negative influence on vitamin D status, data is less extensive regarding the role of acute on chronic inflammation over the calcium homeostasis at both renal and intestinal level in IBD patients. One study using a mouse model of CD ileitis with a deletion in the tumor necrosis factor (TNF) AU-rich elements (ARE), characterized by elevated TNF-alpha levels identified that in spite of maintaining a normal calcium level, ileitis with increased TNF-alpha expression lead to a disturbed calcium homeostasis. This was characterized by reduced duodenal and renal calcium transporters, diminished 1,25(OH)2D3 levels, and increased bone resorption associated with profound bone abnormalities (Zeng, 2009). These findings might suggest that normal serum calcium level does not necessarily reflect normal calcium metabolism in IBD patients; therefore, measuring hair calcium concentration might represent an option for better reflecting calcium status in this setting, in order to ensure an optimized monitoring.

Selenium represents an important trace element in modulating the anti-inflammatory and antioxidant response, considering that it is part of glutathione-peroxidase and thioredoxin reductase. Moreover, the selenium deficiency can negatively influence DNA repair and cell cycle regulation (Bera et al., 2012; Avery, Hoffmann, 2018; Geerling et al., 2000). We identified statistically significant lower selenium concentrations for both UC and CD patients compared to the control group, results which are in accordance with other study evaluating selenium deficit in IBD patients compared to healthy controls, referring to serum concentration (Lomer et al., 2019). However, these results are opposed to those reported by Cho and Yang, who did not identify statistically significant lower selenium concentration for IBD patients compared to healthy controls, neither when evaluating serum level, nor when evaluating hair concentration of this trace element (Cho, Yang, 2018).

Since there are conflicting results for evaluating selenium status in IBD patients, considering various methods used to determine its concentration, but also considering the

multiple potential causes of variation of its concentration, it is difficult to underline an optimum approach for evaluating selenium status in this patient category.

The common encounter of zinc deficiency reported in patients with IBD (15 to 40% of the patient population) (Ohashi et al., 2019**)** is not surprising, considering the chronic diarrhea and malabsorbtion during disease flares and the key role zinc plays in the maintenance of intestinal barrier integrity (Vagianos et al., 2007). On the other hand, zinc deficiency has also been reported for patients in remission, reaching up to one third of patients (Alkhouri et al., 2013; Jin et al., 2019). While zinc is generally accounted to play an important role in fighting oxidative stress, it is also important for keratinocytes proliferation in hair ( Siva et al., 2017). These roles could explain the presence of zinc deficiency among IBD patients with long standing disease course. In addition, a study including IBD patients highlighted that low serum zinc concentration might be associated with poor disease outcome, reflected by increased rate of complication, increased need for hospitalization and surgery 90 (MacMaster et al., 2012)

However, in our study, we did not identify statistically significant differences between hair zinc concentration between IBD patients and the control group. This particular finding in spite of the important involvement of zinc deficiency in oxidative stress and intestinal permeability might be partly related to the low number of patients included in the study. Our results are consistent with the ones reported by Cho and Yang, who did not identify significant differences between hair zinc concentration between IBD and controls, although when evaluating serum concentrations of this trace elements, the differences were significant and pointed towards a zinc deficit among IBD patients (Cho, Yang, 2018). Corroborating these findings, we might presume that there is a relative serum zinc deficiency related to the inflammatory process, without affecting the zinc deposits, such as the zinc incorporated in the hair, therefore potentially overestimating the degree of zinc deficiency in this patient category. Still, lower serum zinc level might be useful in predicting increase of disease activity, considering that one recent study investigating micronutrient deficiency on CD outcome identified a significant association between low serum zinc concentration and shorter time until disease flare for patients who were in remission at the moment of inclusion in the study (Kempson et al., 2021). Consequently, there is a need for further prospective studies evaluating both serum and hair zinc concentration in order to identify the best way to integrate these findings in the clinical setting.

Hair copper concentration was lower for IBD patients compared to healthy controls in our study, but the difference reached the level of statistical significance only for CD patients, compared to the control group. This finding might be explained by the impaired copper absorption among CD patients with ileal involvement. Moreover, taking into account previous research highlighting that copper incorporated from endogenous sources is securely bound within the hair, without being lost upon exposure to the environment (Horning et al., 2015), we can assume that low copper concentration in the hair of CD patients might reflect a true deficit in this context.

With regard to manganese status, we identified a statistically significant higher concentration of this trace element among UC patients, but not in CD patients, compared to the control group. There was no statistically significant correlation between hair manganese concentration and parameters reflecting inflammation and disease activity scores, neither for UC nor for CD patients in the study group. These findings might be due to the low number of patients included and further studies involving larger patient group could offer better insight regarding the status of manganese among IBD patients. More data on manganese status among IBD patients could be useful, considering that currently

manganese is a less studied trace element for this patient category, although it has an important antioxidant role, as part of manganese-superoxide dismutase and also as a cofactor of xantinoxidase, arginase, pyruvate decarboxylase and glutaminsynthetase (Choi et al., 2020). A study on mouse model highlighted that manganese deficiency exacerbates intestinal injury and inflammation, promoting increased intestinal permeability, through alteration of tight junction expression (Trumbo et al., 2001). These data together with the identification of manganese deficiency in a study including pediatric patients recently diagnosed with IBD (Cho, Yang, 2018) support the opportunity to further investigate the role of this trace element in the pathogenesis of IBD. Furthermore, it is also important to find the optimum method to assess patients' nutritional manganese status, especially for making recommendations regarding dietary habits to those at a high risk of IBD. Currently, no formal recommended dietary allowance for manganese is available for IBD patients, where the need for this trace element might be higher than in healthy individuals, for whom the Institute of Medicine's Dietary Reference Intake cites ~2 mg manganese per day as adequate for adults (Malepfane, Muchaonyerwa, 2017).

Another chemical element for which we found increased hair concentration in IBD patients compared to healthy control is sulfur. Since the main hair structural component is keratin, which includes disulfide bonds (Ogawa, 2018) we can presume a change in hair incorporation of sulfur among IBD patients. On the other hand, increased sulfur concentration in the hair of IBD patients might be attributed to inflammation, since in the studied group we identified a statistically significant correlation between sulfur concentration and CD activity. Moreover, increased hair sulfur concentration can be related to the consequences of oxidative stress, namely the increased expression of metallothioneins, a type of cysteine-rich Zn/Cu-binding proteins, which is amplified at keratinocyte level (Coroaba et al., 2020; Seguí et al., 2004).

The oxidative stress in the context of IBD might be related to the deficit of copper we identified among IBD patients compared to the control group, considering that copper is part of the copper-zinc superoxide-dismutase. This activity of this enzyme has been previously reported to be reduced in IBD patients, although the mechanism of this decreased activity is very intricate and involves both chronic inflammation and zinc deficit (Mohammadi et al., 2017; Ojuawo, Keith, 2002) As far as other reported results on copper status in IBD patients is concerned, there are conflicting data on comparing copper concentration in IBD patients to healthy controls (Malavolta et al., 2015)

However, there are several factors which might be incriminated in influencing the micronutrient status and hair composition, such as diet, nutritional status or the time of disease evolution. To avoid significant influence of general nutritional status on micronutrient concentration, we have studied the correlation between BMI and the concentration of the studied micronutrients and found no statistically significant correlation, neither in the control group, nor in the study group. Since the groups did not differ with a statistical significance with regard to BMI and this parameter was within comparable ranges among the studied groups, it is difficult to shape further conclusions with regard to the influence of BMI on the status of hair micronutrients.

*Originality, and applicability of study results in the management of IBD*

The identification of some nutritional deficiencies, including vitamin deficiencies (which have not been evaluated in our study) and their influence on the evolution of patients may constitute arguments for the need of specific dietary recommendations in patients with IBD, possibly supplementing some minerals and microelements. This type of supplementation together with an adequate pharmacological treatment may contribute to an integrative and thorough management of the patients with IBD.

Arguments for dietary supplementation with some microelements are also found in some studies in the literature. For example, based on the impact of zinc deficiency on oxidative stress, zinc supplementation has been considered necessary for patients with IBD. (Li et al., 2017). Regarding selenium deficiency which is also involved in oxidative stress in IBD, it has not been extensively investigated clinically but, reviewing the available data from animal models, a benefit of selenium supplementation in IBD has been hypothesized (Dubuquoy et al., 2006).

However, given that we did not find in the literature a reference range for the normal values of the microelements in the hair, the clinical applicability of the results of this study at the present time is limited. Consequently, further studies are needed to make progress in standardizing the method, but also to validate the method, respectively to establish cut-offs for each microelement, so that this method becomes potentially diagnostic.

*Limitations of the study*

The study we conducted was the first of its kind conducted in patients with IBD in Romania and among the few available at European level. Although the evaluation of the microelements in the skin appendages has begun to raise scientific interest in chronic inflammatory pathology, especially autoimmune, predominantly in rheumatological pathology, this method is not yet standardized and consequently is not widely used.

The fact that a series of biological parameters reflecting the activity of the disease have been assessed simultaneously, the hair microelement determination is an advantage, as it is possible to study correlations in the research undertaken.

Also, another original element of our study is the fact that this research included the evaluation of a large panel of microelements, some of them little or not studied for patients with IBD to date, such as copper and manganese.

On the other hand, in any research there are a number of difficulties and limitations.

A limitation is represented by the fact that the quantification of the microelements was carried out only at the level of the skin appendages, without a concomitant assessment of their concentration in the serum, in order to be able to benchmark and highlight possible agreement or differences between assessments. It would have been useful to assess the values of fecal calprotectin as reflecting the inflammation at intestinal level, so that in the study of the correlation between the concentration of microelements and the inflammatory status, there is a more specific method that reflects the association with inflammation in the intestine.

*Future directions*

This research opens up multiple perspectives of study. These include the opportunity to further investigate the correlations between the deficiencies of microelements and the type of medication followed by the patient, respectively with the history of medication, given the potential influence of azathioprine in particular on the metabolism of some of the microelements, but also the influence that biological therapy can have through the immunological changes it induces on the concentration of microelements. Another element of interest would be the study of the correlation between possible deficiencies of microelements and the presence of extraintestinal manifestations in patients with IBD, respectively the presence of comorbidities, given the pleiotropism of the action of some of these microelements and their involvement in the etiopathogenesis of various extradigestive pathologies, especially considering the roles of selenium, zinc and manganese. Also, a potential predictive value of the deficits of some of the microelements on the evolution of IBD patients can be followed, by subsequent monitoring of the evolution of the disease, using activity scores and biological parameters.

### *1.2.2.4. Predictive value of some clinical-biological markers on the course of inflammatory bowel disease*

An essential step in the management of inflammatory bowel diseases is the monitoring of patients. Clinical, biological and endoscopic criteria are available for this purpose and are included in disease activity scores, the most used being the Mayo score for UC and CDAI for CD, respectively.

The concept of non-invasive monitoring is developing, and markers of systemic inflammation such as C-reactive protein (CRP), fibrinogen, erythrocyte sedimentation rate (ESR), but also markers of intestinal inflammation (fecal calprotectin) are routinely used. However, these parameters provide information about the degree of disease activity at a given point in time (Sandborn et al., 2001). Although a series of tools have been developed to assess the severity of a flare, including clinical, history, biological and endoscopic parameters, with their summation in activity scores, these do not reflect the long-term evolution of the disease either. The presence of a severe IBD flare is not a certainty of severe colitis and, on the other hand, a less severe onset of the disease does not guarantee a benign evolution of the inflammatory process in the long term.

Identifying some predictors of the adverse mid/long term progression of IBD would be extremely useful, as it would facilitate the adaptation of therapy and would indicate the subgroup of patients requiring tight monitoring (Liverani et al, 2016).

Personal contributions

Based on the results obtained in our research previously exposed in this habilitation thesis, I participated as lead author, together with a research team interested in the study of IBD, in a study that aimed to assess the medium-term (6 months) predictive value of clinical elements (presence of extraintestinal manifestations, alteration of psychological status, presence of sleep disorders) and biological parameters (serum iron, cholesterol, CRP/albumin ratio, neutrophil/lymphocyte ratio) for the evolution of disease activity (Gîlcă-Blanariu et al, 2020). Also, similarly to the objectives set out in the previous studies, we aimed to determine cut-off values for parameters that have a predictive value for the accentuation of disease activity and to identify possible correlations between the parameters evaluated and the classic biological markers of systemic inflammation (CRP, fibrinogen).

**Material and Methods of Study**

We conducted a prospective, observational study, in which we initially included 82 adult patients diagnosed with inflammatory bowel disease from which 59 patients completed the study (41 patients with UC and 18 with Crohn's disease), admitted on an inpatient or one-day hospitalization in the Institute of Gastroenterology and Hepatology, Iași. The study was conducted from September 2019 to February 2020.

Patients enrolled in the study were followed up prospectively, evaluated at baseline and then - according to the national IBD follow-up protocol - at 6 months. As with the other studies conducted, patients who refused to sign informed consent, those with infectious colitis, patients with significant associated pathologies (neoplasia, psychiatric pathology, severe cardiopulmonary disorders), patients on medication that may interfere with the microbiota, psychological status or sleep (analgesics, psychotropics, muscle relaxants, thyroid hormones, etc.) were excluded.

For each patient included in the study, the demographic data, the complete diagnosis of the underlying disease: UC/CD, the location of the lesions, the degree of activity of IBD, the number of previous hospitalizations, the clinical-biological picture, the presence of comorbidities, the presence and type of extra-intestinal manifestations, the treatment followed at the time of inclusion in the study and the previous one, the history of surgery have been recorded.

The diagnosis of UC, respectively of CD was stated on clinical, biological and endoscopic criteria**.** For the assessment of disease activity, the Mayo score for UC and the CDAI score for Crohn's B were used.

*The biological assessment* included, besides the markers of systemic inflammation (CRP, ESR, fibrinogen), complete blood count, with the calculation of the neutrophil/lymphocyte ratio (NLR), serum iron, ferritin, serum albumin, cholesterol, serum triglycerides.

*The assessment of sleep* quality was performed using the Pittsburgh Sleep Quality Index (PSQI) questionnaire, while the *assessment of depression and anxiety* was performed using the Hospital Anxiety and Depression Scale (HADS) questionnaire. An altered quality of sleep was defined by a PSQI score ≥5 (Buysse et al 1989); and the alteration of psychological status was defined by obtaining subscores ≥ 8 to the HADS questionnaire when assessing at least one of the 2 HADS components-anxiety (HADS-A) or depression (HADS-D) (Zigmond, Snaith, 1983).

All the above-mentioned data were collected during the initial assessment and at the 6-month assessment the clinical and historical data and biological samples (blood count, CRP, fibrinogen and calculation of Mayo/ CDAI activity score calculation) were recorded.

*The statistical analysis* of the recorded data was performed using the SPSS version 25.0. Variables were reported as mean values with standard deviation or as median and interquartile range (Q25 – Q75). Comparisons between the groups analyzed were performed using Student's t-test, or Kruskal Wallis test for continuous type variables, depending on the homogeneity of the value series.

The qualitative variables were presented as absolute (n) and relative (%) frequencies, and the comparison between the groups was based on the results of the Chi-square test.

The univariate correlational analysis was performed based on the results of the Pearson test. The predictive value for several parameters was assessed using logistic regression, and the variables with significant results were subsequently included in the multivariate analysis.

The analysis of the ROC curve has been used to assess the predictive power of some variables. The univariate and multivariate analysis by logistic regression was used to study the correlation of some variables with the increased disease activity at 6 months.

Ethical issues

The study protocol and all procedures included in the study were in accordance with ethical standards and the 1964 Declaration of Helsinki and subsequent amendments.

**Results**

Of the 82 patients eligible for inclusion in the study evaluated during the designated period, 59 completed the study (6 did not consent to participate in the study, 3 patients did not meet the inclusion criteria: 2 had acute infections, 1 patient had a history of neoplasia and 14 patients did not attend the 6-month assessment).

For the 59 patients who completed the study (41 patients with UC and 18 patients with CD), a range of demographic and clinical-biological parameters were recorded, without showing statistically significant differences by disease subtype (Crohn's disease/UC) (Table 1.XVI)

In order to identify whether the parameters tracked have predictive value for exacerbation of disease activity in the medium term (6 months), we assessed possible correlations with parameters such as anemia (defined as Hb <12g / dL for female patients and <13g / dL for male patients), hyposideremia (serum iron <50 mg / dl), hypercholesterolemia (serum cholesterol > 200 mg / dl), presence of extraintestinal manifestations, sleep disorders (PSQI ≥5), changes in psychological status (HADS-A and / or HADS-D ≥ 8).

Disease activity was asserted in the presence of elevated CRP and fibrinogen values. The Pearson test revealed several statistically significant correlations: *elevated CRP values* at the 6-month assessment correlated with the presence of hypercholesterolemia ($p = 0.015$) and the altered psychological status ($p = 0.06$) at baseline. *Increased fibrinogen values* at the 6-month

assessment was correlated with hyposideremia (p = 0.023), hypercholesterolemia (p = 0.01) and sleep disorders (p = 0.039) identified at baseline (Table 1.XVII).

**Table 1.XVI.** Descriptive statistics of the evaluated parameters

| **Patient characteristics** | **Study Group n=59 (100%)** | | **Statistic test** | ***p*-Value O** |
|---|---|---|---|---|
| | **Ulcerative colitis n=41 (69.5%)** | **Crohn's Disease n=18 (30.5%)** | | |
| Active disease, n (%) | 13(22%) | | 0.133 † | 0.716 |
| | 8(19.5%) | 5(27.8%) | | |
| Anemia, n (%) | 19 (32.2%) | | 2.864 † | 0.091 |
| | 16 (39%) | 3(16.7%) | | |
| Disease activity scores median (Q25;Q75) | Mayo score 3(1;7) | CDAI score 129.5 (40.75; 198.5) | NA | NA |
| Hemoglobin, median (Q25;Q75) | 13.1 (11.9; 14.3) | | 1.669# | 0.101 |
| | 12.6(11.7:14.25) | 13.8(12.45;14.52) | | |
| Serie iron level median (Q25;Q75) | 59 (23; 86) | | 1.029# | 0.308 |
| | 51 (23; 80) | 66.5 (20.75; 97) | | |
| Ferritin median (Q25;Q75) | 75(36; 174) | | -0.167 # | 0.868 |
| | 75 (32.5; 182.5) | 62 (39.5; 172.25) | | |
| CRP median (Q25;Q75) | 0.82 (0.24; 2.09) | | 0.743 # | 0.461 |
| | 1.07(0.2:2.07) | 0.61 (0.33:2.84) | | |
| Albumin (mean ± SD) | 4.02 ± 0.51 | | 0.410 # | 0.683 |
| | 4 ± 0.55 | 4.06 ±0.41 | | |
| Fibrinogen (mean ± SD) | 419.49 ±67.1 | | 1.173# | 0.246 |
| | 412.41 ± 59.62 | 435.88 ±81.65 | | |
| ESR median (Q25;Q75) | 5 (3; 10) | | -0.377 # | 0.708 |
| | 5 (3.25; 9.5) | 6 (3; 11) | | |
| Cholesterol (mean ± SD) | 179.71 ±47.21 | | -0.95 # | 0.346 |
| | 183.84 ±45.81 | 171 ±50.25 | | |
| Triglycerides median (Q25;Q75) | 86 (70.25;120.5) | | 0.354 # | 0.725 |
| | 89.5(69.75; 120.75) | 81.5(69.75; 121) | | |
| Extraintestinal manifestations n (%) | 12 (20.3%) | | 0.133 † | 0.481 |
| | 11(26.8%) | 1 (5.6%) | | |

# - T-test, † Chi-Square test, (*) marked effects arc significant for p < 0.05

**Table 1.XVII**. Correlations between various parameters at baseline and increased inflammatory markers (CRP, fibrinogen) at 6 months

| **Parameters evaluated at baseline** | **Increased CRP value at 6 month evaluation IBD patients (p value)** | **Increased fibrinogen value at 6 month evaluation IBD patients (p value)** |
|---|---|---|
| Anemia | 0.118 | 0.852 |
| Hyposideremia | 0.054 | 0.023* |
| Hypercholesterolemia | 0.015* | 0.01* |
| Extraintestinal Manifestations | 0.198 | 0.274 |
| Impaired sleep | 0.960 | 0.039* |
| Altered psychological status | 0.006* | 0.256 |

Pearson correlations, (*) marked effects are significant for $p < 0.05$

Considering the results of the correlation tests, we investigated the predictive value for increased inflammatory bowel disease activity at 6 months for the parameters included in the previous analysis. We also studied the predictive value for other parameters: neutrophil/lymphocyte ratio (NLR), CRP/albumin ratio (CRP/alb), HADS-D value, HADS-A,PSQI. We considered that the disease showed an increase in activity at 6 months if there was an increase in the Mayo score for UC and CDAI score for CD, respectively, compared to the score from the initial assessment.

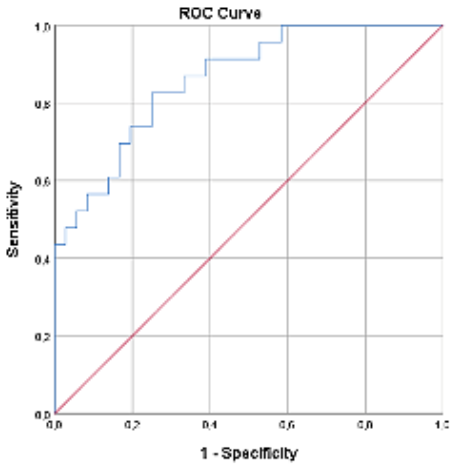
Univariate analysis demonstrated the predictive value for increased disease activity of hyposideremia (OR 13.09; 95% CI, 1.57-109.11), hypercholesterolemia (OR 5.18; 95% CI, 1.25-21.44), NLR (OR 3.83; 95% CI, 1.87-7.83) and HADS-D score (OR 1.56; 95% CI, 1.22-2.01). (Table 1.XVIII).

The parameters for which significant results were obtained in the univariate analysis were entered in the multivariate analysis to identify the parameters with predictive value on the increase of IBD activity at 6 months. In the multivariate analysis, significant values were identified only for hyposideremia (OR 9.63; 95% CI, 1.05-87.75) (Table 1.XVIII).

**Table 1. XVIII**. Evaluation of the prediction power of various parameters on increase in disease activity at 6 months

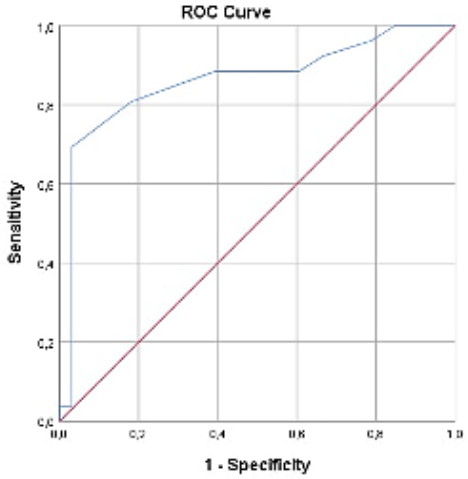
| Parameter | Univariate analysis Study Group OR 95% CI | Multivariate analysis Study Group OR 95% CI |
|---|---|---|
| Anemia | 1.77 (0.42-7.39) | |
| Hyposideremia | 13.09(1.57-109.11) | 9.63 (1.05-87.75) |
| Hypercholesterolemia | 5.18(1.25-21.44) | 3.38 (0.71-15.95) |
| Extraintestinal Manifestations | 3.77 (0.44-32.36) | |
| Impaired sleep | 2.47 (0.59-10.31) | |
| NLR | 3.83(1.87-7.83) | 0.54 (0.25-1.15) |
| CRP/ALB | 0.31 (0.04-2.08) | |
| HADS-D | 1.56 (1.22-2.01) | 0.99 (0.79-1.25) |
| HADS-A | 0.96 (0.79-1.15) | |
| PSQI | 1 (0.85-1.18) | |

Considering the fact that in the univariate analysis the NLR and HADS-D parameters were identified as having a predictive value for increasing the disease activity at 6 months, we performed the analysis of the ROC curve and identified cut-off values in this regard. Thus, for NLR the value of the cut-off with predictive value is 2.7, respectively for HADS-D 8.5 (Figure 1.19).




(a)

| | |
|---|---|
| Cut off value | 2.7 |
| AUC | 0.862 |
| Sensibility | 0.826 |
| Specificity | 0.722 |
| p value | 0.001 |




(b)

| | |
|---|---|
| Cut off value | 8.5 |
| AUC | 0.853 |
| Sensibility | 0.808 |
| Specificity | 0.82 |
| p value | 0.001 |

**Fig. 1.19.** ROC curve evaluating the prediction power of (**a**) NRL and (**b**) HADS -D at baseline on disease activity at 6 months

**Discussion**

Taking into account the fact that IBD is a pathology with evolution in activity spikes alternating with periods of remission, affecting the quality of life of patients but also with a major socio-economic impact, (GBD 2020) the efforts made to identify potential predictors of the severity of the disease and the occurrence of activity spikes are justified.

In the study we conducted, we analyzed a series of parameters used in daily practice, with the aim of identifying a potential predictive value for the evolution of IBD in the medium term (6 months). Among the items studied, we have identified a number of factors that can contribute to the prediction of disease activity at 6 months and which are easy to assess: hyposideremia, hypercholesterolemia and increased NLR.

An interesting finding in our study is that hyposideremia at baseline correlated with the increased disease activity and increased fibrinogen at 6 months, but these correlations were not found in the case of anemia, possibly because the etiology of anemia in IBD is mixed. This could also be due to the coexistence of inflammation (clinical or subclinical) with histological changes, which play an important role in iron deficiency, probably mediated by the hepcidin-ferroportin axis. Early identification of hyposideremia and its correction remain important pillars in the management of IBD patients, (Reinisch et al., 2013; Çekiç et al, 2015).

Of the other biochemical parameters evaluated, NLR was investigated as a marker of subclinical inflammation, being a promising element in this regard, all the more so as it is easily accessible, being calculated from parameters available within the blood count. Acaturk et al studied the usefulness of the NRL for assessing IBD activity and identified a NLR cut-off for active disease of 3.2 for CD and 3.1 for UC, respectively. In our study the cut-off value of NLR was 2.7 for the entire group of patients with IBD. (Acaturk et al., 2015)

Another biochemical parameter evaluated which we found to have predictive value for disease activity is hypercholesterolemia. From a pathophysiological point of view, this may be explained by the proinflammatory effect it can have by its accumulation in macrophages and other immune cells, generating toll-like receptor (TLR) signaling and inflammasome activation. (Tall, Yvan-Charvet, 2015)

Regarding other clinically assessable parameters, as in one of the studies detailed above, we looked at the potential predictive value of altered psychological status and sleep disorders. In the studied group, we identified a correlation between the depression score and the exacerbation of the inflammatory syndrome at 6 months. Instead, we did not obtain significant correlations between the worsening of the inflammatory syndrome and the presence of sleep disorders or anxiety.

The importance of psychological comorbidity on the evolution of IBD is generally recognized, by promoting inflammation and by associating sleep disorders, which can increase the effects of altered psychological status in patients with IBD and further maintain the pro-inflammatory status (Marinelli et al., 2020). Moreover, a bidirectional relationship between psychological status and inflammatory activity in IBD is reported in literature, especially in terms of depression. (Keefer, Kane, 2017).

Regarding this aspect we identified for the HADS-D score a cut-off value of 8.5 which is predictive for disease activity at 6 months.

**Conclusions**

Identifying predictors of disease activity in patients with IBD is a priority to optimally tailor disease monitoring and adjust treatment. Among readily accessible parameters, hyposideremia, hypercholesterolemia, increased NLR and presence of depression represent, according to our study, potential predictive factors that could be easily monitored in daily clinical practice.

*Originality and applicability of study results in the management of IBD*

Hyposideremia, hypercholesterolemia, increased NLR and presence of depression are readily accessible parameters, have been identified as predictive factors for disease activity in patients

with IBD and can be easily monitored in daily clinical practice.
Also, calculating the cut-off value for the HADS-D score that is predictive for disease activity at 6 months can be an argument for the screening of depression in IBD patients and at the same time can be a tool to identify depression for early referral to the psychiatrist and optimization of integrative therapeutic management.
*Research limits*
Although in the univariate analysis the previously mentioned parameters were identified as having a predictive value on the IBD activity, their interpretation still requires caution, given that following the multivariate analysis we did not obtain statistical significance for their predictive value (except for hyposideremia) probably due to the small sample size. Also, following the evolution in the study only for 6 months can be considered a limitation of the research.
*Future directions*
Prospective studies on a large number of patients are needed to assess as accurately as possible the predictive value of these markers, possibly for their inclusion in prognostic scores.

***

## 1.3. SUMMARY OF THE RESULTS OBTAINED ON TOPICS DERIVED FROM INFLAMMATORY BOWEL DISEASES

### 1.3.1. Results obtained and elements of novelty

By critically analyzing the results obtained in the studies carried out and summarizing the discussions by reference to the literature, we can formulate the following conclusions of this research path:

- ***Oxidative stress profile in IBD patients:***
  - patients with inflammatory bowel diseaseshowed increased levels of peripheral antioxidant enzymes (SOD and GPX) during disease flare compared to the control population, while patients in remission not only had significantly lower levels of SOD and GPX than patients with active disease, but also showed a statistically relevant decrease in these antioxidant enzymes compared to the control group. This deficiency may be present prior to the onset of the disease, thus constituting a trigger of inflammation.
  - Lipid peroxidation is significantly increased in patients during disease flare compared to controls, with a higher value existing in those in remission.
  - There are no significant differences in the markers of peripheral oxidative stress between UC and CD, thus supporting the hypothesis of a common etiopathogenesis.
  - The correlation of oxidative stress with clinical and endoscopic activity scores is poor, emphasizing the complexity of the mechanisms involved and the lack of utility of these determinations as biomarkers.
- ***Psychopathological and sleep disorders in patients with inflammatory bowel disease:***
  - There is a much-increased level of anxiety and depression among IBD patients compared to the general population
  - There are no significant differences in the psychological distress between UC and CD patients
  - Anxiety and depression scores are significantly higher during disease flare than in remission. At the same time, patients in remission show significantly higher level of these scores compared to the controls, which argues a chronic level of stress of these patients.
  - The presence of anxiety and depression among this patientcategory isnot influenced by the duration of the disease, since the newly diagnosed patients do not have

significantly higher scores compared to patients with longstanding disease. This evidence supports the fact that emotional disorders are not only present after diagnosis, as it has often been considered but are present throughout the course of the disease.

- ***Status of micronutrients in patients with inflammatory bowel disease:***
  - The hair concentration for most of the studied microelements was significantly different in patients with inflammatory bowel disease compared to the control group, as follows: concentrations of magnesium, selenium, silicon, copper, calcium and iron were statistically significantly lower in patients with IBD compared to the control group. Hair concentration of manganese and sulphur was superior in patients with IBD compared to the control group.
  - The hair concentration of selenium and zinc was not statistically significantly different between patients with CD compared to patients with UC.
  - In patients with IBD, there was a trend towards an inverse correlation between the values of zinc, selenium, calcium and magnesium dosed into the hair and the values of some markers reflecting the inflammatory syndrome
  - Measuring the hair concentration in various minerals and trace elements could be a reliable method of assessing their status among patients with IBD and contribute to a better integration of micronutrient supplements into disease management.

## 1.3.2. Dissemination of results and scientific visibility

The scientific activity carried out in this direction of research (documentation and clinical research), as presented in Tables 1.XIX, resulted in 7 articles published in extenso in ISI peer-reviewed journals (5 main author and 2 co-author), 2 articles in ISI journals proceedings, 3 articles in BDI-indexed journals, 7 chapters in specialist books published in CNCSIS-recognized publishing houses and numerous posters and oral communications.

**Table 1.XIX:** Other scientific papers published *in extenso* in peer-reviewed journals

| No | Title/ authors/journal/ IF | WOS citations | Google Scholar citations |
|---|---|---|---|
| **Scientific papers published *in extenso* in ISI-listed journals** | | | |
| 1. | DIFFERENT PROFILE OF PERIPHERAL ANTIOXIDANT ENZYMES AND LIPID PEROXIDATION IN ACTIVE AND NON-ACTIVE INFLAMMATORY BOWEL DISEASE PATIENTS. Achitei D, Ciobica A, Balan G, Gologan E, Stanciu C, Stefanescu G. *Dig Dis Sci*. 2013;58(5):1244-1249. **IF= 2,550** | 65 | 86 |
| 2. | NEW INSIGHTS INTO THE ROLE OF TRACE ELEMENTS IN IBD. Gîlcă-Blanariu GE, Diaconescu S, Ciocoiu M, Ștefănescu G. New *Biomed Res Int*. 2018; 1813047. Published 2018 Sep 6. **IF = 2.197** | 21 | 32 |
| 3. | SARCOIDOSIS ASSOCIATED WITH INFLIXIMAB THERAPY IN ULCERATIVE COLITIS. Gîlcă GE, Diaconescu S, Bălan GG, Timofte O, Ștefănescu G. *Medicine (Baltimore)*. 2017;96(10):e6156. **IF=2,028.** | 15 | 13 |
| 4. | SLEEP IMPAIRMENT AND PSYCHOLOGICAL DISTRESS AMONG PATIENTS WITH INFLAMMATORY BOWEL DISEASE-BEYOND THE OBVIOUS. Gîlcă-Blanariu GE, Ștefnescu G, Trifan AV, et al. Sleep *J Clin Med*. 2020;9(7):2304. Published 2020 Jul 20. **IF – 4.242** | 1 | |
| 5. | HAIR EDX ANALYSIS - A PROMISING TOOL FOR MICRONUTRIENT STATUS EVALUATION OF PATIENTS WITH IBD?Gîlcă-Blanariu GE, Coroabă A, Ciocoiu M, Ștefnescu G. Hair EDX. *Nutrients*. 2021;13(8):2572. Published 2021 Jul 27. **IF – 5.719** | | |

| No | Title/ authors/journal/ IF | WOS citations | Google Scholar citations |
|---|---|---|---|
| 6. | SPECIFIC FEATURES OF CLOSTRIDIUM DIFFICILE COLITIS IN PATIENTS WITH INFLAMMATORY BOWEL DISEASE. Mihai, C; Stefanescu G, Gogalniceanu P, Anton C, Dranga M, Jigaranu O, Balmus IM, Timofte D , Ciobica A , Stefnescu G, Prelipcean CC. *Arch Biol Sci* 2015 (67);1: 147-153. **IF=0,367** | 1 | 2 |
| 7. | DERMATOLOGICAL MANIFESTATIONS IN PEDIATRIC INFLAMMATORY BOWEL DISEASE. Diaconescu, S.; Strat, S.; Balan, G.G.; Stefanescu, G.; Ioniuc, I.; Stanescu, A.M. *Medicina* 2020, *56*, 425 **IF=2.43.** | | 1 |
| **Scientific papers published *in extenso* in ISI proceedings journals:** | | | |
| 1. | FUNGAL DYSBIOSIS IN INFLAMMATORY BOWEL DISEASE- WHERE ARE WE ?. Gîlcă G-E, Ştefănescu G*, Ciocoiu M. Neurogastro 2017 - Meeting Of The Romanian Society Of Neurogastroenterology With Rome IV Regional Central East European Meeting, 2017: 222-227 | 1 | |
| 2. | DYSPLASIA IN INFLAMMATORY BOWEL DISEASE: PERSPECTIVE FROM A TERTIARY CENTRE IN NORTHWESTERN ROMANIA. Popa I V, Drug V L, Popa R, Savin A, Cardoneanu A, Bărboi O, Ştefănescu G. *Filodiritto Proceedings XXXVI National Congress of Gastroenterology, Hepatology and Digestive Endoscopy Cluj-Napoca,* 8-11 June 2016; p 346-350. | | |
| **Scientific papers published *in extenso* in BDI indexed journals:** | | | |
| 1. | EVALUATING PREDICTIVE FACTORS FOR DISEASE ACTIVITY AMONG PATIENTS WITH INFLAMMATORY BOWEL DISEASE Gîlcă-Blanariu G.E, Ştefănescu G*, Afrăsânie VA, Gologan E, Mitrică DE, Timofte O, Bălan G.G, Olteanu AV, Ciocoiu M. *Med. Surg. J.*. Soc. Med. Nat., Iaşi – 2020 – vol. 124, no. 3, 367-373. | 1 | 1 |
| 2. | ULCERATIVE COLITIS ASSOCIATED WITH VITILIGO AND IGA DEFICIENCY IN A YOUNG GIRL. Naumcieff I, Burlea I, Diaconescu S*, Chiriac MI, Olaru C, Gimiga N, Ciubotariu G, Mihăilă,D, Ştefănescu G, Trandafir LM. *Arch Clin Cases 2017; 4(1):41-46* | | 1 |
| 3. | CLINICAL, BIOLOGICAL AND EPIDEMIOLOGICAL ASPECTS OF INFLAMMATORY BOWEL DISEASES IN NORTH-EAST ROMANIA Achitei D, Gologan E, Stefanescu G, Balan Gh:, *Rev Med-Chir Soc Med Nat,* vol.117, no2 /2013. | | 8 |
| **Total citations** | | **105** | **144** |
| **Cumulative impact factor/direction** | | **19,533** | |

The visibility of the research on topics derived from inflammatory bowel diseases is reflected in the large number of citations (WOS- 105 citations and Google Scholar – 144) as well as in the cumulative impact factor of the journals in which articles were published (cumulative IF overall/editorial = 19.533).
Also, the awards obtained (4 articles awarded by UEFISCDI and one paper awarded by RCCC) are a guarantee of the quality of this research.

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I believe that the studies I have carried out in this direction of research and the results that we have obtained can contribute to the shaping of new strategies for an integrated and at the same time personalized approach to the patient with inflammatory bowel disease, and the results obtained can form the basis for designing prospective clinical studies in order to identify and prioritize parameters that could be used in surveillance or for the development of other therapeutic principles.

# Chapter 2.
# BETWEEN PEDIATRIC GASTROENTEROLOGY DEPARTMENT AND ADULT HEALTHCARE

## 2.1. CONTEXT AND RATIONALE OF THE RESEARCH TOPIC

The transition of adolescents from pediatric to adult care is a crucial moment in the management of chronic diseases. Improved medical treatment and availability of new drugs and surgical techniques have improved the prognosis of many pediatric disorders, prolonging survival, thus making the transition to adult care possible and necessary. An inappropriate transition or the incomplete transmission of data from the pediatrician to the adult Gastroenterologist can dramatically decrease compliance to treatment and worsen prognosis of a young adult patient, particularly in the case of severe disorders (Elli et al., 2015)

In 2013, experts representing four Italian Gastroenterology Societies (Italian Society of Pediatric Gastroenterology, Hepatology and Nutrition, Italian Society of Hospital Gastroenterologists and Endoscopists, Italian Society of Endoscopy, Italian Society of Gastroenterology) gathered for a panel discussion and elaborated a text representing the official position (official statements) on transition medicine in Gastroenterology (Elli et al., 2015)

The gastroenterological concept of transition initially took into consideration several gastrointestinal and hepatic chronic disorders (inflammatory bowel disease, hepatic cirrhosis, liver transplant, viral and autoimmune chronic hepatitis, Wilson's disease, celiac disease (Elli et al., 2015) but the management of functional disorders (chronic constipation, gastroesophageal reflux disease) (Brooks et al., 2017) is drawing increasing interest.

Transition represents a continuous process involving the patient, his / her parents but also the health provider through the medical services provided for adults and children. The process of transition of the pediatric patient with digestive pathology presupposes the transfer of medical responsibility from the parent to the child and the education of the adolescent with regard to the medical system dedicated to adults.

Current guidelines recommend that medical assistance for adolescent patients on record with chronic digestive pathology should be provided through the cooperation of the gastroenterology specialist with pediatric competency as well as the adult gastroenterologist. The transition process has a variable duration according to disease severity. (Elli et al., 2015; Brooks et al., 2017)

My interest in pediatric and transition gastroenterology developed starting with the collaboration with the research team made up of specialists in pediatric gastroenterology from the Santa Maria Hospital Iași. In 2012, upon participating in a series of scientific events dedicated to gastroenterology and digestive endoscopy, we identified areas of common interest and initiated several shared research projects. The studies were initially related to the management of oesophageal foreign bodies, area of interest in which both the team from the Institute of Gastroenterology and Hepatology of the Saint Spyridon Hospital and the Pediatric Gastroenterology Clinic of the Saint Mary Hospital in Iași have been recognized for their vast experience. Subsequently, we collaborated in other areas of interest including digestive endoscopy, GI functional disorders, inflammatory bowel disease, etc.

## 2.2. SCIENTIFIC ACHIEVEMENT IN THE FIELD OF RESEARCH

As I mentioned previously, this collaboration resulted in an extensive research activity that has continued from 2012 until today and produced a series of scientific articles including general survey as well as original articles grouped by related themes in this area of research. The general survey covered different subjects: digestive endoscopy (informed consent in endoscopic practice and other pre-procedural aspects – psychological preparation, sedation and anaesthesia, recommendations in interventional digestive endoscopy in the pediatric patient (Gimiga et al., 2016; Diaconescu et al., 2015; Diaconescu et al., 2019) as well as other areas of interest for transitional gastroenterology – dermatological manifestations in patients with IBD (Diaconescu et al., 2020), particularities of Hp infection in different age groups – child, adolescent and the elderly (Ștefănescu G et al., 2018), pancreatic cancer in the pediatric patient vs adult (Diaconescu et al., 2021). I have also published in collaboration with pediatric specialists a series of clinical cases of digestive pathology (Diaconescu et al., 2019; Naumcieff et al., 2017). The subject matter of the original articles included rare lesions such as oesophageal polyps, diagnosis and management of ingested foreign bodies, bowel movement disorders, anorectal malformations (Diaconescu et al., 2016; Olaru et al., 2016; Ciongradi et al., 2016).

At the same time, I have collaborated with the Pediatric Gastroenterology Clinic in a research project entitled "The role of melotherapy and pscyhotherapy in the relief of anxiety around the endoscopic exploration of the digestive tract in the child and adolescent" and have published theoretical data as well as preliminary results of the investigations.

**Relevant publications in the field of the second research direction**

In the following, I will present succinctly the results of the main studies carried out in collaboration with pediatric gastroenterology specialists that were published in the original articles, with subject matter in this area, articles to which I have contributed as principal author or co-author (reproduced with permission).

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### 2.2.1. Digestive Endoscopy in the Child, Adolescent and Young Adult

Pediatric digestive endoscopy had its beginnings in the 70's (Cremer M et al.,1974; Ottenjann et al., 1970). Initially, it involved devices used in adults but with the dynamic development of this method, instruments were created adapted to children as well as endoscopic accessories that have permitted increasingly more sophisticated diagnostic and therapeutic manoeuvres. (Ottenjann, 1970; Cremer et al.,1974; Triangli et al., 2017; Thomson et al., 2017).

Currently, several international forums have developed guidelines for the practice of pediatric endoscopy, namely the European Society of Gastrointestinal Endoscopy, the European Society of Pediatric Gastroenterology, Hepatology and Nutrition, the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition and the American Society for Gastrointestinal Endoscopy.

According to the most recent guidelines, current indications for superior digestive endoscopy in the child are similar to those in the adult and are subdivided in diagnostic indications (abdominal pain suggestive for an organic disorder, unexplained loss of weight, refractory anemia of unspecified etiology, recurrent vomiting, hematemesis, hematochezia, chronic recurrent diarrheal, dysphagia, odynophagia, corrosive substance ingestion for the evaluation of severity of the lesions, monitoring of rare cases of Barrett oesophagus, etc.) or therapeutic (percutaneous endoscopic gastrostomy or jejunostomy, foreign body extraction, food bolus

impaction, oesophageal stenosis dilation, hemostasias in variceal or non-variceal upper GI bleeding, etc.) (Diaconescu et al., 2016; Adhicari et al., 2007). Colonoscopy has in turn diagnostic indications such as rectorrhage, chronic diarrheal of unspecified etiology, unexplained anemia, intestinal polyposis syndromes, abscesses and perianal fistulas, radiological suspicion of ileocolic stenosis, post-intestinal transplant complications) and therapeutic (polypectomy, colic stenosis dilation, hemorrhagic lesion treatment, foreign body removal, sigmoid volvulus reduction).

**Personal contributions in the field of Digestive Endoscopy in the Child, Adolescent and Young Adult**

In the beginning of the collaboration with the pediatric gastroenterologists, the initial subjects taken under scrutiny in this area of research were digestive endoscopy, the management of foreign bodies and oesophageal polypoid lesions as well as preparation of the pediatric patient for endoscopic exploration (Table 2. I.).

**Table 2. I.** Publications in the field of Digestive Endoscopy in the Child, Adolescent and Young Adult:

| |
|---|
| FOREIGN BODIES INGESTION IN CHILDREN-EXPERIENCE OF 61 CASES IN A PEDIATRIC GASTROENTEROLOGY UNIT FROM ROMANIA.<br>Diaconescu Smaranda, Gimiga Nicoleta, Sarbu Ioan, **Stefanescu Gabriela**, Olaru Claudia, Ioniuc Ileana, Iulia Ciongradi, and Marin Burlea<br>o *Gastroenterology Research and Practice*, 2016, **IF – 1.863** |
| UNUSUAL ENDOSCOPIC FINDINGS IN CHILDREN: ESOPHAGEAL AND GASTRIC POLYPS. THREE CASES REPORT.<br>Diaconescu Smaranda, Miron Ingrith, Gimiga Nicoleta, Olaru Claudia, Ioniuc Ileana, Ciongradi Iulia, Sarbu Ioan, **Stefanescu Gabriela**<br>o *Medicine*, 2016, **IF – 1.804** |
| EFFECTIVE COMMUNICATION AND PSYCHOTHERAPY IN REDUCING ANXIETY RELATED TO DIGESTIVE ENDOSCOPY PROCEDURES FOR PEDIATRIC PATIENTS. Gimiga Nicoleta, Bors Alexandra-Mihaela, **Ştefănescu Gabriela**, Iorga Magdalena, Diaconescu Smaranda.<br>o *International Journal of Medical Dentistry,* 2016. |
| ALTERNATIVE THERAPIES IN REDUCING ANXIETY AND PAIN FOR INVASIVE PROCEDURES IN PEDIATRIC PRACTICE.<br>Diaconescu Smaranda, Iorga Magdalena, Bolat Maria, Stanca Raluca, **Ştefănescu Gabriela**<br>o *Romanian Journal of Oral Rehabilitation,* 2015 |
| CURRENT RECOMMENDATIONS IN PEDIATRIC INTERVENTIONAL GASTROINTESTINAL ENDOSCOPY<br>Diaconescu Smaranda, Donea Lorenza, Nichita Andreea, Strat Silvia, Rosu Oana Maria, Gimiga Nicoleta, Olaru Claudia, Ghiga Gabriela, Rotaru Bogdan, Bozomitu Laura, Balan Gheorghe G, **Stefanescu Gabriela**<br>o *Romanian Journal of Pediatrics,* 2019. |

### *2.2.1.1. Diagnosis and Management of Ingested Foreign Bodies in Children*

The first article, in chronological order, in which I participated along with the team of pediatric gastroenterologists presented an original scientific research regarding the diagnosis and management of ingested foreign bodies in children and adolescents.

My contribution to this article was as an expert given the large number of endoscopic explorations for the extraction of foreign bodies that I have carried out during my professional life. More specifically, I contributed to the design of the study, the necessary scientific documentation and the redaction of the discussions.

If in the case of adults, the specialty institutions have elaborated as early as 1987 recommendations regarding the management of ingested foreign bodies, which they have regularly updated (last guidelines 2021), European Society of Gastrointestinal Endoscopy (ESGE) and European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) have specified their position only recently (2017) regarding the diagnosis and management of foreign body ingestion in children. (Diaconescu et al., 2016; Adhicari et al., 2007; Anderson et al., 1987) As in the case of adult patients, it is recommended that the presentation to the hospital should involve the emergency room and that initial imaging should include a thoracoabdominal X-ray but also CT in case of ingestion of radiotransparent foreign bodies.

Endoscopy should be carried out as early as possible in the case of a foreign body remaining lodged in the oesophagus. As far as dull objects, coins and impacted food bolus are concerned, these should be extracted in the first 24 hours even if the patient is asymptomatic. Special attention should be given to symptomatic patients or in the case of foreign bodies represented by batteries, in which case endoscopic instrumentation should be carried out within the first two hours.

If the object is located at the level of the stomach or duodenum, extraction is recommended in symptomatic children or in the case of objects measuring more than 2.5/6cm; in all other situations monitoring is preferred, extraction being necessary only if spontaneous elimination does not appear within 4 weeks.

Sharp objects should be extracted in the first two hours, regardless of localization (oesophagus, stomach, proximal duodenum). Magnets will be extracted in the first 24hours if they are found at a level where the endoscope may reach; in contrary cases monitoring will be carried out by a multidisciplinary team which must include the pediatric surgeon. Impacted food boluses will be extracted in the first 2hours in symptomatic children and in the first 24hours in those who are asymptomatic. Recommended endoscopic accessories are retrieval net, polypectomy loops, rat tooth forceps. (Diaconescu et al., 2016; Adhicari et al., 2007)

**Patients and method**

The previously mentioned article presented the results of a retrospective study carried out over a period of 5 years which included 61 patients with ages between 10 months and 17 years (average age 3.25 +/- 4.7 years). Sex distribution showed an approximately equal ratio, namely 32 boys (52.45%) and 29 girls (47.54%). 40.98% of the patients were institutionalized children who were not under direct parental supervision. (Susy Safe Working Group, 2013).

**Results**

The clinical picture at presentation was varied. (Table 2.II)

As far as types of ingested foreign bodies are concerned, these were quite varied (coins, bolt nuts, batteries, etc.) as described in Table 2.III. Of the total number of children, 38 (62.35%) presented to the hospital in the first 24 hours following ingestion, 16 (26.33%) within 24-48hours and 7 (11.32%) at over 72 hours following the ingestion.

Multiple regression analysis was carried out on variable related to patient (age, sex), type of ingested object (dimensions, shape, material) and the time to presentation to the hospital, in order to establish corelations between these variables and the clinical picture. The results of this analysis are presented in Table 2.IV. Positive correlations were found between the clinical symptomatology and the object shape (r partial = 0.56, $p < 0.01$), time to presentation to the hospital (r partial = 0.45, $p < 0.01$) and patient age (r partial = 0.34, $p < 0.000557$).

**Table 2.II:** Clinical Picture of Ingested Foreign Bodies in Children in the Study Group

| Clinical presentation | Number of patients | % |
|---|---|---|
| Asymptomatic | 18 | 29.50 |
| Abdominal pain | 34 | 55.73 |
| Vomiting | 21 | 34.42 |
| Foreign body sensation | 7 | 11.47 |
| Hematemesis | 2 | 3.27 |
| Drooling and food refusal | 1 | 1.64 |
| Stridor and cough | 1 | 1.64 |

**Table 2.III:** Types of Foreign Bodies Identified

| Foreign body | Number of patients | % |
|---|---|---|
| Coins | 16 | 26.23 |
| Other metal objects | 8 | 13.11 |
| Bones | 5 | 8.19 |
| Batteries | 4 | 6.55 |
| Buttons | 4 | 6.55 |
| Large seeds | 3 | 4.91 |
| Alimentary boluses | 3 | 4.91 |
| Glass, marbles, toothpicks, magnets, and unidentified plastic objects (toy parts) | 2 each | 3.27 each |
| Needles, screws, nails, keys, hair pins, pencils, plastic lenses, and shattered glass | 1 each | 1.64 each |

**Table 2.IV.** Multiple regression analysis

| Multiple correlation | Estimated value |
|---|---|
| Multiple correlation coefficient *r* | 0.92344 |
| Multiple $r^2$ | 0.85274 |
| F(6. 1161) | 20.68154 |
| *P* | 0.00000 |
| Std. err. of estimate | 0.32562 |

| Partial correlation | Correlation interval (beta) | Std. err. | *T* | *P*<br>95% confidence interval |
|---|---|---|---|---|
| Intercept | | | -6.54998 | 0.000000 |
| Patient factors | | | | |
| Age | 0.348487 | 0.070137 | 3.54287 | 0.000557 |
| Sex | 0.093035 | 0.065924 | 1.41125 | 0.160654 |
| Institutionalization | -0.022719 | 0.065342 | -0.34769 | 0.728660 |
| Foreign body factors | | | | |
| Size | -0.102692 | 0.066169 | -1.55198 | 0.123196 |
| Material | -0.010585 | 0.067656 | -0.15646 | 0.875925 |
| Shape | 0.565067 | 0.066849 | 6.95704 | 0.000000 |
| Time to presentation | 0.450815 | 0.062466 | 5.61612 | 0.000000 |

Routine chest X-ray identified only 42 of the foreign bodies while 19 children had ingested radiotransparent objects. EGD carried out within 24-72hours following ingestion especially due to delayed presentation to the hospital or lack of appropriate apparatus in the territory clinics; other factors contributing to the delay of endoscopic procedures were end of the week presentations, lack of emergency endoscopic facilities and team and presentation post-ingestion of foods. Endoscopy was positive in 36 children which permitted identification of 3categories of patients: X-ray and endoscopy positive, X-ray negative but positive endoscopy (identification of the foreign body or mucosal lesions caused by it) and positive X-ray with negative endoscopy (all of these patients had ingested dull metal objects that had rapidly and spontaneously passed the level accessible by endoscope. The correlations between endoscopy and X-ray are summarized in Table 2.V

**Table 2.V:** Correlations between Radiographic and Endoscopic Exploration in the Study Group

<table>
<tr><td colspan="2"></td><td colspan="3">Cases with positive X-ray<br>42</td></tr>
<tr><td colspan="2">Negative X-ray and endoscopic findings</td><td colspan="2">Positive X-ray and endoscopic findings</td><td>Positive X-ray and negative endoscopy</td></tr>
<tr><td>Mucosal injuries<br>5</td><td>Foreign bodies<br>14</td><td>Foreign bodies<br>22</td><td>Mucosal injuries<br>8</td><td>12</td></tr>
<tr><td></td><td colspan="2">Cases with foreign bodies identified using endoscopy<br>36</td><td colspan="2"></td></tr>
</table>

Endoscopic extraction was carried out successfully in 19 (31.14%) of the 36 children with the identified foreign object using a polypectomy snare (Figure 2.1). The extraction was not successful in 17 (27. 86%) cases. 28 patients eliminated the foreign bodies naturally in an interval ranging between 3 and 20 days and in 14 cases they were not found in spite of close parental monitoring passing probably unobserved.

**Fig. 2.1:** Examples of extracted foreign bodies from patients who participated in the study

**Discussion**

The results of our study match the data in the literature as far as distribution by age and sex of the patients (Arms et al., 2008; Abbas et al., 2013). At the same time, we found differences in terms of the clinical picture: abdominal pain (55.73%), vomiting (34.42%) and an important percentage (29.50%) of asymptomatic children.

In the reported data by other authors, the percentage of asymptomatic children was situated between 25-55% (Abbas et al., 2013; Conners, 2000; Rybojad et al., 2012). Using multiple regression analysis, we identified positive correlation between the clinical picture and the shape of the objects – the sharp ones being associated with more severe clinical manifestations (r

partial = 0.56, p <0.01), time to hospital presentation (r partial = 0.45, p <0.01), and patient age (r partial = 0.34, p <0.000557). Other authors found significant correlations between object location, its size and the time passed since ingestion (Litivitz et al., 2010; Shastri et al., 2001). Most frequently, the children swallowed coins (26.23%) similar to existing data in the literature. In our study, the CXR (chest X-ray) was positive in 68.85% cases matching the existing data from other authors (64% -96.04%) (Abbas et al., 2013; Yang et al., 1991; Pokharel et al., 2008). The CXR (chest X-ray) may be negative in the case of ingestion of radiotransparent objects or may be due, in the case of late hospital presentation, to elimination of the object (without it being observed) before presentation. In 31.14% of children, endoscopic extraction was carried out successfully; the existing literature reports percentages ranging between 23% and 98.06% (Yang et al., 1991; Pokharel et al., 2008; Palta et al., 2009). These differences are owed to the differing times to presentation, the type of ingested object but also to the degree of technical capabilities of the endoscopic services. This latter aspect also explains the large number of failed endoscopic extractions (27.86%), since it is known that the diversity of endoscopic accessories and adaptation of the instruments to the type of foreign body increases the rate of success of the intervention.

All objects that could not be extracted were eliminated spontaneously in an interval ranging between 3 and 20 days; to these are added 11 cases in which endoscopy was negative but the object was found subsequently in the stool. Thus, of the total 61 patients, the endoscopic extraction was successful in 19, 28 foreign bodies were eliminated spontaneously and 14 were not found; our study reports rather negative results that can be explained by the absence of an emergency endoscopy service as well as the suboptimal technical capabilities. We did not encounter complications in the study group with the exception of a 2-year-old girl in whom an alkaline battery impacted in the superior oesophagus in spite of being extracted caused the formation of a tracheoesophageal fistula associated with bronchopneumonia and subsequent respiratory failure followed by the constitution of an oesophageal stenosis which necessitated endoscopic dilation.

**Conclusions**

In conclusion, we consider that active monitoring of children by their parents and the personnel in kindergartens, rehabilitation centers for patients with neuromotor disabilities and psychiatric services as well as careful adherence to instructions associated with various toys offered to children are efficient methods of prevention. At the same time, introducing national guidelines and protocols as well as establishing emergency endoscopy services in tertiary centers is a necessity which may modify significantly the prognosis for these patients since, as we have shown in our study, the symptomatology and subsequent progression depends on the time interval from the accident to the endoscopic intervention.

*Originality and applicability*

As mentioned at the beginning of the subchapter, unlike the adult situation, international guidelines for the management of oesophageal foreign bodies in children have recently come into use. In Romania, at the time of publication of the study, there were very few studies on this pathology in the pediatric population, performed in small groups. Our study included a great variety of swallowed objects.

*Limitations of the study*

The limitations of this study are mainly that this was a retrospective-descriptive review providing data from a single pediatric gastroenterology center.

*Future directions*

We will set out in the near future to carry out a comparative study of this pathology in adults vs children and adolescents, with the intention to study the type of swallowed foreign bodies, the impaction area, underlying lesions and the presence of eosinophilic esophagitis.

### *2.2.1.2. Unusual Endoscopic Lesions*

The second pathology diagnosed by digestive endoscopy that constituted a subject of interest in collaboration was represented by gastric and oesophageal polyps (Diaconescu et al., 2016).
Polypoid lesions at the level of the upper digestive tract are identified in 0.6-6% of upper digestive endoscopies carried out. These are localized most frequently at the level of the stomach (Bulur et al., 2020; Choong, Meyers, 2003; Archimandritis et al., 1996; Morais et al., 2007; Jalving et al., 2006). Polypoid lesions and polyps are those lesions that protrude into the lumen often arising from the level of the mucosa, typically of small sizes, asymptomatic and discovered incidentally during endoscopies.
If oesophageal and gastric polys do not represent a rare pathology in the adult, these structures are encountered in less than 1% of upper digestive endoscopy carried out in children; data in the literature are isolated and refer, as in our article, to small cohorts of patients (Septer et al., 2014; Attard et al., 2002; Cakir et al., 2014; Fleury et al., 2015).

**Patients and method**

Through retrospective analysis over a period of 5 years (2011-2015) of patient notes, endoscopic data and morphopathology results, we have identified out of a number of 2140 patients, 3 cases of oesophageal and gastric polyps in adolescents with ages between 13 and 17 (0.14%). All patients presented with pyrosis, vomiting and epigastric pain.
The first patient, an adolescent of age 17 with a 3-year history of pyrosis and epigastric pain who received intermittent PPIs, upper digestive endoscopy identified lesions of esophagitis A (Los Angeles), a sessile polyp measuring 1cm in diameter and juxtacardial location, antral gastritis and duodenal bulb mucosal congestion, without associating infection with H. pylori (Figure 2.2.A).

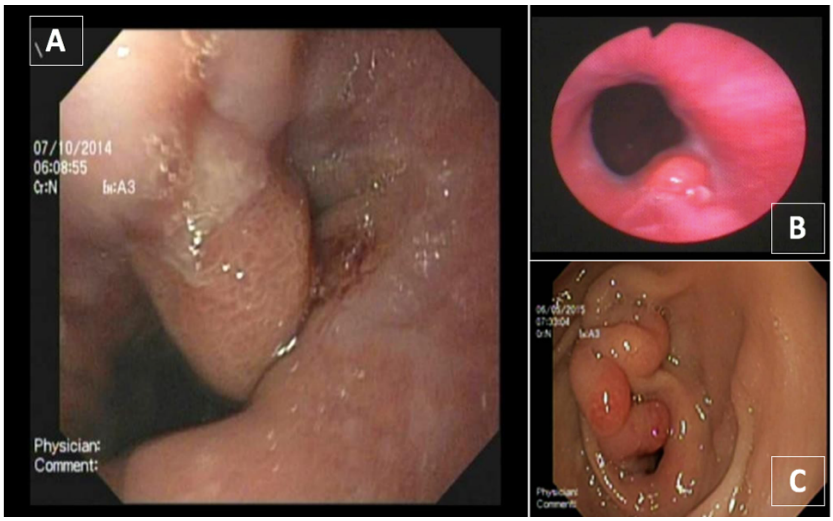


**Fig. 2.2: Endoscopic Appearance of Identified Lesions: A –** Juxtacardial sessile polyp in a 17-year-old male adolescent; **B –** Juxtacardial sessile polyp in a 13-year-old female adolescent; **C –** Giant Prepyloric Fold with Double-Headed Polyp in a 15-year-old adolescent male.

The second case, an adolescent girl 13 years of age, a relatively short history (3months) of epigastric pain worsened in spite of treatment with PPIs. Upper GI endoscopy showed esophagitis A (Los Angeles) lesions, a sessile polyp measuring 7mm, juxtacardial location and antral gastritis aspect (Figura 2.2.B). This case also did not associate H. pylori infection.
The last patient in this series was a 15 year old adolescent with ESKD (end-stage kidney disease) undergoing peritoneal dialysis who presented with intense epigastric pain and incoercible vomiting. The endoscopic exploration revealed a normal oesophagus, gastric mucosa without modifications down to the prepyloric region where a giant fold presented a

double-headed polyp which underwent intermittent protrusion into the duodenum; the patient was confirmed with *H. pylori* infection.
The histology of the lesions was varied: hyperplastic lesions in the first and third case and an entirely distinct aspect in the second with proliferation of surface and glandular epithelium on vasculoconjunctive pedicle, moderate inflammatory infiltrate and the presence of intestinal type cylindric epithelium with caliciform cells presenting intracytoplasmatic vacuoles positive for Alcian Blue dye indicating acidic mucopolysaccharides. (Figure 2.3- A, B second case, C- third case)
The first two patients were discharged with PPI treatment with oral administration for two months; the control endoscopy did not show macro- or microscopic modifications of the lesions. Both cases were monitorized endoscopically and histologically. In the third case, triple antibiotic therapy was initiated in adapted dose due to the ESKD (end-stage kidney disease); the clinical and biological status of the patient did not permit therapeutic endoscopy.

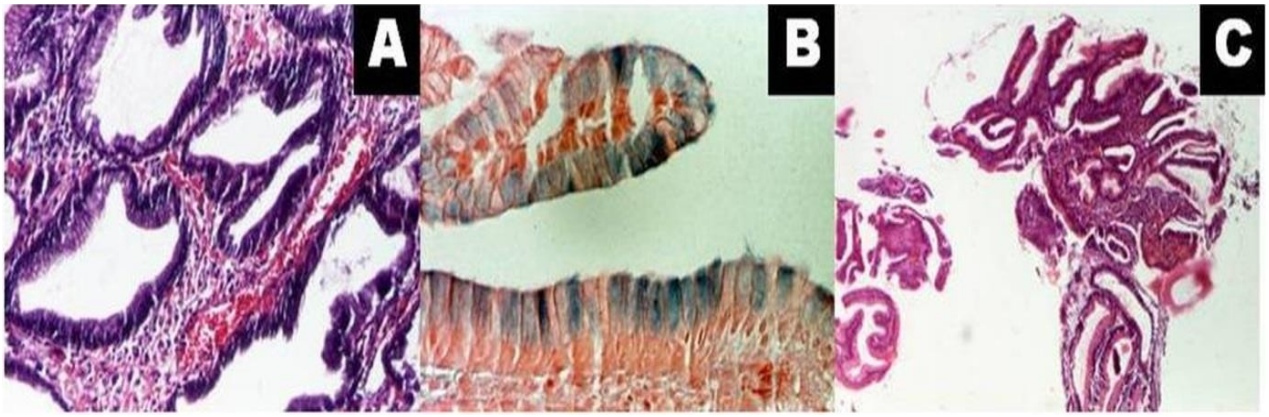


**Fig. 2.3:** Histopathological aspects (**A:** HEx100, **B:** ABSx200, **C:** HEx40)

As in the case of the adult patients, the oesophageal polyps were frequently associated with gastro-oesophageal reflux disease, hiatal hernia, Barrett oesophagus, eosinophilic esophagitis, Crohn's disease and type 1 Neurofibromatosis while the gastric polyps with Helicobacter pylori infection and chronic administration of PPIs (Bishop et al., 2002; Pashankar, Israel, 2002; Grynspan et al., 2008; Jain, Chetty., 2009). From a histologic standpoint, the oesophageal polyps were inflammatory and fibrovascular; there were also described the so-called IPFC (inflammatory polyp-fold complex), squamous papillomas and hamartomatous lesions. Gastric polyps were hyperplastic-inflammatory, hamartomatous, adenomatous, polyps developed at the level of the fundic glands and heterotopic lesions. In the first case, we considered the inflammatory lesion development in the context of the gastroesophageal reflux disease suggested by the clinical signs and the endoscopic appearance of the oesophageal mucosa; in agreement with findings by other authors, treatment with PPIs did not lead to modifications in polyp dimensions or histology. (Septer et al., 2014) In the second case, the distinct histology and the fact that none of the bioptic samples showed appearance suggestive of Barrett oesophagus and the clinical history was of short duration lead us to the conclusion that goblet cells are embryonic rests of ciliated cylindrical epithelium, described in the specialized literature. (Bani-Hani, Bani-Hani, 2006) In the third patient, the described hyperplastic appearance was associated with *H. pylori* infection.

**Conclusions**

In conclusion, we can state that the rarity of these lesions requires a histologic diagnosis of high accuracy which will guide subsequent therapy. Clinical, endoscopic and histologic monitoring is required in all such cases and endoscopic or surgical polypectomy may be taken into

consideration in certain cases according to patient age, morphopathological appearance, lesion dimensions and localization.

#### *2.2.1.3. Study of Alternative Methods of Anxiety Reduction Related to Endoscopic Procedures*

Another related subject with digestive endoscopy in the child and adolescent which we dealt with in collaboration with pediatric specialists was pediatric patient preparation for endoscopic explorations.

In the following, we will refer to the research project entitled „The role of melotherapy and psychotherapy in the reduction of anxiety related to endoscopic exploration of the GI tract in the child and adolescent" obtained in a private grant competition organized in the summer of 2015 by the ART Foundation 2017 and which produced two articles published in BDI journals including a review type and original which had a significant number of citations.

Endoscopic explorations are known to be invasive, sometimes painful and for this reason anxiety provoking with a particular emotional impact. In order to reduce the discomfort felt by patients in the case of colonoscopic exploration, in the majority of Western countries, the standard is conscious sedation associated with retrograde amnesia induced with midazolam or sedative-analgesics. (Friedman, 1998; Costa et al., 2010; Uman et al., 2013; Klassen et al., 2008) These methods are applied in the case of adult patients and more so in pediatric patients in both colonoscopy and upper digestive endoscopy. (Triangli et al., 2017; Thomson et al., 2017, Hartling et al., 2013) Analgesic-sedation, besides the potential adverse effects determined by administration of the pharmacological substances also has the disadvantage of high costs and time consumption. (Uman et al., 2013) Therefore, there are numerous studies aimed at identification of alternative methods of reducing patient discomfort among which is mesotherapy. (Matsota et al., 2013; Sunitha Suresh et al., 2015; Bampton, Draper, 1997; Bechtold et al., 2009; Biddiss et al., 2014).

The benefits of music therapy have been demonstrated by numerous meta-analyses however the degree to which these effects may be attributed directly to mesotherapy are difficult to quantify. (Bechtold et al., 2009, Rudin et al., 2007; Tam et al., 2008)

There are studies which demonstrate that the level of adrenaline and cortisol are reduced with the help of relaxing music. (Tazakori et al., 2007; Smolen et al., 2002) Other investigations into the role of mesotherapy in the reduction of discomfort during colonoscopy suggest a beneficial role in the reduction of pain and increased post-procedural satisfaction.

At the same time there are authors who report modified perception of the medical staff regarding the difficulty of the investigation. (Costa et al., 2010). A meta-analysis of 39 studies which included 3394 children with ages between 2 and 19 who underwent psychological interventions for the reduction of stress and pain related to venepuncture and vaccination revealed the efficacy of hypnosis and cognitive behavioural therapy. (Uman et al., 2013) There were also studies of the effect of music on the reduction of anxiety in children related to stomatological interventions and admission to emergency care units in pediatric hospitals. (Klassen et al., 2008; Hartling et al., 2013) Recent investigations suggest that music may be used as a complementary method for the reduction of pain and anxiety in the post-anaesthesia period, post-operative as well as in the neonatal intensive care units. (Matsota et al., 2013; Sunita Suresh et al., 2015).

Starting from these data, we published a review regarding the influence of mesotherapy and psychotherapy in the preparation of patients for endoscopic exploration. (Diaconescu et al., 2015)

We have also published an original article in which we have set out to study the emotional impact of endoscopic procedures (superior digestive endoscopy) in the child and adolescent and the influence of adequate communication and of psychotherapy on the somatic and

psychological parameters which characterize patient status before, during and after endoscopic exploration. (Gimiga et al., 2016).

**Patients and method**

The study involved patients with indications for upper digestive endoscopy who were directed to the Digestive Endoscopy Department of St Mary's Pediatric Hospital. The patients were evaluated both psychologically for pre-procedural anxiety and clinically for somatic parameters before, during and after endoscopic exploration. The subjects were randomized in two groups: the first group was evaluated endoscopically in standard conditions while the second group underwent a preparatory discussion with the clinical psychologist for 10-15 min with the aim of reducing anxiety regarding the intervention and preparing the patient psychologically for the procedure.

After the exploration, the degree of patient satisfaction was evaluated as well as the degree of cooperation during the procedure. For each group, the demographic data, somatic parameters (HR, BP, SaO2), exploration duration were analysed separately and the information in questionnaires completed by the patients, parents and endoscopist were interpreted.

Preliminary investigation was realized on a group of 232 patients who underwent various endoscopic investigations. (Gimiga et al., 2016)

Anxiety was evaluated through direct observation of behaviour before the procedure, monitoring of the external signs of anxiety – feelings of intense fear and unrest, clinical parameters (pallor, perspiration, tremor, tachypnoea, tachycardia) and certain behavioural modifications (crying, parental dependence). At the same time, parents were questioned regarding their experience related to the endoscopic procedures.

**Results**

Direct observation identified physical signs and behaviours related to anxiety regarding the medical procedure in 36 patients representing 15.5% of the group studied. Of the 36 cases in which anxiety was present, 52.8% were female patients and 47.2% were male patients.

In terms of age group, anxiety is more common in the age group between 6-12years (69%) with a slight increase in anxiety in female patients (N = 14). In the 1-5 age group, anxiety was present in 22% of patients and in the 13-18 age group were included 8% of patients. (Figure 2.4).

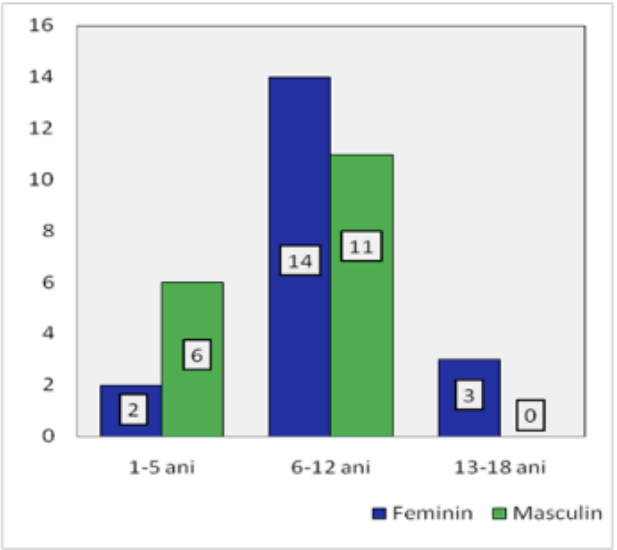


**Fig. 2.4:** Distribution of Patients with Signs of Anxiety by Age and Sex

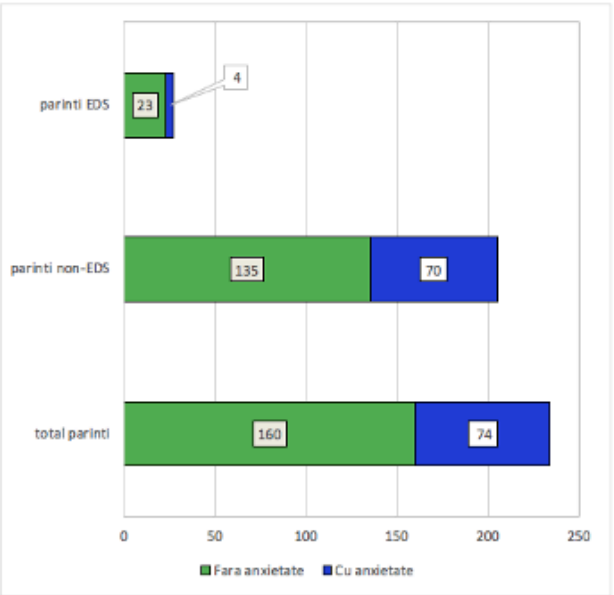


**Fig. 2.5:** Distribution of Parents with Signs of Anxiety by Personal Experience of EGDS

The group of family members included 232 parents and the anxiety related to endoscopic investigations in their children was present in 31.9% of cases. 27 (11.63%) parents had themselves been exposed to digestive endoscopy investigations at pediatric age, fact which had a positive influence on reducing preprocedural anxiety in the children, only 14.8% presented anxiety before the procedure. (Figure 2.5).

**Conclusions**

We can conclude that a parent informed regarding a medical procedure can explain themselves to their children the investigation thusly contributing to the reduction of anxiety in the young patient.

Parents of children who had endoscopy under general anaesthesia did not present anxiety related to the investigation itself but rather to the anaesthesia compared to the parents of children who had endoscopic investigations under various other types of sedation and who had presented anxiety with regard to the procedure.

*Originality and Applicability*

Our study was, to our knowledge, the first in Romania to assess anxiety related to upper gastrointestinal endoscopy exploration in both children and their relatives. The obtained results can lead to the elaboration of some conclusions that are the basis of the utility and applicability of the psychological training of the small patients but also of their parents in the pediatric endoscopy centers from Romania.

*Future directions*

For the future, we have planned to continue research into melotherapy as an adjunct method during endoscopic exploration and, as in the case of foreign bodies, we have set out to evaluate the role of psychotherapy and melotherapy on a group of adult patients, the results obtained pending publication most likely in the first part of next year.

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## 2.2.2. Functional Digestive Disorders

In digestive pathology, functional disorders of the GI tract are often encountered in both adults and children. On a global scale, the prevalence of constipation in the general adult population varies between 2% and 28% similar to that reported in children (0,7% and 29,6%). (Hyams**.**, 2016; Mugie et al., 2006) Most studies reported prevalence rates similar for boys and girls. (Higgins, Johansson, 2004). In a Dutch study regarding morbidity in children, it was observed that constipation incidence decreases with age. In breastfed babies, the incidence was 40/1000 per year, in children with ages between 2 and 4 it was 22/1000 per year and after the age 11, the incidence was 5/1000 per year; percentages are on the rise in well developed countries as a consequence of changes in culinary habits. (Linden et al., 2005).

Morphophysiological particularities of the child make the structural causes and the clinical progression of constipation different from that in the adult therefore requiring a specific diagnosis and treatment.

Until the present time, diagnosis of functional constipation in pediatric patients was based on a complex of symptoms in the absence of underlying organic causes. (Rasquin et al., 2006) In the past, many studies used the Iowa criteria in order to define constipation in the child. (Loening Baucke, 1990) Today, similar diagnosis in the adult is made according to the ROMA IV criteria (Table 2.VI) These take into account, the frequency and consistency of the stool but also disorders secondary to constipation.

**Table 2.VI:** Definition of Functional Constipation in the Older Child and Adolescent according to Rome IV Criteria (Drossman et al.,.2016)

| **Functional Constipation** – presence of at least two of the following, developed at least once per week, for minimum 1month of insufficient criteria for an irritable bowel syndrome diagnosis | |
|---|---|
| 1 | Two or less bowel movements per week in a child at least age 4 |
| 2 | At least one episode of fecal incontinence per week |
| 3 | History of fecal retention via posture or voluntary effort, history of difficult bowel movements. |
| 4 | History of large diameter stool that plugs the toilet. |
| 5 | Presence of a large fecal mass at the level of the rectum. |
| 6 | Symptomatology that does not fulfil the criteria for any other pathology after adequate medical evaluation. |

The effects of chronic constipation reach into adult age leading to a loss of productivity. Similar with the situation encountered in adult patients, in children social activity is diminished and some authors report nearly 30% of pediatric patients were less productive in school and had a higher rate of school absenteeism. Other studies revealed that in school children there was a loss of 2,4 productive days per month due to symptoms. (Johanson, Kralstein, 2007) A national health study also reported a significantly greater percentage of time loss due to health issues (9.08% compared to 5.20% in the control group) and a greater depreciation of time allotted to daily activities (46.58% compared to 33.90% in the control group). (Sun et al., 2011). The global impact of absenteeism was estimated to an average period of absences of 0.4 days/year. (Belsey et al., 2010).

**Personal contributions**

Several years ago, when we established collaboration with the team of pediatric specialists interested in digestive pathology, the number of studies regarding functional constipation in children in Romania was scant. During the doctoral period and shortly after defending my doctoral thesis, I was interested in functional pathology, for which reason I decided to join the team of pediatric gastroenterologists which studied this pathology in the pediatric patient, and following our collaboration, I published two studies ISI on this theme (Table 2.VII).

The two studies published in journals indexed ISI with impact factor which I will present succinctly in the following, tried to identify the predisposing factors of chronic constipation in the child particularly the psychosocial aspects of chronic constipation and encopresis in the child.

**Table 2.VII.** Publications in the field of functional disorders in the child and adolescent:

| |
|---|
| SOME RISK FACTORS OF CHRONIC FUNCTIONAL CONSTIPATION IDENTIFIED ÎN A PEDIATRIC POPULATION SAMPLE FROM ROMANIA.<br>Olaru Claudia, Diaconescu Smaranda, Trandafir Laura, Gimiga Nicoleta, **Ştefănescu Gabriela**, Ciubotariu Gabriela, Burlea Marin.<br>o *Gastroenterology Research and Practice* 2016, **IF -1.863.** |
| CHRONIC FUNCŢIONAL CONSTIPATION AND ENCOPRESIS ÎN CHILDREN ÎN RELATIONSHIP WITH THE PSYCHOSOCIAL ENVIRONMENT<br>Olaru Claudia, Diaconescu Smaranda, Trandafir Laura, Gimiga Nicoleta, Olaru Radian, **Ştefănescu Gabriela**, Ciubotariu Gabriela, Burlea Marin, Iorga Magdalena.<br>o *Gastroenterology Research and Practice* 2016, **IF -1.863.** |

### *2.2.2.1. Predisposing Factors of Chronic Functional Constipation*

In the first article mentioned, we reported the results of a study aimed at identifying the potential predisposing factors of functional constipation and tried to establish certain

correlations with diet and socio-familial factors via characterization of the family milieu of the patient, analysis of the family history and identification of a positive history of functional constipation among the parents, as well as development of a nutritional profile of the patients. (Olaru et al., 2016)

**Patients and method**

For data gathering, both parents and school-age children were asked to respond to a questionnaire which included data referring to: the moment of onset of the bowel movement disorder, characterization of the stool in terms of quantity and quality; moment of onset of signs and symptoms of the disease and association with extradigestive pathological events or with introduction of certain foods in the child's diet; a dietary inquiry aimed at establishing the type of diet before the onset of constipation and after, the presence and type of functional digestive disorders in their parents or other close relatives.

The adults were diagnosed with functional constipation according to Rome III criteria for adults (in use at the time).

The effect on the growth curve including weight and height was quantified via calculation of the BMI (body mass index) according to the formula: BMI ($kg/m^2$ = W (kg)/H ($m^2$). Excess weight was considered when the BMI was the 85th percentile of the reference standard, obesity as a BMI greater than the 95th percentile and underweight was defined as a BMI under the 5th percentile.

Parents were also evaluated in terms of BMI. They were considered underweight with a BMI ≤ $18.5kg/m^2$; normal weight with a 18.5 <BMI<24.9 $kg/m^2$, overweight with 25,0 <BMI <29.9 $kg/m^2$ and obese with BMI≥$30kg/m^2$.

At the same time, we assessed the degree of physical activity, the amount of time spent before the TV or computer screen and data were obtained regarding the socio-familial milieu.

Relevant family history helped identify disorders in first-degree relatives. Statistical analysis of the data was carried out using the SPSS 20.0 program.

The clinical study was realized on a group of 234 children with age between 4 and 18 years diagnosed with chronic constipation defined according to the Rome III criteria (which were in use at the time). The control group was made up of 112 children. Before the initiation of the study, we obtained consent from the parents and minors passed 14 years of age.

**Results**

142 out of the 234 children who had finalized the study were males (60.73%) and 92 (39.27%) were females. In the control group there were no notable differences between sexes, the male to female ratio being 1:1.

With regard to the familial environment, there were no significant differences between the two groups.

In our study, the majority of constipated children presented with onset of the disorder in the first two years of life (Figure 2.6).

The average age of onset of symptomatology was 26.39 months, with minimum values of 9 months and maximum 36 months. It should be noted also that 63.14% of the cases entered into the study, the onset of constipation was in the age interval between 24-36 months, average calculated as 26,39 months.

Regarding the frequency of bowel movements, we found that their frequency was significantly smaller in children in the study group with an average of 1 bowel movement every 4.59 days compared to 1 bowel movement every 1.13 days in the control group. (Figure 2.7)

A risk factor identified in our study was a positive family history for constipation. This was found in 38.49% of cases.

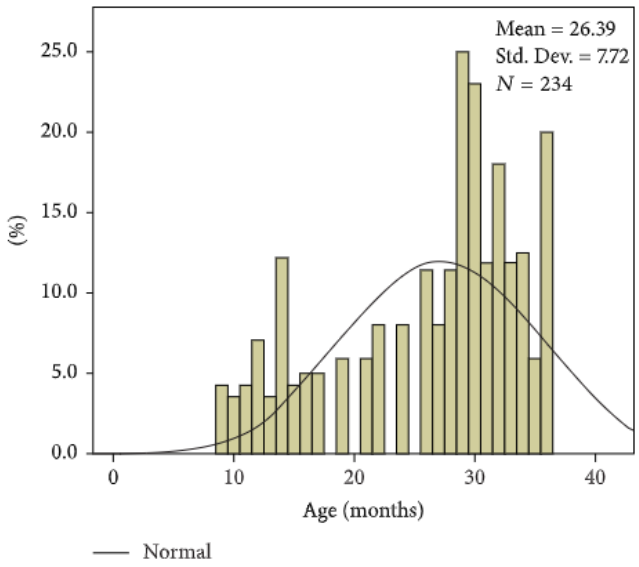


**Fig. 2.6.** Histogram of the age of onset of constipation

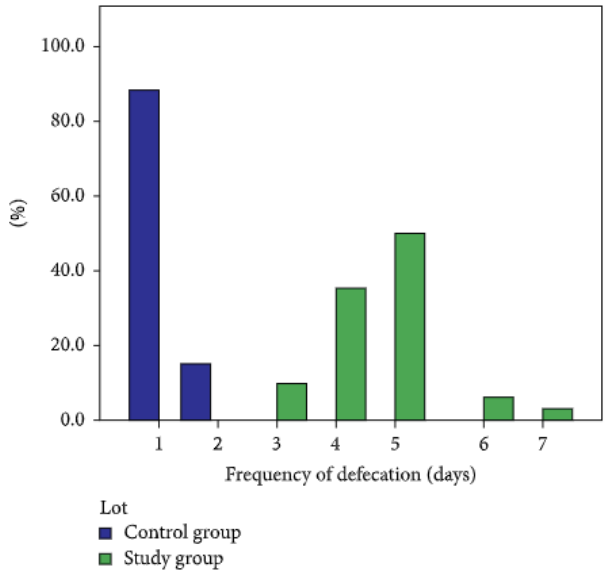


**Fig. 2.7.** Distribution of Patients by Group in Terms of Bowel Movement Frequency

Of the children in the group with constipation, 80 (34.2%) lived at the grandparents, 33 (14.1%) came from monoparental families, 16 children (6.8%) were in the care of a maternal assistant and 6 (2.6%) came from an orphanage. The remaining 99 patients (42.3%) lived with both parents, a significantly smaller percentage compared to the children in the control group who lived with their parents in proportion of 75%. (Figure 2.8).

Another possible factor involved in the development of constipation in the child may be represented by toilet training. The age when toilet training was begun in the study group varied between 1 year of age to 3.5 years of age with an average of 2.4 years compared to 1.8 years in the control group.

A statistical correlation may be observed between the medium age at the beginning of toilet training and the age of onset of constipation ($F = 70.749$, $p<0.001$, 95% Cl). (Figure 2.9).

The alimentary questionnaire has shown that in our study group, a low percentage of children were breastfed, namely 26.07% (60.71% in the control group) and 40.60% received cow milk. In the control group, the proportion of formula / cow milk was only 7,14%. ($\chi^2 = 55.60$; df=3; $p<0.00$).

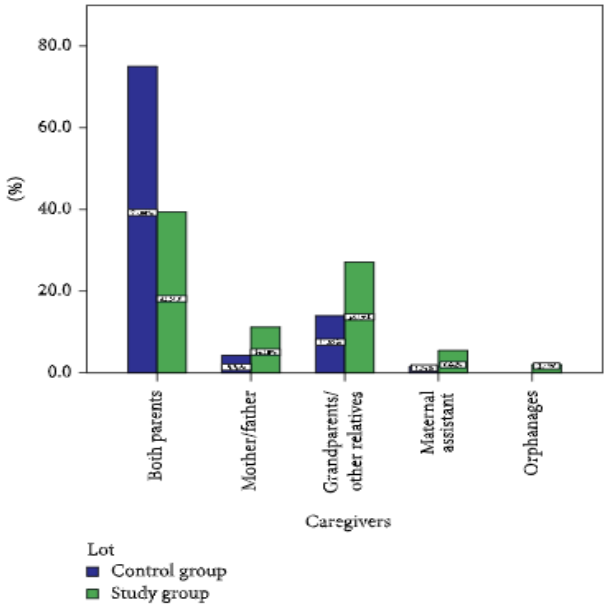


**Fig. 2.8.** Group Assignment by Socio-Familial Milieu

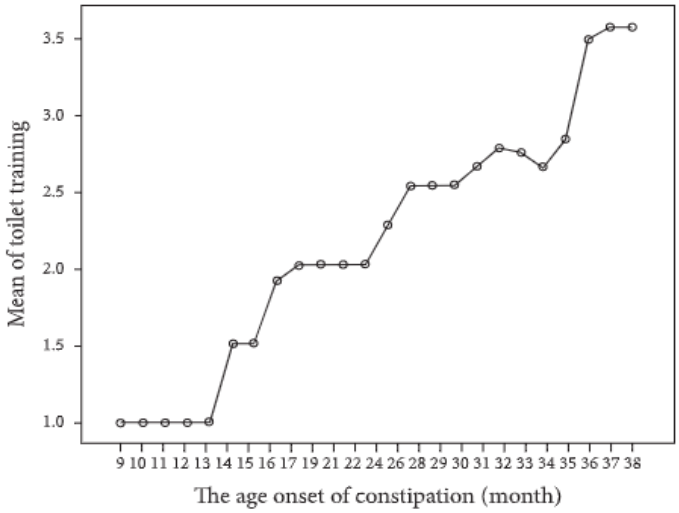


**Fig. 2.9.** Corelation between age of onset of constipation and the beginning of toilet training

Another parameter assessed in the study subjects included in the comparative study with the control group was actual nutritional status and alimentary habits. The following features were recorded: of the children with constipation, 69 patients were overweight (29.49%), compared to 10 (8.93%) in the control group; 19 children (8.12%) were underweight compared to 12 (10.71%) in the control group. The remaining patients were normal weight.

We assessed also the nutritional status of all parents. 60.68% of the parents of children in the study group were overweight or obese compared to 26.79% in the control group. At the same time, 69.57% of the overweight / obese children in the study group had parents who were also overweight / obese. Only 56.85% of parents of children in the control group were overweight / obese ($\chi^2 = 63.49$; df=4; $p<0.001$).

In order to evaluate the alimentary schedule we investigated the hour of the last meal. This varied between 19:00PM and 23:00PM in the study group compared to 19:50 for the control group.

We set out to identify a nutritional profile of the patient with constipation. To this aim, we analysed the frequency of consumption for the principle food groups. We evaluated the dietary fibre intake in fruits and vegetables.
Among the study group we observed a low intake of these compared to the control group, feature correlated with an increased intake of animal protein. (Figure 2.10)

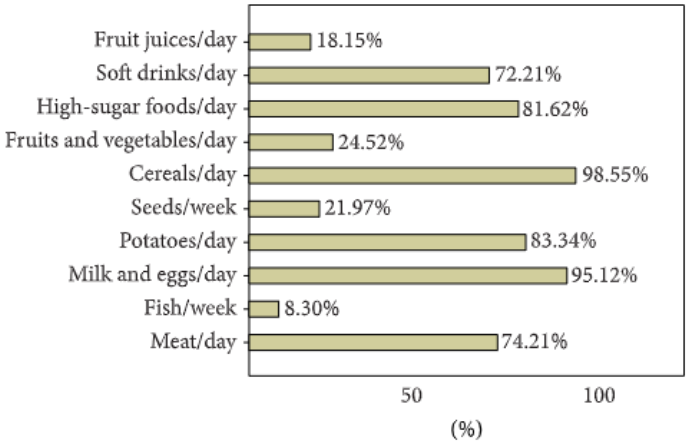


**Fig. 2.10.** Dietary Profile of Children with Constipation

With regard to physical activity, in the study group, 52.99% of children are sedentary and 39.32% practice some kind of sport at most 2hours/week while in the control group 33.93% are sedentary and 59.82% declared some form of moderate physical activity. ($\chi^2$ = 18.419; df=3; p<0.001). In the study group, we observed a greater period of time that the patients spent before the TV or computer screen. 50% of children in the study group spend between four and six hours per day before the TV or computer screen compared to 15.18% recorded in the control group. The majority of children in the control group (84.82%) spend between one and three hours per day in this mode compared to 50% of children in the study group. Thusly, we found a significant statistical correlation between the number of hours spent before a TV or computer screen and the presence of constipation. (F= 92.162, p<0.001, 95%Cl).

**Discussions**

There are few epidemiological data available regarding the age of onset of constipation in breastfed infants. (Stewart, Schroeder, 2013). There are studies which reported the onset of constipation during the first year of life, in approximately 50% of infected children. (Del Ciampo et al., 2002). Loening-Baucke consider the prevalence rates for constipation in the first and second years of life to be 2.9% and 10.1%, respectively. (Loening-Baucke, 2005).
Regarding the frequency of bowel movements, our results agree with those reached in other studies; Nyhan and collaborators realized a study on a group of 800 children and reported a frequency of 1 bowel movement every 4.4. days. (Nyhan, 1952, Metaj et al., 2003)
The observed increased frequency of constipation in relatives of children included in this study may be explained by genetic factors as well as eating habits common to all the members of the family. (American College of Gastroenterology Chronic Constipation Task Force, 2005; Longstreth et al., 2006)
Also, regarding the family history, our study revealed that a large number of grandparents fulfil the role of surrogate parents for their grandchildren. There is little data in the literature on how children living with grandparents are affected. It has been observed that psychological stress in grandparents leads to a higher degree of maladaptation in grandchildren. (Smith et al., 2008).

The literature suggests that, in many countries, toilet training is started at an increasing age, possibly as a result of introduction of disposable diapers. (Michael, 1999; Wong, Wong, 2007). Schor reported that 50% of children obtained bowel control at age 3,5 years, a significantly greater age compared to our study. (Schor, 2004)

Another factor of constipation induction may be the use of powdered milk formulas which can facilitate the development of constipation. Moreover, feeding cow milk plays an important role in slowing intestinal peristalsis in breastfed infants and little children.

The home environment of the patients influenced significantly their alimentary habits in the first months of life: thusly, as expected, a greater percentage of children fed cow's milk was found in those who came from rural environments (78.95%). Patients fed milk formula were found in proportion of 78,26% in the urban environment.

Unfortunately, diets based on cow's milk in the first year of life is a relatively common practice in poor, rural communities in Romania. Most studies in the specialty literature refer to the relation between constipation and allergy to cow milk's protein. It has been proven that bowel disorders can be the result of hypersensibility to cow milk's protein. Even though not proven clinically, excessive consumption of cow's milk may lead to the development of constipation even though there is no intolerance to cow's milk proteins, the underlying mechanism being the fact that cow's milk determines a slowing of intestinal peristalsis and triggers satiety in the child thereby reducing intake of other liquids and fibre rich foods. (Irastorza et al., 2010) For this reason the recommended intake of cow's milk must be restricted. The data analysed showed that sucklings and little children with chronic constipation in the study group consumed a greater quantity of cow's milk (563.25 ml / day) compared to children in the control group (365.63 ml / day).

Regarding actual nutritional status and alimentary habits, a significantly greater prevalence of obesity in children in the study group was identified compared to the control group ($\chi^2$ =104.94; df=2; $p<0.001$). Some authors reported obesity as being one of the risk factors for development of peristalsis disorders. (Pashankar, Loening-Baucke, 2005)

Given that obesity is the result of alimentary habits, physical activity level and hormonal influences, we set out to analyse these factors.

The nutritional behaviours in children are strongly influenced by the home environment. Parents determine the development of alimentary habits in children through their own alimentary habits and their meal schedule. (Cutting et al., 1999; Johnson, Birch, 1994).

In order to identify the risk factor for inadequate diet we took into consideration the hour of the last meal and the fast food type meals encountered in both subjects in the study group and those in the control group. In our study, in children with functional constipation, the hour of the last meal was later compared to the control group.

There was also a significant difference in the frequency of fast food meals. Thusly, in the study group, the monthly average was 3.65 meals / month compared to the monthly average of 1.36 fast food type meals / month in the control group.

Constipated subjects consumed more frequently the following foods: meat products, milk, concentrated sweets, soda drinks. Those in the control group consumed all of these less frequently showing a greater consumption of fruits and vegetables.

Our results are in concordance with other data in the literature referring to dietary recommendations for children with constipation. (Rowan-Legg et al., 2011)

With regard to physical activity, our results vary from those obtained in a group of adolescents in Taiwan but are in concordance with another study involving children whose physical activity was under 1hour per day was associated with constipation. (Chien et al., 2011; Driessen et al., 2013)

**Conclusions**

In conclusion our study revealed an increased prevalence of functional constipation in males, children from rural environments, dysfunctional families with inadequate alimentary habits. Alimentary habits during the first year of life as well as subsequent – especially the type of foods – play an important role as well as lifestyle of children with respect to physical activity. Early dietary guidance and intervention most definitely plays an important preventive role against the development of constipation.

*Limitations of the study*

This is a single center report that only included children admitted to our gastroenterology unit.

*Future directions*

We believe that the prevalence of chronic functional constipation among the children in our country might be higher; further population-based studies are necessary. At the same time, considering the reports of the World Health Organization, Romanian Health Ministry, and other studies regarding defective dietary habits, obesity, and sedentary behaviour of children in our country, their association with functional constipation requires nationwide investigation.

### *2.2.2.2. Somatization and Behavioural Problems in the Child with Chronic Constipation and Encopresis*

Functional constipation is characterized by rare bowel movements, stools of modified consistency or painful defecation without an underlying organic cause. Up to 84% of children with chronic constipation presented episodes of fecal incontinence. (Loening Baucke, 1987) Chronic constipation and secondary fecal incontinence are a source of distress for both the child and his/her family. Symptoms are often persistent and with frequent recurrences. (Bernard-Bonnin et al., 1993). Fecal incontinence can also elicit feelings of guilt and discomfort and is associated with social withdrawal, anxiety and depression. Encopresis prevalence was assessed in approximately 1-3% of the general pediatric population. (Hunt et al., 2007). Data in the literature show that this rate is higher (4%) in developing countries. (Sun et al., 2011). In Romania, no studies on encopresis were carried out before the study in which I participated. The long term results and factors influencing prognosis are controversial. While some studies reported development of behavioural disorders as predictors of poor results, Montgomery described a negative correlation with progression and prognosis factors. (Montgomery, Navarro, 2012)

In this line of inquiry, I participated in a study carried out by the team of pediatric gastroenterologists in which I contributed in the elaboration of the study protocol as well as the obtained result interpretation and integration.

The aim of this study was to identify the socio-demographic characteristics of encopresis patients and their families, development of certain behavioural problems, as well as identification of anxiety and depression disorders that appear in this group. (Olaru C et al, 2016) With this aim we concentrated on the following: identifying home and social environment conditions through determination of the educational level and current profession of the parents, study of the modifications of somatization and behaviour of the patients and we set out to establish certain correlations between the severity of the clinical manifestations and the psychosocial impact on the patients as well as their families.

**Patients and method**

With this aim we carried out a study on a group of 57 patients, over a period of 20 weeks. We included in the study, children with ages between 6 and 15 years. During history taking we obtained information regarding the level of education of the parents and their professional qualifications. At the same time we recorded data regarding the schooling rate of every child, as well as the number of school absences recorded in the last semester of school. For the

evaluation of clinical manifestations of encopresis, the children and their parents were asked to record the frequency of symptoms in a journal for a week. During this week, the laxative treatment was interrupted. In order to evaluate the impact of the encopresis disorder, all patients were subjected to a psychological exam.

**Results**

In our study subject distribution according to sex indicated the presence of 75,44% (n-43) boys and only 24.56% (n=14) girls (M:F ratio = 3.07:1). The patients had ages between 6 and 15 years, the medium age being 10.82 years. (Figure 2.11)

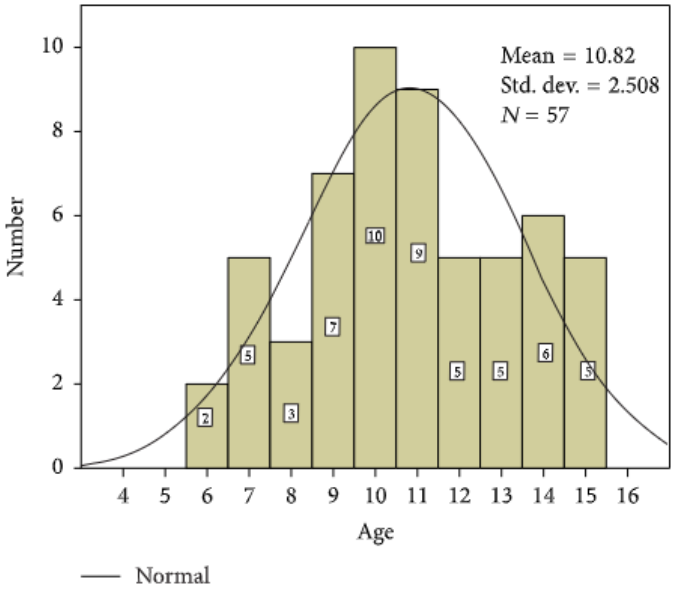


**Fig. 2.11.** Distribution of patients by age

In the study group, frequency of encopresis episodes varied between 18 episodes / month and 41 episodes / month with an average of 28.3. We did not observe a correlation between the number of encopresis episodes and the home environment. Another variable scrutinized was the average number of years of education that the parents had completed.

Thus, we identified an educational level of 11.23 ± 5.56 years in mothers while the fathers had an average number of 9.35 ± 4.53 years of study. Analysis of the current occupation and professional status showed that parents included in the study worked in various fields of work. We identified 48 unqualified workers, 23 intellectuals, 28 persons without a current place of work and 15 individuals who had entered retirement for medical reasons. Interpretation of the Anova test revealed the fact that the number of encopresis episodes per month was influenced by the level of education of the female parent. (F = 2.684, p = 0.008, 95% CI). (Figure 2.12)

We centralized data regarding education during interviews with the relatives and conversations with parents. We quantified the level of education and the number of days of school absenteeism in the study group. Of the 57 children included in the group, 9 (15.78%) abandoned school and 5 (8.77%) had to repeat a year of studies. (Figure 2.13)

21 children had a number of 0-10 absences, 31 children declared a number of 11-40 absences and 5 of them had between 41 to 100 absences. Interpretation of the Anova test showed that the number of encopresis episodes per month correlated with the number of absences. (F = 7.968, p = 0,001, 95% CI). (Figure 2.14)

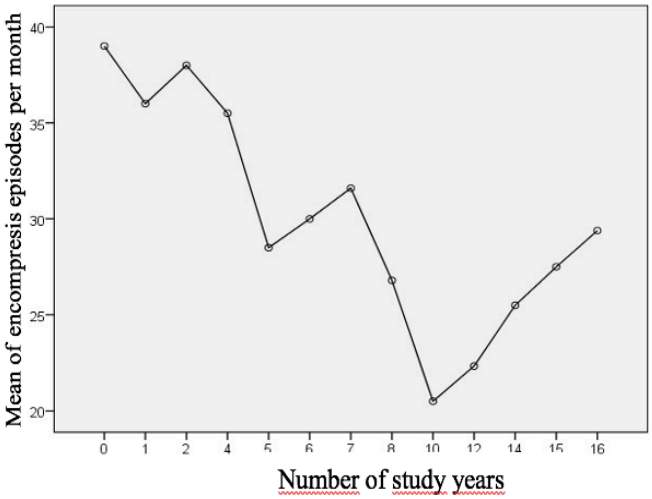


**Fig 2.12.** Average Frequency of Encopresis Episodes Relative to the Level of Education of Female Parents

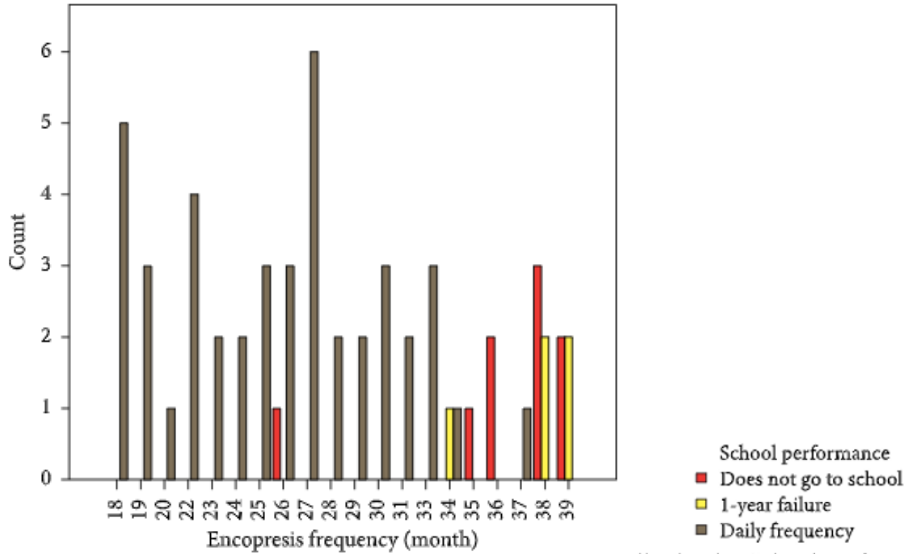


**Fig. 2.13.** Patient Distribution by School Performance and Number of Encopresis Episodes per Month

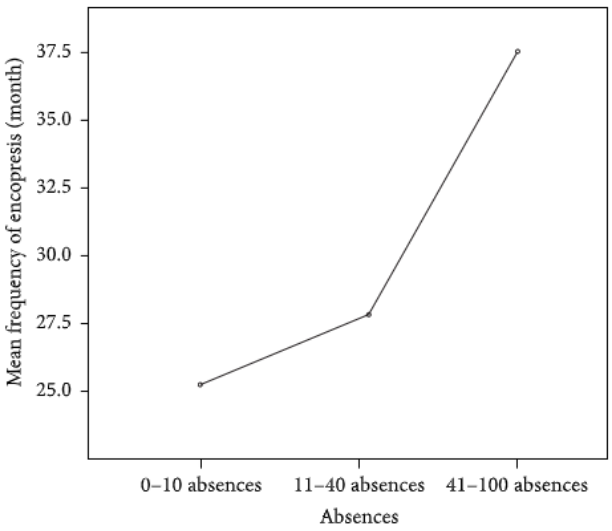


**Fig. 2.14** Average Frequency of Encopresis Episodes Relative to the Number of School Absences

Psychological data were collected with the aim of creating a profile of children with problems related to encopresis and constipation. Psychological evaluation identified (in various associations) psychomotor agitation (N = 9; 15,79%), anxiety (N = 22; 38,59%), affective deprivation (N = 30; 52,63%), social adaptation difficulties (N = 13; 22,81 %), introversion (N = 12; 21,05%), reduced tolerance to frustration (N = 11; 19,29%), depression (N = 8; 14,03%), speech disorders (N = 5; 8.76%), and emotional stress (N = 5; 8.76%). (Table 2.VIII)

**Table 2.VIII.** Psychological Modification Distribution

| Psychological examination | No. of cases | Percentage |
|---|---|---|
| Psychomotor agitation | 9 | 15.78% |
| Anxiety | 22 | 38.59% |
| Panic attack | 1 | 1.75% |
| Tic disorder | 2 | 3.5% |
| Affective deprivation | 30 | 52.63% |
| Social adjustment difficulties | 13 | 22.81% |
| Low average IQ | 2 | 3.51% |
| Negativism | 7 | 12.28% |
| Irritability, irascibility | 6 | 10.52% |
| Acute reaction to stress | 1 | 1.75% |
| Depressive syndrome | 8 | 14.03% |
| Shyness | 12 | 21.05% |
| Low tolerance to frustration | 11 | 19.29% |
| Speech disorders | 5 | 8.76% |
| Emotional distress | 5 | 8.76% |
| Hypochondriac tendencies | 1 | 1.75% |

**Discussions**

The data obtained differed from other data in the specialty literature which indicated a higher prevalence in small children. (Van der Wal et al., 2005) Similar results were obtained in the population in South East Nigeria. The authors of the study showed that encopresis had a prevalence of 3% in children age 4 and of 1.6% in children age 10 and affected predominantly males. (Roberts et al., 1992) Concerning the environment from which the subjects came, we observed that the majority came from the urban environment. The low frequency of patient in rural zones in our study may be a result of delayed diagnosis due to reduced access to medical services in disadvantaged communities as well as ignoring symptomatology by patients or their parents with a lower level of education. This idea is supported by the fact that the average duration between the onset of the disorder and the first presentation for medical consultation was greater in the rural environment compared to the urban environment (11.7 weeks / 7.8 weeks). An important aspect that we identified is the fact that only 59.7% of patients live with both parents. Data in the literature confirm the fact that family structure in which the child develops influences their subsequent cognitive and psychoaffective development and physical health. Studies involving children have concluded that those born in families with married parents had fewer psychoaffective and general health problems and higher values of cognitive scores. (Brown, 2004).

Parental education was reported as a competence marker for education of toilet training as well as a protective factor against stress related to living in a dysfunctional family. (Cavanagh et Huston, 2006). In our study, children whose mothers had a high education level declared a

smaller number of encopresis episodes per month. An important question was if frequency of encopresis episodes influences school performances.
Analysis of the schooling rate, school absenteeism and the capacity of children to complete the school year showed that the subjects with encopresis have learning disabilities, weak school performances and increased rate of absenteeism. The relationship between the state of health and academic performances is more complex than would appear at first glance. Risk factors include attitude and beliefs of the parents, models of mother-child interaction, maternal education level, socioeconomic status, familial / social support, family size, stressful life events and cognitive capacity of the child. It has been proven that there are psychosocial factors such as emotional development which affect academic achievements. Children exposed to these factors present an increased risk of developing emotional and behavioural problems as well as school failure. (Stern et al., 1988)
The most frequent modifications were anxiety and social adaptation difficulties; we show an increased rate of somatic and behavioural disorders. Data in the literature show that the association of encopresis with behavioural disorders has led to an unfavourable prognosis of this illness. (Zaky et al., 2016) Levine has concluded that patients with encopresis who did not respond to treatment had a higher rate of aggressive antisocial behaviours before initiation of treatment. A series of studies have demonstrated reduced social abilities and a degree of social isolation greater in children with encopresis. (Zaky et al., 2016; Mellon et al., 2013)
As far as treatment is concerned, attention deficit and behavioural problems may be a cause of treatment failure; improvement of these parameters through specific interventions may increase compliance to treatment and may prevent development of domestic conflicts. (Zaky et al., 2016) On the other hand, familial factors such as depression and / or symptoms of maternal anxiety are associated with bowel movement disorders at school age and factors related to family functioning should be included in the psychological intervention. (Van Dijk et al., 2015)

**Conclusions**

The conclusions of this study permit an outline of the profile of patients with encopresis as far as psychosocial factors are involved in the multifactorial determinism of this disorder.
Parent education regarding the associated somatic and behavioural disorders can lead to a better diagnosis and a better response to treatment for children with constipation and fecal incontinence.

*Originality and applicability of the results in medical practice*

Prior to the publication of our study, no studies related to encopresis were performed on the pediatric population in Romania.
Screening of behavioural disorders in patients with encopresis may be useful for their therapeutic management. A more aggressive treatment for constipation may be justified for these patients. In the cases associating serious behavioural disorders, early diagnosis and multidisciplinary treatment could be useful for both the child and the family.

*Limitations of the study*

This is a single center report and is limited to children addressed to our gastroenterology center. It is not clear whether the results would hold for a nationally representative sample of children in Romania. Because the period covered by the study was also relatively short, it is not clear whether the disparities between children of parents with more or less education remain as children age and how they affect a larger set of outcomes.

*Future directions*

Our analysis leaves many questions for future research. The behavioural disorders noticed in children with encopresis could be either a result of their excessive concern with uncontrollable encopretic accidents and the resulting social tension or a result of some developmental delays that could ultimately play a part in the development or persistence of encopresis. Another

critical question is the extent to which specific policies and programs dedicated to children can help address the observed deficits (improvement of access to health care services and various means of cognitive development). Future research is necessary in this direction.

***

## 2.3. SUMMARY OF THE RESULTS OBTAINED ON TOPICS DERIVED FROM THE RESEARCH DIRECTION

**Results obtained and novelty elements**

Following the research carried out in this direction, we have obtained a series of important results which I present below in summary, structured according to the research sub-directorates:

– ***Digestive Endoscopy in Child, Adolescent and Young Adult**:*

- Regarding esophageal foreign bodies, our study presented the experience of a tertiary center of Pediatric Gastroenterology and performed an analysis of the patient s symptoms, the type of foreign bodies, the therapeutic management and the evolution of patients. Based on these data, it can be concluded that it is necessary to standardize the diagnostic and therapeutic behavior in this pathology and in the pediatric gastroenterology services. Also, a permanent emergency digestive endoscopy service would be very useful in pediatric department.
- Regarding the polypoid lesions identified endoscopically at the level of the upper digestive tract in children, we published an article in which we presented 3 distinct cases from a series of over 2000 endoscopic explorations. Although exogastric polypoid lesions are relatively common in adults, these structures are encountered in less than 1% of the upper digestive endoscopies carried out in children; data in literature is isolated and refer, as in our article, to small cohorts of patients
- An aspect published for the first time by a group of Romanian authors described the anxiety related to upper gastrointestinal endoscopy exploration in both children and their relatives. We also analyzed the role of psychological counseling of young patients and their parents before endoscopic exploration.

– ***Functional Digestive Disorders***

- We analyzed the factors that promote constipation in children and, in addition to diet, BMI, environment and the age from which the child begins to use the toilet independently, our study has shown that there are socio-familial and environmental risk factors, as well as a positive history of constipation in relatives.
- We analyzed the profile of the pediatric patient with encopresis and showed that behavioral disorders in children as well as anxiety or depression in the mother are frequently associated with this pathology and influence the evolution and response to treatment.

**Dissemination of the results and scientific visibility**

From the scientific activity carried out on this research direction (documentation and clinical research), as presented in tables 2.IX, resulted 7 articles published in extenso in ISI listed journals, 1 article in journals ISI proceedings, 7 articles in BDI indexed journals.

The visibility of the research in this direction is reflected in the number of citations (WOS- 57 citations and Google Scholar = 110) as well as in the cumulative impact factor of the journals in which the ISI articles were published (cumulative global FI / direction = 16.641). One article was also awarded by UEFISCDI.

As I mentioned before, the interdisciplinary collaboration in this direction brought me many satisfactions: in addition to publications in international journals and a significant number of citations, I participated in the design of research grants (a research project funded by a private

foundation, which won through competition, and a well-ranked cross-border project after evaluation, which did not qualify for funding), I collaborated on a video atlas of digestive endoscopy (now in the editing stage), I was lead author or co-author of numerous papers presented at national and international scientific events.

**Table 2.IX.** Scientific Articles published *in extenso* on themes of Pediatric Gastroenterology

| Nr. crt. | Title / Author / Journal / Impact Factor | WOS Citations | Google Scholar Citations |
|---|---|---|---|
| **Scientific Articles published *in extenso* in ISI ranked journals** | | | |
| 1. | UNUSUAL ENDOSCOPIC FINDINGS IN CHILDREN: ESOPHAGEAL AND GASTRIC POLYPS. THREE CASES REPORT. Diaconescu S, Miron I, Gimiga N, Olaru C, Ioniuc I, Ciongradi I, Sarbu I, **Stefanescu G**: *Medicine*, 2016, DOI: 10.1097/MD.0000000000002539 , ISSN: 0025-7974, Online ISSN: 1536-5964<br>**IF=1,804** | 11 | 14 |
| 2. | CHRONIC FUNCTIONAL CONSTIPATION AND ENCOPRESIS IN CHILDREN IN RELATIONSHIP WITH THE PSYCHOSOCIAL ENVIRONMENT-A PROSPECTIVE COHORT STUDY: Olaru CA, Diaconescu S*, **Stefanescu G**, Gimiga N, Olaru RA, Ciubotariu G, Burlea M, Iorga M. *Gastroenterology Research and Practice*, vol. 2016, Article ID 7828576, 7 pages, 2016. doi:10.1155/2016/7828576<br>**IF=1,863** | 16 | 24 |
| 3. | FOREIGN BODIES INGESTION IN CHILDREN-EXPERIENCE OF 61 CASES IN A PEDIATRIC GASTROENTEROLOGY UNIT FROM ROMANIA. Diaconescu S, Gimiga N, Sarbu I, **Stefanescu G**, Olaru C, Ioniuc I, Ciongradi I, Burlea M. *Gastroenterology Research and Practice*, vol. 2016, Article ID 1982567, 6 pages, 2016. doi:10.1155/2016/1982567<br>**IF=1,863** | 11 | 34 |
| 4. | SOME RISK FACTORS OF CHRONIC FUNCTIONAL CONSTIPATION IDENTIFIED IN A PEDIATRIC POPULATION SAMPLE FROM ROMANIA. Olaru C, Diaconescu S*, Trandafir L, Gimiga, N **Stefanescu G,** Ciubotariu G, Burlea M. *Gastroenterology Research and Practice*, vol. 2016, Article ID 3989721, 8 pages, 2016. doi:10.1155/2016/3989721<br>**IF=1,863** | 9 | 25 |
| 5. | DERMATOLOGICAL MANIFESTATIONS IN PEDIATRIC INFLAMMATORY BOWEL DISEASE. Diaconescu S, Silvia Strat, Gheorghe G Balan, Carmen Anton, **Stefanescu G**, Ioniuc I, Stanescu AA. *Medicina* 2020, 56(9),425; https://doi.org/10.3390/medicina56090425<br>**IF=2.43** | | 1 |
| 6. | CONSIDERATIONS ON CHILD ABUSE FROM A MEDICAL AND PSYCHOSOCIAL PERSPECTIVE. Iorga M, **Ştefanescu G**, Gimiga N, Olaru C, Ion L, Kantor C, Russo M, Diaconescu S. *Revista De Cercetare Si Interventie Sociala* , 2018, 61, 231<br>**IF=1.076** | | 1 |
| 7. | COULD THE BURDEN OF PANCREATIC CANCER ORIGINATE IN CHILDHOOD? Diaconescu S, Gîlcă-Blanariu GE, Poamaneagra S, Marginean O, Paduraru G, **Stefanescu G .** *World J Gastroenterol* 2021; 27(32): 5322-5340.<br>**IF=5.742 (2020)** | | |
| **Scientific Articles published *in extenso* in ISI proceedings:** | | | |
| 8. | ANOREXIA NERVOSA AT A YOUNG MALE PATIENT-CLINICAL AND THERAPEUTIC APPROACHES. CLINICAL CASE PRESENTATION. Bolos, A; **Stefanescu, G**; Barzu, A; Szalontay, AS., *Neurogastro* 2017 - Meeting Of The Romanian Society Of | 1 | |

| Nr. crt. | Title / Author / Journal / Impact Factor | WOS Citations | Google Scholar Citations |
|---|---|---|---|
| | Neurogastroenterology With Rome Iv Regional Central East European Meeting, 2017: 177-181. | | |
| **Scientific Articles published *in extenso* in BDI indexed journals:** | | | |
| 9. | RARE CAUSES OF ACUTE ESOPHAGITIS WITH SEVERE DYSPHAGIA IN CHILDREN. Diaconescu, S; Schiopu, CG; Gimiga, N; Moisa, SM; Ghiga, G; Donea, L; **Stefanescu, G**; Rosu, OM) *Romanian Journal Of Oral Rehabilitation* Volume: 11 Issue: 2 Pages: 49-54 Published: APR-JUN 2019. WOS:000472600400007 | | |
| 10. | APPROACH TO *HELICOBACTER PYLORI* INFECTION IN SPECIFIC AGE GROUPS. **Ştefănescu G**, Bălan GG, Gîlcă-Blanariu GE, Olaru C, Timofte O, Gimiga N, Roşu OM, Anton E, Ion LM**,** Diaconescu D. *International Journal of Medical Dentistry* • issue 2 April / June 2018 • pp. 113-121 | 1 | 2 |
| 11. | ULCERATIVE COLITIS ASSOCIATED WITH VITILIGO AND IGA DEFICIENCY IN A YOUNG GIRL. Naumcieff I, Burlea M, Diaconescu S*, Chiriac MI, Olaru C, Gimiga N, Ciubotariu G, MihăilăD, **Ştefănescu G**, Trandafir LM. *Arch Clin Cases 2017; 4(1):41-46* DOI: 10.22551/2017.14.0401.10092 41 | | 1 |
| 12. | EFFECTIVE COMMUNICATION AND PSYCHOTHERAPY IN REDUCING ANXIETY RELATED TO DIGESTIVE ENDOSCOPY PROCEDURES FOR PEDIATRIC PATIENTS. Gimiga N, Bors AM, **Ştefănescu G**, Iorga M, Diaconescu S . *International Journal of Medical Dentistry*, 2016; 6 (4) 255-260 | 8 | 3 |
| 13. | ANORECTAL MALFORMATIONS IN A TERTIARY PEDIATRIC SURGERY CENTER FROM ROMANIA: 20 YEARS OF EXPERIENCE Ciongradi I, Aprodu G, Olaru C, **Ştefănescu G**, Ioniuc I, Gimiga N, Iorga M, Sârbu I, Diaconescu S. . *Journal of Surgery [Jurnalul de chirurgie].* 2016; 12(2): 131-137 DOI:10.7438/1584-9341-12-1-21 | | 5 |
| 14. | ALTERNATIVE THERAPIES IN REDUCING ANXIETY AND PAIN FOR INVASIVE PROCEDURES IN PEDIATRIC PRACTICE Diaconescu S, Iorga M, Bolat M, Stanca R, **Stefanescu G.** *Romanian Journal of Oral Rehabilitation.* 2015; 7 (4)78-83. | | |
| 15. | CURRENT RECOMMENDATIONS IN PEDIATRIC INTERVENTIONAL GASTROINTESTINAL ENDOSCOPY<br>Diaconescu S, Donea L, NichitaA, Strat S, Rosu OM, Gimiga N, Olaru C, Ghiga G, RotaruB, BozomituL, Balan Gh, **Stefanescu G.** *Romanian Journal of Pediatrics* . 2019, Vol. 68 Issue 3, p166-170. | | |
| **Total citations** | | **57** | **110** |
| **Cumulative impact factor / direction** | | **16.641** | |

The success of the collaboration with the team of pediatric gastroenterologists and with psychologists and psychiatrists is also proved by the invitations to contribute with original or review articles in journals with high impact factor. Some of the invitations were accepted and materialized by publishing articles (Diaconescu et al, 2021; Gîlcă-Blănariu 2020).

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In view of all these aspects, I consider that the research direction presented in this chapter has a consistent scientific coverage and is a guarantee that all the research that we will continue to carry out in this field will benefit from the acquired expertise.

# Chapter 3
# BETWEEN MEDICAL AND SURGICAL PROCEDURES

## 3.1. CONTEXT AND RATIONALE OF THE RESEARCH TOPIC

Endoscopic Retrograde Cholangiopancreatography (ERCP) is the most complex interventional method in gastroenterology, assimilated to surgical procedures and is a challenge for any gastroenterologist.

First ERCP procedures are mentioned as far as 1968 (ASGE, 2016) and since then it has become an indispensable procedure for almost all interventional endoscopists. To date, more than 500000 ERCP procedures are performed each year in the United States providing a less invasive treatment for pancreatic and biliary diseases (Anderson et al., 2012). In the last decades the role of ERCP has evolved from a mainly diagnostic one to a highly therapeutic one (ASGE, 2016), allowing endoscopists to perform endoscopic sphincterotomy, insert biliary or pancreatic stents, remove bile duct stones, perform dilation of stenoses or to take brush cytology samples. Nevertheless, ERCP is well known for its risks, complications and adverse effects, therefore being considered the most difficult to perform endoscopic procedure. All these potentially negative aspects can be classified as pre-procedural, intra-procedural or postprocedural conditions. Early recognition and prompt management of adverse effects and complication is a key factor to the optimal management of ERCP-related mortality and morbidity. The last several years have been marked by the interest for ERCP-related adverse effects and complications.

In this context, in the last 5 years, together with the interventional endoscopy team that performs ERCP in the Institute of Gastroenterology and Hepatology in Iași and in collaboration with two research teams (one from Gheorghe Asachi Technical University and another from "Petru Poni" Research Institute from Iași), I participated in a series of researches that focused on two of the most common complications encountered in patients who underwent ERCP: infections and acute post-ERCP pancreatitis.

We have addressed issues related to pre-procedural activities (obtaining informed consent), equipment preparation, and we focused on identifying and managing risk factors for the two complications mentioned.

## 3.2. SCIENTIFIC ACHIEVEMENT IN THE FIELD OF RESEARCH

**Relevant publications in the field of the third research direction**

Next, in the habilitation thesis, I will present the results published in the main articles in which I participated as main author or co-author (reproduced with permission). These are systematized in two sub-directions - ERCP-associated infections and post-ERCP pancreatitis (PEP).

The studies undertaken on each of the two directions had on the one hand the analysis of some risk factors for the occurrence of these complications and, on the other hand, the identification of useful solutions in their medical management.

***

### 3.2.1. ERCP-associated infections

In the former years post-ERCP pancreatitis, followed by bleeding or perforation were well studied complications (Andriulli et al.,2007; Kochar et al., 2015). Recently ERCP-related

infections became a dominant point of interest for a great part of the medical community because have clearly become a worldwide issue. Moreover, infections after other various gastrointestinal endoscopic procedures were found to be substantially more common than previously thought (Wang et al.,2018), thus requiring prompt and effective administrative interventions not only on the reprocessing protocols of devices, but on the design and technology of endoscopes.

In the United States, the FDA and the Centers for Disease Control and Prevention (CDC) have taken a strong position against the duodenoscope-related nosocomial infections by releasing, on February 26, 2018, new standardized protocols for duodenoscope surveillance sampling and culturing that have had great visibility and impact worldwide, however the incidence and prevalence of such infections seems to remain almost unchanged (Calderwood et al., 2018).

The problems related to duodenoscope-associated infections and their human-to-human transmission despite thorough reprocessing methods have stated new challenges for cleaning and disinfection of such medical devices worldwide (Humphries et al., 2015).

Duodenoscopes are different from standard digestive endoscopes by their complex design involving the presence of an elevator channel located at the tip of the endoscope that permits manipulation of different accessories primary used in endoscopic retrograde cholangiopancreatography (ERCP) (Kim, Muthusamy, 2016). Duodenoscopes are multiple use devices requiring cleaning, high-level disinfection (HLD) or sterilization and subsequent drying dependent on the recommendations of the manufacturer (Mcdonnell et al., 2016).

*Duodenoscope-related infections*

First duodenoscope-related infection was presented around almost 30 years ago (Allen et al., 1987); historically, endoscope related infections were characterized by the constant thread of reprocessing errors or lack of adherence to the reprocessing protocoles indicated by the producers (Gastmeier, Vonberg, 2014; Aumeran et al., 2010). Transmission of such infections despite producer reccomanded reprocessing protocols was recognized only recently, the main cause being considered the difficult-to-clean duodenoscope devices which are able to select, harbor and move multidrug-resistant bacteria. Most importantly, as current studies state, such infections occur despite recognizable breaches of standard reprocessing protocols (Alrabaa, 2013; Epstein et al., 2014).

Traditionally, since 1968, medical devices have been categorized in three general classes tributary to the Spaulding classification (Spaulding,1968): 1. *Critical devices* – medical devices entering sterile tissue or blood vessels. These devices should always be sterile; 2. *Semicritical devices* – medical devices that come in contact with mucous membranes and/or non-intact skin. These devices require high-level disinfection (HLD). Digestive endoscopes and duodenoscopes have been considered semicritical devices; 3. *Noncritical devices* – medical devices that come in contact only with intact skin. These devices are considered safe if at least cleaned with usual surface disinfectants.

It has been clearly shown that even if endoscopes are semicritical devices, associated and conjunct devices like biopsy forceps and sphincterotomes are critical devices that should be sterilized after each procedure (Kovaleva et al., 2013). Therefore, a problem not resolved by the Spaulding classification is that of semicritical devices in need of being used in conjunction with critical devices (Rutala, Weber, 2008; Petersen et al., 2016). Whether or not HDL is still a gold-standard for these devices is debatable.

*Reprocessing duodenoscopes*

As the literature of the field has always stated, the efficacy of world widely used HLD practices was time-validated and time-tested within a very narrow safety margin for eradication of endoscope contaminating microorganisms (Schaefer et al., 2010). While sterilization can be defined as the process leading to complete absence of any type of contaminating microorganism, as stated by the US FDA, HDL is a reprocessing method aiming at inactivating a large amount of microorganisms (such as bacteria, viruses or fungi) to a extent of $10^6$ to $10^9$

reduction of the endoscope bioburden (Mcdonnell et al., 2012; Andriulli et al., 2007 Petersen et al., 2016).

In the most recent context of duodenoscope related infection bursts most producers reviewed the standard protocols for endoscope reprocessing making them harder and harder to comply, monitor, validate or even follow-up. Nevertheless, unfortunately, there are still no consistent, intensive and validated methods for monitoring HLD and duodenoscope reprocessing (Petersen et al., 2011). As stated by the vast majority of recent studies, HLD in duodenoscopes needs to be achieved not only for the outer surface of the medical device but also to the inner working channels and wire channels, especially in what the older models of duodenoscopes with no sealed elevator channel are concerned. Moreover, the recess from under the elevator is usually concealed and isolated during the pre-HLD steps in this way formation of resistant microbial biofilms seem to occur (Kim et al., 2016). Any unanticipated damage in the proximity of the duodenoscope tip may lead to persistent bacterial colonization (Kim et al., 2016) and resistant bacteria selection.

**Personal contributions in the field of ERCP-associated infections**

The results obtained from research in this field have been published in 3 ISI-listed articles, which can be found in the table 3.I.

**Table 3.I.** Publications in the field of ERCP-associated infections :

| |
|---|
| PRELIMINARY STUDY ON EROSION OF POLYMER COATINGS OF DUODENOSCOPES.<br>Balan G Gheorghe , Pavel Laura , Sandu Andrei Victor, **Stefanescu Gabriela***, Trifan Anca Victoriţa<br>o *Materiale plastice* 2016, **, IF – 0.778.** |
| DUODENOSCOPE-ASSOCIATED INFECTIONS BEYOND THE ELEVATOR CHANNEL: ALTERNATIVE CAUSES FOR DIFFICULT REPROCESSING.<br>Balan G Gheorghe, Rosca Irina, Ursu Elena Laura, Fifere Adrian, Varganici Cristian Dragoş, Doroftei Florica, Turin-Moleavin Ioana Andreea, Sandru Vasile, Constantinescu Gabriel, Timofte Daniel, **Stefanescu Gabriela,** Trifan Anca Victoriţa, Sfarti Cătălin Victor.<br>o *Molecules* 2019, **IF – 3.267** |
| PLASMA-ACTIVATED WATER: A NEW AND EFFECTIVE ALTERNATIVE FOR DUODENOSCOPE REPROCESSING .<br>Balan G Gheorghe , Rosca Irina, Ursu Elena Laura, Doroftei Florica, Bostanaru Andra-Cristina, Hnatiuc Eugen, Nastasa Valentin, Sandru Vasile, **Stefanescu Gabriela,** Trifan Anca Victoriţa, Mares Mihai.<br>o *Infection and drug resistance* 2018, **IF – 3.000.** |

### *3.2.1.1. Preliminary Study on Erosion of Polymer Coatings of Duodenoscopes*

Based on these considerations, in 2016 we participated in an experimental study together with a team of researchers from Gheorghe Asachi Technical University of Iasi, Faculty of Materials Science and Engineering through which we set out to verify whether through normal use, the duodenoscope suffers microscopic damage to the outer shell that may be associated with the formation of a possible biofilm responsible for some of the infectious complications of ERCP.

The study consisted of a preliminary assessment of the polymer coatings of duodenoscopes, which have multiple functions. The duodenoscope is in contact with all liquids from saliva, gastric juices and gall liquids, next to erosion. Direct imaging was used to identify the superficial erosion with the help of an optical microscope

**Materials and methods**

Two standard duodenoscopes were involved in the study, one new – as reference and one after appreciatively 1 year of normal use. The optical micrographs were obtained using a Zeiss Imager A1m microscope, using dark field and bright field filters, at magnifications between 50X and 200X, which is attached to a camera and specialized software AXIOCAM. Further

studies will involve scanning electron microscopy. Three areas were selected next to the interface between two of them: A – the top end area which is the most subjected to friction; B – the intermediate area which requires the most elasticity; C – normal polymeric coating; A-B – the interface area between A and B where a polymeric binder is used (figure 3.1.).

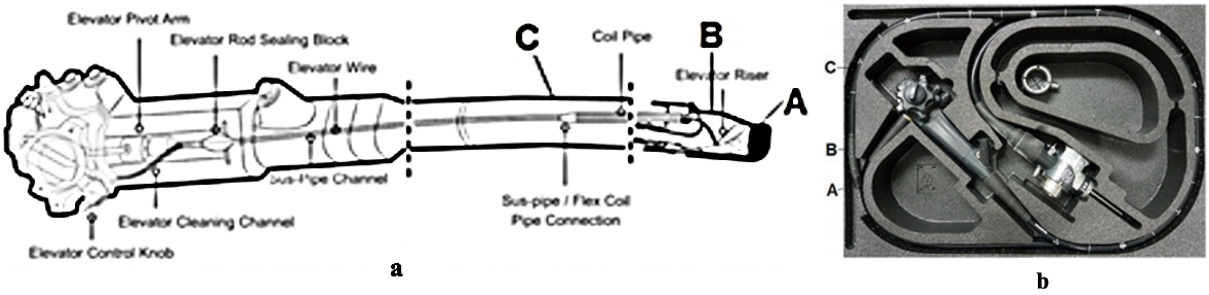


**Fig. 3.1** The duodenoscope: **a** - constructive scheme (31) and **b** – image of a duodenoscope with the analysed areas A, B and C

Reprocessing protocol of duodenoscope (model acquired in 2014, 150 ERCPs) is presented in table 3.II. After these treatments the duodenoscopes are dried and than stored or used.

**Table 3.II.**The reprocessing protocol

| Operation | Time | Solution composition | pH |
|---|---|---|---|
| Cleaning | 15 minutes | decilmetilamonium propionate, polyhexamide, surfactants, enzyme complex, 0.5% dilutions, in room temperature water | ~ 7 |
| Disinfection | 15 minutes | decilmetilamonium propionate, polyhexamide, surfactants, enzyme complex, 0.5% dilutions, in room temperature water | ~ 7 |
| HLD | 15 minutes | Stabilized glutaraldehyde 20% | ~ 6 |

## Results and discussions

Using the optical microscopy the superficial layer can be clearly observed in figure 3.2.

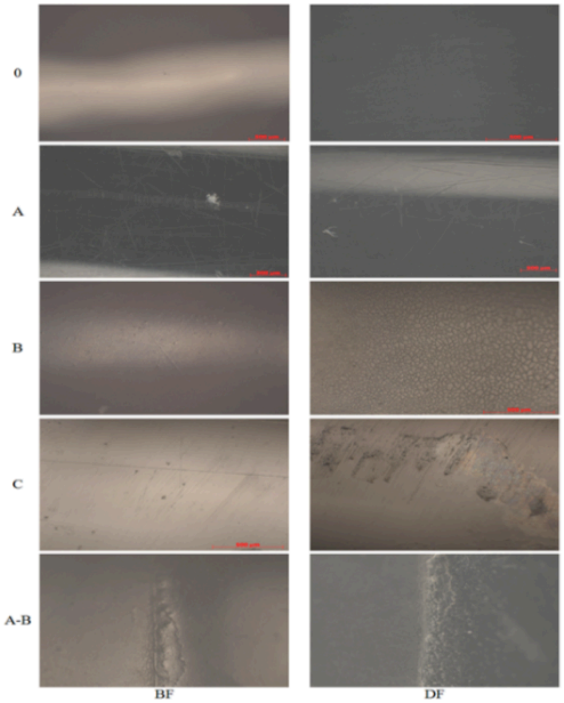


**Fig. 3. 2.** The optical micrographs on the analysed areas using bright field (BF) and dark field (DF) filters at magnification of 50 and 100X to infection.

The areas selected for the analysis are made from different composition and texture, having different functions and due to this were analysed in both dark field, which shows real color of materials, and also bright field. The polymer coating of the duodenoscopes presents multiple structures and composition, from here the different deterioration of it. The experimental part of the study clearly showed that even normal day to day usage of duodenoscopes may lead to surface damages, probably making them prone to harbouring multiresistent extremely adherent bioburden, capable to defy HLD (Pajkos et al., 2004). Such biofilm is seen as difficult to eradicate as harbored bacteria are 10-1000 times more resistant to antibiotics than planktonic cells (Mah et al., 2001). It has been reported that concentrations of antibiotics required to achieve bactericidal activity against such adherent organisms can be three to four orders of magnitude higher than for planktonic bacteria, depending on the species-drug combination (Schierholz et al., 1999).

Consequently, as to what the cause of such bioburden is concerned, according to a recent review article, a consistent number of studies concluded that many of the duodenoscope-transmitted infections occurred independently on any breach in reprocessing protocols or device quality (Gastmeier et al., 2014). Moreover, similar endoscope transmitted infections have been demonstrated also in gastroscopes and colonoscopes (Ribeiro, De Oliveira, 2012; Kinney et al., 2002), therefore also the outer surface of the medical device could be incriminated, despite the complex technical structure of the duodenoscope. Cross-contamination of endoscopes during reprocessing could also play an important role (Lubowski, Newstead., 2006). Findings of a recent study show that such endoscope related infections occur independently on the producer of the medical device, on the HLD protocol used or on the endoscope model studied (Mcdonnell et al., 2016). These results may once more generate the idea that the potential multidrug resistant biofilm is a diffuse bioburden on endoscopes. Such bioburden load may be promoted by surface alterations of medical devices which even if associated with normal use may promote aggregation and adhesion of microorganisms (Chhaya et al., 2015). As it is normal, the unused coating (the new one) presents no scratch or deterioration on the surface, compared to the used ones in various locations, as the one selected by us and marked with A, B and C. A zone is the area with the most friction, being at top end of the device. The second area, B, is the one with the most elasticity required – presenting a texture with superficial microcracks. The C area presents longitudinal scratches due to the erosion on insertion and removal of the device from the tract. The interface between A and B is made of a binder polymer with small superficial porosity, which can be susceptible to biofilm formation.

**Conclusions**

Duodenoscope-related infections may be seen as a major health issue world-wide. Conjunct effort is made on sorting out its causes and on finding solutions in order to assure the best possible standard of care and outcomes for patients undergoing ERCP. Thorough assessment through experimental studies of duodenoscope structure may generate new ideas on potential weak points related to duodenoscope biofilms. Regular usage of duodenoscopes lead to surface damages. The properties of the coatings are very important, requiring high elasticity and microhardness, next to antimicrobial activity. The occurred degradation by erosion or the chemical interactions influence the surface susceptibility.

*Originality and applicability of the results in medical practice*

The study is one of the few of its kind performed for devices used in digestive endoscopy and, to our knowledge, the first in Romania.

The results obtained by the experimental study showed that a duodenoscope used under normal conditions for about 1 year shows microscopic damage that can predispose to the development of resistant germs. These findings are arguments for further research in order to produce / use materials with increased resistance to mechanical or chemical erosion, which may have

antimicrobial properties, and which are integrated into the composition of the endoscopic devices.

*Limitations of the study*

Extending our investigation to the internal elements of the duodenoscope (working channel, air / water channel, elevator) as well as identifying potential residues and carrying out a microbiological analysis would have added value to our study. Such aspects were addressed in the following studies in which I participated, as detailed below.

*Future directions*

The aims of future studies should include comparative analyses of duodenoscopes with varying degrees of wear and tear as well as comparative analyses of the impact that different chemicals used for reprocessing have on the degree of duodenoscope wear and tear.

*

Taking into consideration the relevance and potential for opening new avenues of research of the preliminary experimental study results, in 2018 we participated in another study in collaboration with a team of researchers from the "Petru Poni" Institute of Macromolecular Chemistry in Iasi. This second study aimed to achieve an advanced characterization of physicochemical properties and antibacterial behaviour of duodenoscope components

### *3.2.1.2. Duodenoscope-Associated Infections beyond the Elevator Channel: Alternative Causes for Difficult Reprocessing*

As I mentioned earlier, multiple recent reports associate multidrug-resistant organisms (MDRO) with duodenoscope-transmitted infections during endoscopic retrograde cholangiopancreatography (ERCP).

Particularly, MDROs, such as carbapenem-resistant enterobacteriaceae (CRE), were associated with ERCP-related nosocomial clusters due mainly to difficulties in the adequate cleaning of the elevator channel and recess (Rutala, Weber, 2015), which are independent of potential breaches in the reprocessing standards (Epstein et al., 2014; Verfaillie et al., 2015). The development of a multidrug-resistant biofilm is thought to be triggered by repeated bacterial contamination of the duodenoscope parts that are difficult to clean or even sealed (Verfaillie et al., 2015).

Hence, manufacturers, such as Olympus America, have repeatedly announced sustained arrangements for the return of scope devices for elevator replacement that would allow consistency with the FDA cleared protocols (Boumitri et al., 2018). Moreover, manufacturers have developed new duodenoscope models that either ensure proper sealing of the less accessible elevator channel (OLYMPUS GmbH), or create detachable and autoclavable (KARL STORZ, Germany) or even single-use disposable elevator devices (PENTAX Medical, DEC™). Furthermore, recent data sustain the idea that most of the inert surfaces of the scopes become more susceptible to contamination because of significant deterioration, due to repeated daily use. (Petersen et al., 2016; Lee et al., 2015) We studied this aspect and reported the results in the preliminary experimental study, previously presented (Balan et al., 2016). MDROs have the capacity to form biofilms that are resistant to both physical cleaning and chemical disinfection (Otter et al., 2015) and such biofilm formation is promoted by the difficulty of accessing surface reprocessing, and also by surface defects from manufacturing or secondary to physical damage due to passing forceps or routine instrument handling (Kovaleva et al., 2013; Chhaya et al., 2015).

Consequently, the aim of this second study, was to describe possible scope surface damages secondary to routine wear that could provide an alternative cause for duodenoscopes being less amenable to proper reprocessing and clearance of bioburden.

As mentioned above, this study was conducted in collaboration with a research team from the Center of Advanced Research in Bionanoconjugates and Biopolymers (IntelCentru), "Petru

Poni" Institute of Macromolecular Chemistry in Iasi.
In addition to the original study, in which only optical microscopy was used for analysis, in this second study, we aimed to analyse the physical characteristics, chemical, thermal behavior, antibacterial activity of both the surface coating along the length of the duodenoscope as well as of its internal structures (work channel, air-water channel, elevator).

**Materials and Methods**:
In order to assess both outer and inner surfaces, a duodenoscope was dismantled and four samples were taken from the outer resin polymer and from the air/water, elevator, and working (biopsy) channels that were characterized by Fourier Transform Infrared Spectroscopy (FT-IR), Differential Scanning Calorimetry (DSC), Thermogravimetric Analysis (TGA), Atomic Force Microscopy (AFM), Scanning Electron Microscopy (SEM) techniques and the antimicrobial activity were tested.

***Duodenoscope Samples***
We selected a duodenoscope from a high-volume tertiary hospital that was previously used in up to 500 ERCP procedures between 2012 and 2014. The duodenoscope was reprocessed, cultured, and quarantined, and then re-reprocessed and recultured with each reprocessing cycle performed following the manufacturer's revised protocol. Both culture cycles were negative. Afterwards, it was dismantled and samples from the resin polymer outer coatings were processed for analysis (first sample in contact with the distal tip, second sample at 20 cm gradation, third sample at 60 cm gradation, and fourth sample at 120 cm gradation). Samples from the air/water, elevator, and working channels were taken at 5 cm from the distal end which was the site considered most exposed to friction secondary to distal tip angulation. The elevator was detached and analysed separately. The dismantling process was performed in a microbiologically controlled environment

***Methods***
*Fourier Transform Infrared Spectroscopy (FT-IR)* offers quantitative and qualitative analysis for organic and inorganic samples. The spectra produce a profile of the sample, a distinctive molecular fingerprint that can be used to screen and scan samples for many different components.
*Differential Scanning Calorimetry (DSC)* is used widely for examining polymeric materials to determine their thermal transitions. The observed thermal transitions can be utilized to compare materials.
*Thermogravimetric Analysis (TGA)* is a method of thermal analysis. This measurement provides information about physical phenomena and can be used to evaluate the thermal stability of a material.
*Antibacterial Activity* The antimicrobial efficacy of the duodenoscope samples was investigated via a slightly modified Japanese industrial standard JIS Z2801:2000 (JIS Z, 2018)
The antibacterial activity was determined against two different reference strains: Escherichia coli ATCC25922 and Staphylococcus aureus ATCC25923. The tested duodenoscope samples were further used in order to be scanned for biofilm formation.
Images from the study samples collected from the surface of the duodenoscope and the biofilms were recorded by *Atomic Force Microscopy (AFM)*.
The surface morphology of the study samples was observed by *Scanning Electron Microscopy (SEM)*
The aforementioned techniques used in the analysis of the samples were carried out by researchers at the "Petru Poni" Institute according to standard procedures. They also interpreted the results.
The funding of the tests was supported by two research grants (European Union's Horizon 2020 research and innovation programme under grant agreement No. 667387 WIDESPREAD 2-2014 SupraChem Lab. This work was also supported by a grant from the Romanian Ministry

of Research and Innovation, CCCDI–UEFISCDI, project number PN-III-P1-1.2-PCCDI-2017-0697/13PCCDI/2018, within PNCDI III.)

## Results and Discussion

### *Fourier Transform Infrared Spectroscopy (FT-IR)*

Figure 3.3 presents the FT-IR spectra of the samples with a higher degree of wear in the reverse order of their numbering. From the fourth to first sample, significant differences occur assigned to carbonyl vibrations, by a major decrease in the carbonyl band intensity. Some authors associated this event with a change in crosslinking density (Xue et al., 2016), which confirms the results obtained by thermal analysis. Since the FT-IR spectra reveal substantial changes in the vibrational band intensities ratio of different chemical bonds from the fourth to the first sample, FT-IR results could explain the morphological changes that determined the variation in thermal properties observed in the DSC and TGA assays.

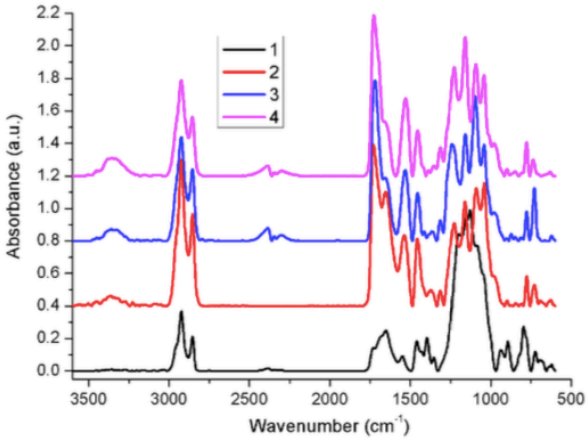


**Fig. 3.3.** FTIR spectra of the studied samples 1–4.

### *Thermal Behaviour*

The thermal behaviour of the studied samples was assessed with the aid of DSC and TGA measurements, since the heat flow generated transitions and thermal decomposition profiles are very useful in establishing general differences in materials aging pathways and/or patterns.
Figure 3.4 shows the DSC second heating scans and their characteristics, recorded up to the onset temperature of thermal degradation.

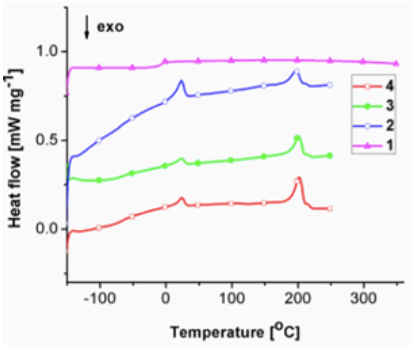


**Fig. 3. 4.** DSC of the studied samples

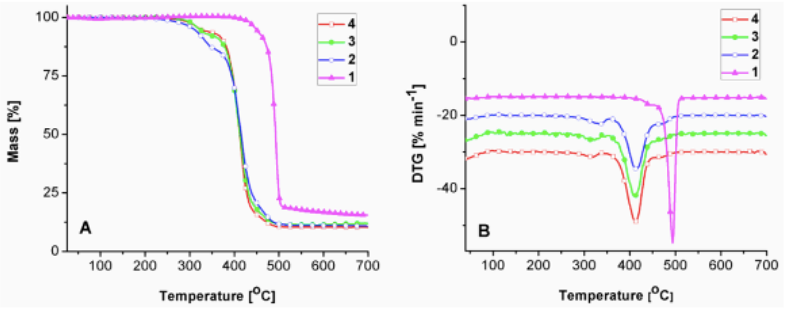


**Fig. 3. 5.** (**A**) TGA and (**B**) DTG curves of the studied samples

Figure 3.5. A shows the thermal behavior of the four areas studied by thermogravimetric analysis and found a higher thermal stability for sample one. Furthermore, the first derivative (DTG) curve peaks indicate three stages of thermal degradation for all the samples, except

sample one (figure 3. 5 B). For sample one the first decomposition stage, most probably attributed to initiation of thermal degradation through scission of weak linkages, disappears leaving only the second stage and the third stage, both at significantly higher onset temperatures.( Varganici et al., 2015).

### *Antimicrobial Activity*

In all the cases, the samples displayed antibacterial properties and did not allow bacterial strains adhesion and growth, There were no differences between the Gram-positive (S. aureus) and the Gram-negative (E. coli) strains and after 24 h of incubation just a few colonies were found in the PCA plates (as compared with the control) suggesting that the antibacterial properties of the endoscope polymer are preserved. As shown in Table 3.III, only the distal sample (first sample) was slightly less effective against both bacterial strains, however, their antibacterial effectiveness was still high. An important acknowledgement in this respect is that the duration of the experimental inoculation and incubation exceeds the usual per-procedure exposure to gut bacteria. Hence, an ERCP procedure usually lasts between 20 min and 1.5 h, afterwards duodenoscopes are pre-cleaned using enzymatic solutions and subsequently reprocessed following the manufacturer issued protocols.

**Table 3. III.** Bacterial contact-killing efficacy against: *S. aureus* and *E. coli*. determined by Japanese industrial standard JIS Z2801:2000

| sample | R factor | |
|---|---|---|
| | *S. aureus* | *E coli* |
| 1 | 4.3 | 4.7 |
| 2 | 5.8 | 4.9 |
| 3 | 5.6 | 5.8 |
| 4 | 5.7 | 5.9 |

### *Atomic Force Microscopy (AFM)*

- *Morphological Characterization*

Figure 3.6 presents AFM topographic images of the four duodenoscope samples. The duodenoscope surface was imaged at five randomly chosen positions from which roughness was calculated. The samples are characterized by an inhomogeneous morphology and even microcracks are observed. The roughness average value varied from 12.8 nm for the fourth sample to 70.2 nm for the first sample, suggesting that the intense usage of the proximal part of the duodenoscope part of the duodenoscope caused, in time, the polymer usage. The results are consistent with our preliminary optical analysis study carried out on similar duodenoscopes (Balan et al., 2016)

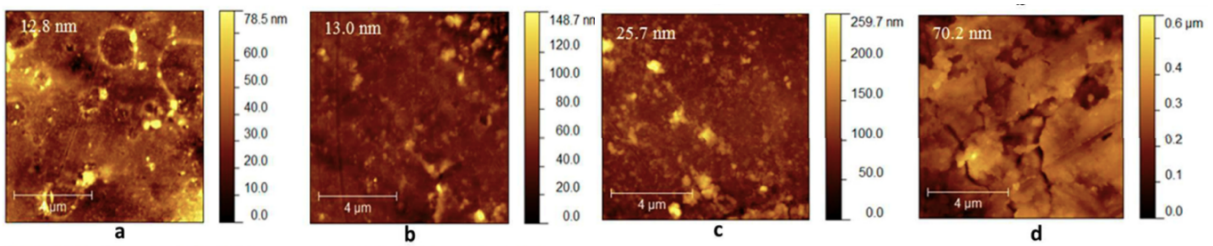


**Fig. 3. 6.** AFM topographic images for the duodenoscope samples: a – 1st sample, b – 2nd sample, c – 3rd sample, d – 4th sample

- *Evaluation of Biofilm Formation on Duodenoscope Samples*

AFM was also used to determine if biofilm is formed on the duodenoscope samples after incubation with Escherichia coli and Staphylococcus aureus (Figure 3.7). In the case of E. coli,

no biofilm formation is observed, however, for the distal segment, the attachment of a few rod-shaped cells (with a length of 2 µm and a width of 0.7 µm) is evident. For S. aureus grown on each tested sample, attached small round-shaped cells (with diameter of 0.7 µm) were individually distributed on the solid surfaces. No aggregates or colonies on tested surfaces were noticed for both bacterial strain, even though usually there were differences found between the antibacterial properties of different surfaces which were caused by the different composition of the Gram-positive and Gram-negative cell walls (Polivkova et al., 2015; Polivkova et al., 2017).

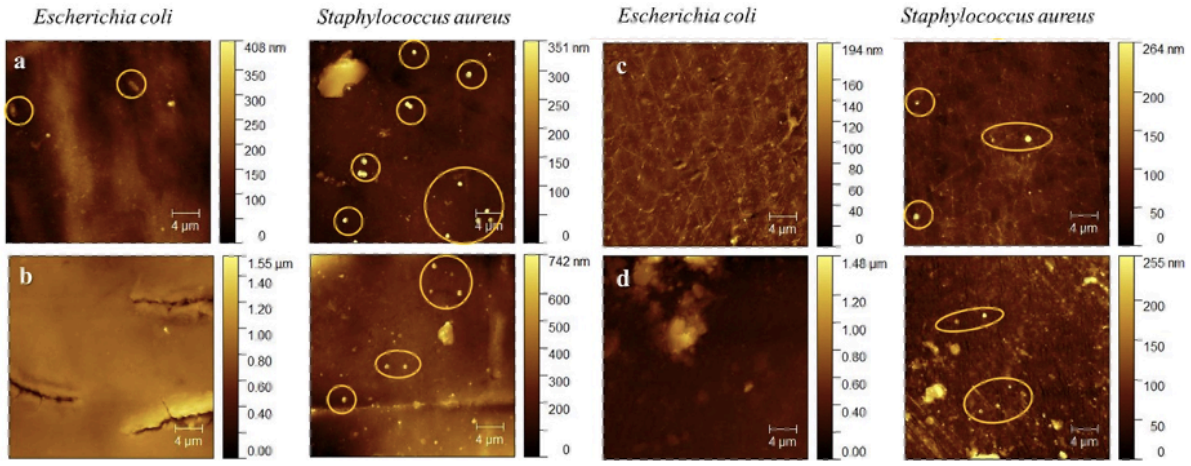


**Fig. 3.7.** AFM topographic images for the duodenoscope samples after incubation with *Escherichia coli* and *Staphylococcus aureus*: **a**—1st sample, **b**—2nd sample, **c**—3rd sample, **d**—4th sample

***Scanning Electron Microscopy (SEM)*** From the SEM images (as shown in Figure 3. 8) it is clearly observed that the coating material displays erosion and deterioration marks induced by the extensive usage and repeated reprocessing. Microscopically detached chips are also visible. Selected areas present different morphologies, resulting in different levels of deterioration.
Figure 3.9 states for similar results inside the air/water, elevator, and working channels, and also on the elevator metallic part.
As expected, the patterns of surface alteration presented in Figure 3.9 are different with respect to the outer and inner (channel) surfaces. While the outer polymers, for both AFM and SEM analysis, show amorphous and irregular patterns of deterioration that could be linked to both repeated reprocessing and use, the working channel shows parallel microrecess formation mainly due to the repeated passage of instruments through the channel. A similar parallel abrasion pattern is observed on the air/water channel. Interestingly, the elevator channel shows some irregular patterns of microabrasion, in contrast to the sealed character of the channel. Nevertheless, microanalysis of the elevator recess shows both microfissures of the metallic material and remnant debris despite thorough reprocessing.

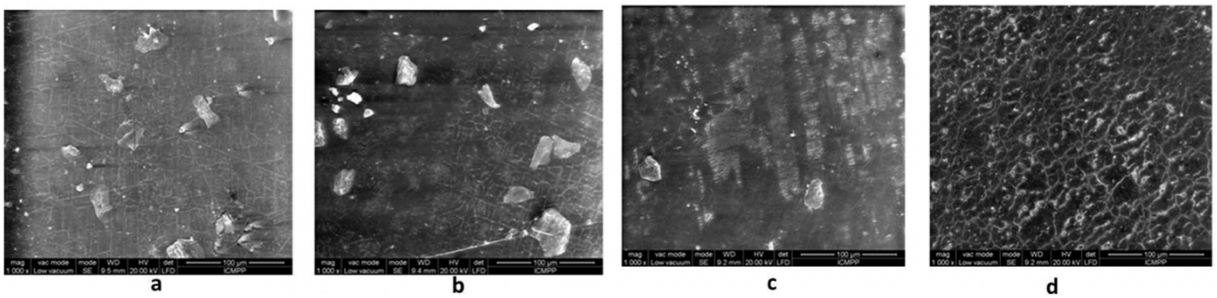


**Fig. 3.8.** SEM micrographs of duodenoscope samples. a—1st sample, b—2nd sample, c—3rd sample, d—4th sample.

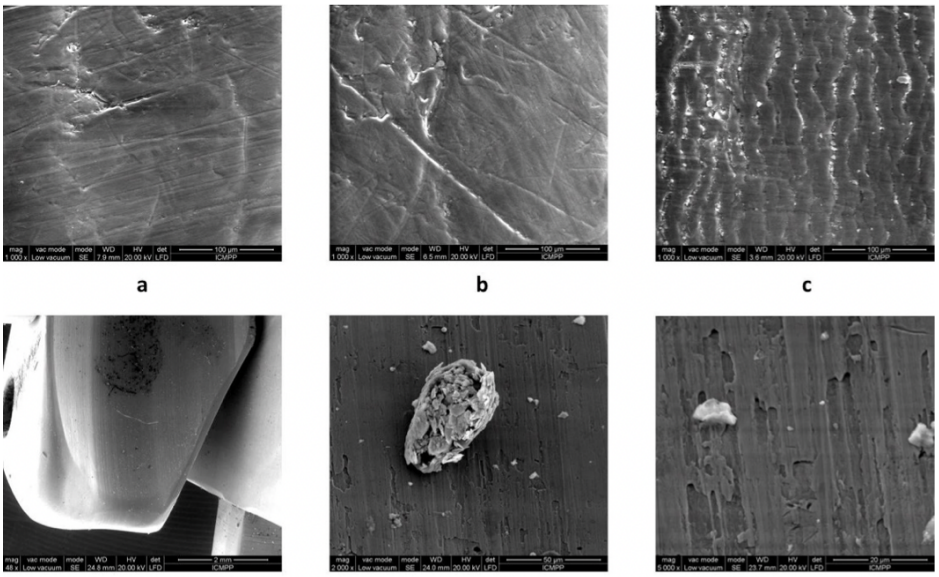


**Fig. 3.9.** SEM micrographs of elevator and channel samples: a—air/water channel; b—elevator channel; c—working channel; d,e,f—elevator recess side.

Concerning the evaluation of the macroscopic aspect of working channel polymers, a recent study allowed optical endoluminal analysis using a specially designed borescope (SteriCam, Sanovas Inc, San Rafael, CA, USA) and proved the presence of numerous scratches with adherent peel and burns, channel bulking, strains, and perforations, as well as associated solid debris and fluid residue on similarly used duodenooscopes. The authors emphasizing the need for further microscopic analysis of such findings (Barakat et al., 2018). Previous preliminary studies associated a higher degree of macroscopically evaluated working channel damages to endoscopes used in interventional procedures (Ofstead et al., 2017, Ofstead et al., 2016). Nevertheless, as most of the up-to-date studies and FDA protocols suggest (Bălan et al., 2018, Humphries et al., 2015, Duodenoscope Surveillance Sampling and Culturing Protocols, 2018), breaches in duodenoscope reprocessing occur due to the difficult-to-clean design of the distal tip. On the other hand, the results of our study show that routine use of duodenoscopes causes microscopic alterations both to the outer surface of the duodenoscope coating polymers and to the inner coating of the air/water, elevator, and working (biopsy) channel. Such surface alterations were previously linked to biofilm formation (Kovaleva et al., 2013) that protects microorganisms from the effects of thermal and chemical reprocessing agents. Therefore, it has long been shown that biofilm is visualized by electron microscopy on the inner lumen of the working and air/water channels of used gastrointestinal endoscopes (Pajkos et al., 2004). The link between duodenoscope surface alterations and positive post-cleaning cultures is also sustained by the fact that, as a recent observational study shows, despite optimized and constant reprocessing protocols there are some duodenoscopes associated with a higher rate of positive cultures while some others are not (Higa et al., 2018). Moreover, the inner channels are the ones harboring resistant bacteria (Gram-positive, spore forming) that persist even after extensive cleaning, alcoholic flush, and continuous channel purge storage conditions (Singh et al., 2018).

## Conclusions

Our study describes the impact of routine procedural use and reprocessing cycles on the duodenoscope. We noticed alterations of both the coating and working channel polymers due to usage, even for a relatively small number of cases. External alterations increase progressively

from the distal to the proximal sample to the elevator sample. However, the coating surface was proven to still be efficient against bacterial adhesion. Changes in terms of surface texture (roughness and cracks due to erosion, chemical resistance, and aging of material) and it was also shown that morphological changes correlated well with the variation in physical properties. Moreover, despite reprocessing and long-term quarantine the elevator harbors remnant possibly organic material suggestive for biofilm formation. All this physical evidence shows that the impact of routine procedural use and reprocessing on the scope possibly makes it susceptible to bacterial contamination and MDRO biofilm formation due to difficult reprocessing of altered surfaces.

*Originality and applicability of the results in medical practice*

The current research is a continuation of the original study conducted in 2016 and which involved analysis by optical microscopy of the physical characteristics of the outer shell of the duodenoscope. In this second study, in addition to the coverage area, we also evaluated the interior elements of the duodenoscope. The analysis was performed by complex and complementary methods and we further monitored the presence of biofilm on the analysed surfaces and the antibacterial behaviour of the constituent materials.

As a result of our research we outline that the industry of duodenoscope manufacturers should also actively participate by developing new materials for endoscope technology. In addition, to date, the FDA's position is to order manufacturing companies to conduct post-market surveillance studies in order to describe the challenges of duodenoscope reprocessing in real-world settings (Postmarket Surveillance (PS) Studies Program, 2018).

Furthermore, as shown by our study, routine use and reprocessing cycles could microscopically impede the integrity of the polymeric materials used in duodenoscope technology, and therefore be a possible alternative cause for duodenoscope-associated infections. Such feedback should provide manufacturers with proof that there is a need for technological alternatives and innovation.

*Limitations of the study*

The activity of PAW against endospores was not tested in our study.

*Future directions*

As a consequence of the results obtained from the two studies presented above, we aimed to identify new methods of reprocessing duodenoscopes. Our goal will be to preserve the antibacterial activity of the duodenoscope surface and to avoid causing injury to the duodenoscope coat.

### *3.2.1.3. New methods of reprocessing duodenoscopes: Plasma-activated water*

This study was performed in addition to the two prior studies detailed in this area of research. We have shown that, under normal use, a duodenoscope undergoes microscopic structural changes, both on the outer surface of the duodenoscope layer and on the inner layer of the air / water channel, elevator and biopsy. Surface changes may favour biofilm formation and may be responsible for ERCP-associated infections.

Most nosocomial pathogens can persist on duodenoscope surfaces for weeks or even months, (Schaefer et al., 2010) and the safety of high- level disinfection of duodenoscope surfaces for controlling nosocomial duodenoscope- transmitted pathogens has been a continuous debate for some time. During the last years, numerous outbreaks of duodenoscope-associated transmission of multidrug-resistant bacteria have been reported worldwide. (Kovaleva, 2013; Muscarella, 2014; Humphries et al., 2015) *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, *Escherichia coli*, and *Pseudomonas aeruginosa* are the bacteria most frequently reported to cause contaminations and duodenoscope-transmitted infections, especially because of their ability to form biofilms. Finding solutions to this issue represents a hot topic in current research.

Usually, the coating materials of duodenoscopes – resin polymers – are heat labile and require disinfection with chemical agents or low-temperature sterilization methods in order to achieve high-level disinfection. The disinfecting agents used for high-level disinfection of duodenoscope can be classified into the following groups: high-level (glutaraldehyde, peracetic acid, ethylene oxide), intermediate-level (ethanol, formaldehyde, phenolic solutions) which do not have sporicidal activity, and low-level (povidone-iodine, cetrimide, benzalkonium chloride) which do not destroy *Mycobacterium tuberculosis*, atypical mycobacteria, and bacterial spores.(ANSI/AAMI ST58:2013; McDonnell et al., 2012) Through their repeated daily use on duodenoscopes, the high-level disinfectants are associated with alterations of duodenoscope resin polymers, despite the low-temperature disinfection process, either by forming fissures in the duodenoscope surface or by affecting its elasticity parameters (Naryzhny et al., 2016) The pre-disinfection procedures are believed to contribute to these alterations too.(Shoop, 2001; Hayakawa et al., 2003) Such results are consistent with our previous findings according to which routine day-to-day usage of duodenoscopes is associated with microfissures, scratches, and increased porosity of the polymer resins mainly secondary to duodenoscope handling and hard-surface contacts during the reprocessing cycles.(Balan et al., 2016)

Nonthermal plasma discharge has recently been widely acknowledged as an effective method for decontamination and is increasingly proposed for biomedical sterilization of various types of equipment. (Morrison et al., 1977; Farin, Grund,1994; Deng et al., 2007; Fridman et al., 2006; Deilmann et al., 2008) Nonthermal plasma proved its valuable properties in surface reprocessing due to its high efficiency in destroying microorganisms causing minimal or no damage to the solid substrates involved. (Lerouge et al., 2001; Moisan et al., 2001; Sladek et Stoffels, 2005).

Plasma- activated water (PAW) is highly active against a large panel of germs, is easy to use, and has the ability to kill microorganisms that otherwise cannot be destroyed by nonthermal plasma discharges (i.e., areas of a device that are not directly exposed or difficult to reach). (Ursache et al., 2014)

Within this context, the aim of our study was to evaluate whether duodenoscopes and their surface components are suited for repeated use of PAW in reprocessing cycles. We also aimed to evaluate the efficacy of PAW in high-level disinfection of endoscopy unit in order to consider PAW as a possible new alternative for duodenoscope reprocessing.

Specifically, the objectives of the research were to analyse both the disinfection capacity of PaW and its effect on the constituent materials of the duodenoscope.

The study was also conducted in collaboration with the research team at the Center for Advanced Research in Bionanoconjugates and Biopolymers (Intel Center), "Petru Poni" Institute of Macromolecular Chemistry.

This project received funding from the European Union's Horizon 2020 research and innovation program under grant agreement number 667387 WIDESPREAD 2-2014 Supra-Chem Lab.

**Materials and methods**

*Duodenoscope samples*

We selected a duodenoscope (TJF-160F; Olympus Corporation, Tokyo, Japan) from Institute of Gastroenterology and Hepatology Iași, that was previously used in up to 500 ERCP and duodenoscopy procedures between 2012 and 2014. The model of duodenoscope we selected for our study has been successfully and extensively used around the world in numerous high-volume centers in the same period, and is regarded as one of the best operating duodenoscopes available on the market. It was dismantled, and samples from the outer resin polymer coating measuring 1 $cm^2$ each were taken for analysis.

We performed two types of experiments: one for the evaluation of the disinfectant properties of PAW in biomimetic conditions and one for the evaluation of PAW compatibility with duodenoscope polymer resins.

*PaW preparation* was performed by chemical researchers according to a standardized procedure.

*PaW disinfectant activity evaluation* was performed under biomimetic conditions.

Four types of strains, namely *Acinetobacter baumannii* ATCC 19606, *E. coli* ATCC 25922, *P. aeruginosa* CIP 82118, and *K. pneumoniae* CIP 53153, were used to evaluate the disinfectant properties of PAW. The coating polymer samples were contaminated in a bacterial suspension and exposed to PAW at various periods (5, 10, 15, 20, and 30 minutes, respectively). Each experiment was done in triplicate, and uncontaminated controls were used each time.

Ten milliliters of standardized bacterial suspension in normal saline solution ($10^8$ cfu/mL) was mixed with 90 mL of fasted-state simulated intestinal fluid (Marques et al., 2011), in order to reproduce the organism's physiological conditions, and then the solution was vortexed. Duodenoscope coating polymer samples were maintained in this environment for 15 minutes with continuous stirring to create friction between surface and simulated environment at 37°C in order to create biomimetic conditions. After 15 minutes of incubation, the samples were removed, left for 1 minute to dry, and afterwards immersed separately in 20 mL of fresh PAW and maintained for 5, 10, 15, 20, and 30 minutes under periodic stirring. The samples were then removed and left for 1 minute to dry. Once dried, all samples were transferred to bottles containing 30 mL of sterile tryptic soy broth, and afterwards they were air-sealed and incubated at 36 ± 1°C for 72 hours. The presence of turbidity after incubation indicated bacterial growth, signifying the presence of viable microorganisms on the samples after disinfection, while the absence of turbidity after incubation indicated the lack of viable microorganisms and the disinfectant efficacy of PAW for the given time of contact.

In order to confirm the absence of viable bacteria, all the incubated bottles, regardless of the media appearance, were checked by subculturing on proper solid media. The absence of turbidity (clear medium) and negative subcultures were consistent with high-level disinfection of samples.

*PaW compatibility with duodenoscope polymer resins*

To assess the impact of repeated PAW treatment on duodenoscope polymer structure, a challenge test was performed. The samples were immersed in PAW for 30 minutes daily, for a 45-day period. The controls were treated similarly, but distilled water was used instead of PAW. All the samples (treated with PAW and untreated) were analysed by scanning electron microscopy (SEM), atomic force microscopy (AFM), and energy-dispersive X-ray spectroscopy (EDX), according to standard procedures, similar to those used in the previous study.

**Results**

The antimicrobial activity was evaluated against four types of strains, that is, *E. coli*, *K. pneumoniae*, *A. baumannii*, and *P. aeruginosa*. After 20 minutes of treatment, the initial burden drastically reduced for *E. coli* and *A. baumannii* only, as these were compatible with a high level of disinfection. After 30 minutes of contact with PAW, complete inactivation occurred in all the tested bacterial strains. This fact was confirmed by the complete absence of microbial growth on subcultures (compatible with high-level disinfection) (Table 3.IV.).

Significant reduction in all bacterial strains was achieved after 30 minutes of PAW contact, proving the effectiveness of this new approach in duodenoscope reprocessing.

Figures 3.10 and 3.11 show the AFM and SEM topographic images of the untreated and PAW-treated samples, respectively. The untreated duodenoscope samples were characterized by an

inhomogeneous morphology and appearance of micro-cracks. After PAW treatment, no remarkable changes occurred in the morphology of the duodenoscope samples compared with the original surfaces, suggesting no changes in the surface structure of the duodenoscope after PAW treatment.

**Table 3. IV.** PaW antimicrobial activity

| **Tested strain** | **5 minutes** | 10 minutes | 15 minutes | 20 minutes | 30 minutes |
|---|---|---|---|---|---|
| *Escherichia coli* | + | + | + | - | - |
| *Klebsiella pneumoniae* | + | + | + | + | - |
| *Adnetobocter baumannii* | + | + | + | - | - |
| *Pseudomonas aeruginosa* | + | + | + | + | - |

**Note:** + indicates the presence of turbidity in the culture media (microbial growth): - indicates no turbidity (confirmed by complete absence of growth on subcultures, that is high-level disinfection).
**Abbreviation:** PAW. plasma-activated water.

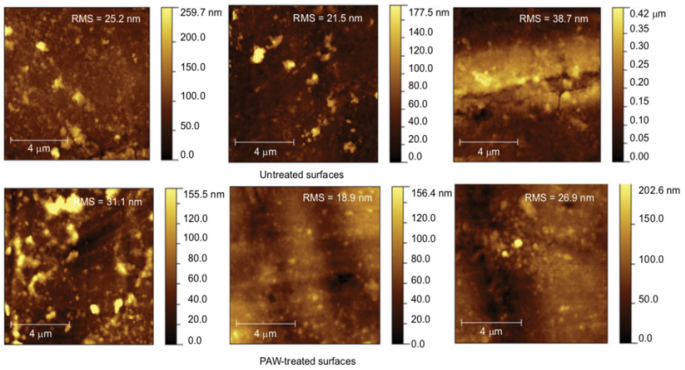


**Fig. 3.10** AFM topographic images of the untreated and PaW-treated samples.

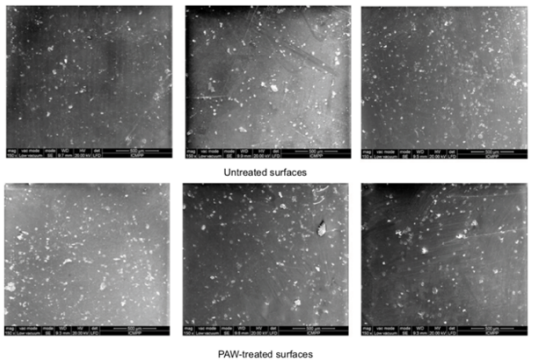


**Fig. 3.11.** SEM micrographs of duodenoscope Samples before and after PaW treatment.

*EDX analysis* was used to determine the differences in the elemental composition between untreated control and treated samples. The results proved no significant differences in the composition between the two types of samples (Table 3.V).

**Table 3.V.** Elemental composition of the duodenoscope surface for the controls and treated samples

| **Element** | **Controls, At%** | **Samples, At%** |
|---|---|---|
| C | 83.40 | 83.36 |
| N | 03.84 | 04.37 |
| O | 12.30 | 12.03 |
| Na | 00.09 | 00.05 |
| Mg | 00.03 | 00.01 |
| Al | 00.05 | 00.02 |
| P | 00.03 | 00.02 |
| S | 00.20 | 00.13 |
| K | 00.07 | 00.02 |

## Discussion

While sterilization can be defined as the process leading to complete absence of any type of viable contaminating microorganism, as stated by the US FDA, high-level disinfection is a reprocessing method that aims to inactivate a large amount of microorganisms (such as bacteria, viruses, or fungi) – at least 6-log to 9-log reduction of the duodenoscope bioburden

which is compatible with it suse in ERCP (Petersen et al., 2016; Andriulli et al., 2007) Therefore, using PAW as a high-level disinfection agent could be a feasible alternative.
Plasma discharge in water generates highly reactive components such as oxygen ions, hydrogen and hydroxyl ions, peroxide, hydrogen peroxide, singlet oxygen, and nitric oxides, primarily in the form of radicals. The treated water is "activated" acquiring new physical, chemical, and biological properties, the most important being the remarkable antimicrobial capacity, as it was proven by our results shown in Table 3.IV. In case of activated water, there is also a "post-discharge" antimicrobial effect manifested until a few days after discontinuation of plasma discharge in water due to both interaction of reactive species with the water (Hnatiuc, 2002) and changes inherent to it. Adding this physical molecular effect to a reprocessing substance resolves the most distressing issue related to duodenoscope high-level disinfection, namely the inaccessibility of many duodenoscope parts to the physical removal of biofilm and bioburden traditionally done by brushing under a chemically active solution followed by high-level disinfection. (Kim et al., 2016). PAW ensures physical removal of biofilm and bioburden through simple contact of activated water with the duodenoscope's most inaccessible areas.
Many Gram-negative species isolated from patients with nosocomial infections, such as *Acinetobacter* spp., *E. coli*, *Klebsiella* spp., *P. aeruginosa*, *Serratia marcescens*, or *Shigella* spp., can survive on inanimate surfaces even for months. Gram-negative bacteria persist longer than Gram-positive bacteria, especially in humid conditions. (Jawad et al., 1996; Williams et al., 2005; Kramer et al., 2006)
Therefore, the antimicrobial activity of PAW was evaluated in our study on four bacterial species that are usually involved in ERCP procedure-related infections: *E. coli*, *K. pneumoniae*, *A. baumannii*, and *P. aeruginosa*. Consequently, we need to acknowledge that viruses do not develop resistance to any type of reprocessing method, as they are easily inactivated during the pre-cleaning or manual washing of duodenoscopes. (Moses, Lee, 2003; Martiny et al., 2004) With regard to fungi, traditionally it has been stated that even if fungal contamination of duodenoscopes may occur, proper inactivation and decontamination is achieved through usual bactericidal reprocessing methods. (Schenk et al.,1978; Singh et al.,1989) Nevertheless, although a possible downside, activity of PAW against endospores was not tested, as exposure to resistant endospores is often achieved over extended exposure times that are not feasible in clinical practice. (Humphries, McDonnell, 2015)
Our results showed that PAW has 100% double-controlled bactericidal effect after 30 minutes of exposure, proving promising properties as a disinfectant for duodenoscope in terms of efficiency, time consumption, and financial aspects.
After repeated PAW treatment of the duodenoscope, no remarkable changes in the micro-morphology and elemental composition of coating materials when compared with the original surfaces.
Overall, with its bactericidal and anti-biofilm effects alongside with excellent safety in what the duodenoscope- structure is concerned, PAW can be considered as a potent high-level disinfectant for duodenoscope reprocessing.

**Conclusion**
Our preliminary study showed several aspects characterized by novelty and usefulness. PAW reprocessing is characterized by significant decrease of bacterial populations, doubled by no surface and composition damage of the duodenoscope polymer resin. It allows skipping the water-rinsing stage of disinfection and minimizes biofilm formation. Therefore, PAW could be considered as a new and effective alternative method of disinfection for duodenoscope reprocessing, to be used after current-standard manual cleaning.
*Originality and applicability of the results in medical practice*

To our knowledge, this is the first study using PAW for duodenoscope reprocessing. PAW was previously reported as an important disinfectant, but its effect was evaluated only in vitro against planktonic bacteria or biofilms, not directly on medical devices.
PAW could be safely used as a high- level disinfectant with biomedical applications. Moreover, high-level disinfection by PAW shortens the overall duration of a reprocessing cycle and makes the duodenoscopes less likely to suffer structural damage secondary to hard-surface friction
*Limitations of the study*
The activity of PAW against endospores was not tested.
*Future directions*
Although promising, such results should be confirmed through case–control tests with current reprocessing standard methods, and also by including other duodenoscope models and in order to assess the efficacy of the system.

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## 3.2.2. Post ERCP-pancreatitis (PEP)

The second sub-direction of research addresses another frequent and severe complication, post-ERCP pancreatitis (PEP).
The average incidence of PEP is estimated at 4-5.5%, with wide variations in different studies, between 0.4% and 40%, depending on the presence of risk factors (Freeman et al., 1996; Andriulli et al., 2007)although transient increase in serum pancreatic enzymes may occur in as many as 75% of patients (Freeman, Guda, 2004).
A brief pathogenesis presentation of the acute post -ERCP pancreatitis (PEP) triggering cascade involves duct obstruction, pancreatic acinar hyperstimulation, release of proinflammatory mediators, added to primary lesions of pancreatic ducts and parenchyma, and later involving local extrapancreatic changes. Only in more severe cases, advanced systemic inflammatory damages, organ disfunctions and multiorganic failure may occur. In such cases a disproportionate systemic inflammatory response is generated, which leads to multiple organ dysfunction through the systemic action of pancreatic enzymes which are released in their activated form. (Milnerowicz et al., 2014; Ye-Chen et al., 2014).
The relatively high incidence and mortality reaching approximately 0.5% are the grounding of the great research interest in studying the pathophysiology, risk factors and potential prophylactic measures. Consequently, there are multiple studies focusing on these objectives throughout the last two decades. (Wang et al., 2009; Donnellan, Byrne., 2012).
Plenty of factors are considered contributors to induction of pancreatic tissue damage alongside ERCP and endoscopic sphincterotomy. Not to be eluted are also personal individual characteristics especially those related to anatomy of the papilla, pancreas and bile ducts and immunological parameters. The experience of the endoscopist and technical parameters of duodenoscopes and sphincterotomes are also not to be neglected.
In this regard, numerous studies have been published over the years that have identified the main risk factors associated with PEP pancreatitis. These are divided into patient-related factors (e.g., age, female gender, history of acute post-ERCP pancreatitis, normal bilirubin values at admission, etc.) and factors that are related to the procedure itself (e.g., difficult cannulation, injection in excess of the contrast agent, excessive use of the guide wire, pre-cut sphincterotomy). (Eiji F et al., 2017). For all these situations associated with the risk of post-ERCP, there is consistent scientific evidence.
However, there are other conditions that are directly or indirectly associated with the risk of PEP and for which studies are less consistent. For example, despite the essential role of deep

duct cannulation in the procedural safety and success, to date there is only limited research concerning the impact of papillary morphology on bile duct cannulation.
On the other hand, various therapeutic regimens have been studied to prevent this complication but the studies have reported conflicting results. Currently, European and American guidelines recommend non-steroidal anti-inflammatory drugs - NSAIDs (diclofenac, indomethacin) in various doses and modes of administration (i.v., intrarectal, etc.). (Kubiliun et al., 2015; Dumonceau et al., 2014; Xingkang et al., 2018). Pharmacological prophylaxis can be routinely administered or in selected cases, where risk factors for the development of pancreatic complications are identified. (Luo et al., 2016; Puig et al., 2014).

**Personal contributions in the field of Post-ERCP- Pancreatitis**
I will further present research results conducted in this subdomain structured as follows: risk factors related to anatomical conditions of the papillary region, related to interventional technique and prophylaxis measures (table 3. VI.)

**Table 3.VI.** Publications in the field of Post-ERCP- Pancreatitis:

| |
|---|
| ANATOMY OF MAJOR DUODENAL PAPILLA INFLUENCES ERCP OUTCOMES AND COMPLICATION RATES: A SINGLE CENTER PROSPECTIVE STUDY**.**<br>Balan GG, Arya Mukul, Catinean Adrian, Șandru Vasile *, Moscalu Mihaela, Constantinescu Gabriel, Trifan Anca, **Stefanescu Gabriela***, Sfarti Cătălin Victor.<br>o *J. Clin. Med.* 2020, **IF- 4.242** |
| SPLIT-DOSE OR HYBRID NONSTEROIDAL ANTI-INFLAMMATORY DRUGS AND N-ACETYLCYSTEINE THERAPY FOR PREVENTION OF POST- RETROGRADE CHOLANGIOPANCREATOGRAPHY PANCREATITIS.<br>Pavel Laura, Bălan G Gheorghe, Nicorescu Alexandra, Gîlcă-Blănariu Georgiana Emmanuela, Sfarti Cătălin, Chiriac Ștefan, Diaconescu Smaranda, Drug Vasile Liviu, Bălan Gheorghe, **Ștefănescu Gabriela**<br>o *World J Clin Cases* 2018, **IF- 1.013** |
| EXPOSURE TO IOPAMIDOL AFTER ENDOSCOPIC RETROGRADE CHOLANGIOPANCREATOGRAPHY. ASSESSING PANCREATIC TOXICITY.<br>BălanGGheorghe, Pavel Laura, Sfarti Cătălin Victor, **Ștefănescu Gabriela,** Bălan Gheorghe, Trifan Anca<br>o *Rev.Chim*. 2016, **IF- 1.232** |
| THE EVOLUTION OF CLINICO-BIOLOGICAL PROFILE OF PATIENTS UNDERGOING PROPHYLAXIS FOR POST-ERCP PANCREATITIS - A PROSPECTIVE STUDY.<br>Pavel Laura, Bălan GGheorghe , Timofte Oana , Bălan, G , **Ștefănescu, G.**<br>o *Medical-Surgical Journal* 2019 |

First, we aimed to study the anatomical conditions that may influence ERCP results and complication rates, as well as to assess the pancreatic toxicity of iodine-based contrast media after ERCP.

***3.2.2.1. Anatomic conditions which may influences ERCP outcomes and complication rates***
Acquiring the skills for a safe and successful bile duct cannulation is an essential step for the overall efficiency of the procedure (Reddy et al.,2017; Hawes, Deviere, 2018), as difficulties in cannulation have often been linked to post-ERCP adverse events or poor outcomes (Testoni et al., 2016; Berry et al., 2019). Difficult cannulation has constantly been regarded as an independent risk factor for post-ERCP pancreatitis (Freeman et al., 1996; Cheng et al. ,2006; Chandrasekhara et al., 2017). Furthermore, inability to cannulate the papilla leads to procedural failure and can require subsequent alternative techniques to be used (Artifon et al., 2015).
Any experienced ERCP endoscopist can differentiate multiple morphologies of the major papilla and their impact on choosing different bile duct cannulation strategies (Halttunen et al., 2014; Löhr et al., 2012). Thus, a well renowned research team from Scandinavia has developed

in 2017 the first interobserver- and intraobserver-validated classification of the endoscopic appearance of the papilla, stressing its role in creating a common reporting system for endoscopists (Haraldsson et al., 2017). Recently, the same team has shown through a prospective multicenter study that the morphology of the major papilla affects bile duct cannulation and an anatomy-based approach should be included in ERCP training programs (Haraldsson et al., 2019).

Given the deemed needed completion of the current validated classification of the papillary morphology, the aims of our study were to firstly expand upon the existing classification including anatomical variations of the papilla that would cover some of its missing morphologies. Secondly, we prospectively evaluated whether such expanded papillary types influence ERCP outcomes and complications rates in a cohort of consecutive patients.

## Materials and Methods

*Patients*

We have prospectively monitored patients referred for therapeutic ERCP within the Institute of Gastroenterology and Hepatology of Iasi, an emergency-based tertiary center in Romania, between 1 January and 31 August 2018.

All patients were managed for both malignant and benign bile duct diseases. Inclusion criteria consisted of: (i) indication for ERCP: bile duct stones (including cholangitis and biliary pancreatitis), cholangiocarcinoma, pancreatic cancer and chronic pancreatitis, bile duct injuries, various extrinsic compressions, primary sclerosing cholangitis and post-liver transplantation strictures; (ii) a native papilla; (iii) age above 18 years; (iv) possibility for follow-up at 15 and 60 days; (v) expressed consent for inclusion in the study. Exclusion criteria were: (i) previous ERCP or sphincterotomy; (ii) presence of ampulary tumors or tumors invading the papilla; (iii) postoperative altered anatomy; (iv) duodenal and/or bilio-pancreatic trauma; (v) cannulation of the minor papilla; and (vi) impossibility for achieving either papilla classification or proper follow-up. Data regarding patient demographics and ERCP indication were recorded in all cases.

*Classification of Papillary Morphology and Procedure Documentation*

During all ERCP procedures morphology of the papilla was assessed. Its appearance was classified in either regular anatomy or one of the four anatomical variations. The classification expansion used within the study was based on a former definition of the papillary anatomical variations previously published by Canard et al. in 2011 (Canard et al., 2011). By adapting the former description of anatomical variants, we obtained an objective and reproducible classification add-on suitable for our study design. A regular papilla was described as presenting the following features: frenulum, orifice, recessus and infundibulum (intraduodenal portion of the common bile duct) as shown in Figure 3.12. Anatomical variations were classified in: Type 1: small and/or retracted papilla, without a recessus and infundibulum; Type 2: papilla with a small infundibulum with no recessus and a poorly defined orifice; Type 3: papilla with a large protruding and/or pendulous infundibulum and visible orifice; Type 4: large papilla with multiple overlying folds over the orifice (commonly referred to as a *hooded* papilla or a *Shar-Pei dog* papilla). Graphical representations of the anatomical variations are illustrated in Figure 3.13. Presence of duodenal diverticula has been documented.

Video documentation of the ERCP procedure was performed in all cases. Bile duct cannulation was monitored. The number of intentional contacts with the papilla for attempted cannulation was noted. The time between the first intentional contact and bile duct cannulation confirmed by fluoroscopy was also measured. Unintentional guidewire passages in the main pancreatic duct were recorded.

Difficult cannulation was defined in accordance with the criteria established by the European Society for Gastrointestinal Endoscopy (ESGE) clinical guideline as: more than 5 contacts with the papilla while attempting to cannulate; more than 5 min attempting to cannulate following

first intentional contact of the papilla; more than one unintended pancreatic duct cannulation or opacification (Testoni et al., 2016).
ERCP procedures were performed by two experienced endoscopists and two senior fellows in advanced endoscopy.

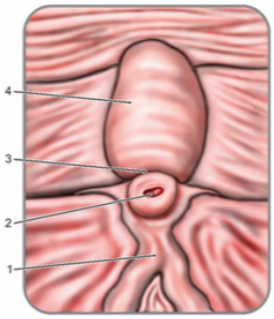


**Fig. 3.12.** Regular anatomy of the papilla:
(1) frenulum; (2) orifice; (3) recessus; and (4) infundibulum..
(2) Adapted after Canard et al., 2011 (17).

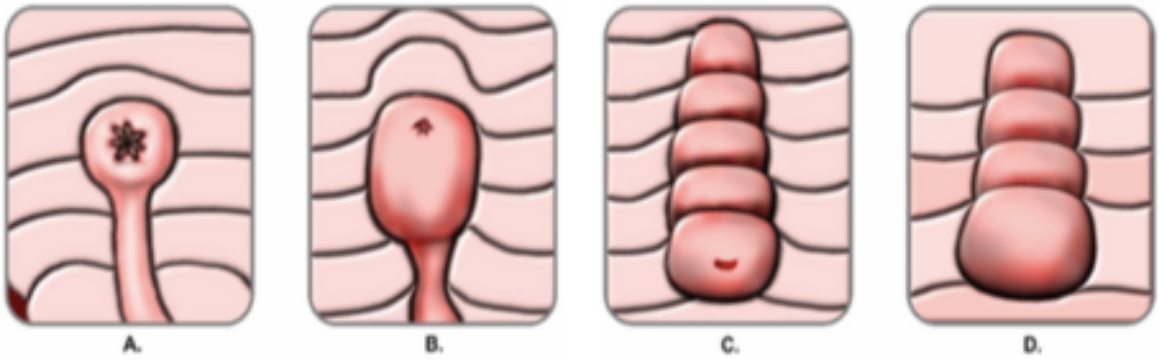


**Fig. 3.13.** Anatomical variations of the papilla (A) Type I; (B) Type II; (C) Type III; (D) Type IV. Adapted after Canard (Canard et al., 2011)

All patients received preanesthetic assessment before ERCP. Administration of intrarectal indometacin or diclofenac and indication of the intravenous hydration protocol with lactated Ringer's solution were approved by the anaesthesia provider for each patient. Intrarectal indometacin or diclofenac has been administered to all patients prior to the procedures.
Data regarding the methods used for cannulation, cannulation attempts and time to successful cannulation, bile duct anatomy, insertion of stents, brush cytology or balloon sphincteroplasty were recorded for univariate analysis and multiple regression.
*Prospective Evaluation*
All patients were hospitalized for at least 24 h after ERCP. A thorough clinical assessment has performed in all patients for at least 24 h after ERCP, including body temperature and vitals, as well as a biochemical panel including complete cell count, C reactive protein levels, lipase levels, liver and kidney function tests. Discharge was decided only by a consultant. Further hospitalization and monitoring of patients was performed depending on the presence of post-ERCP complication or on the overall recovery of patients. All patients were prospectively followed up by physical examination and assessment of symptoms at 15 and 60 days following the procedure in the ambulatory outpatient clinic. Adverse events including post-ERCP pancreatitis, bleeding, infection and perforation were defined according to consensus criteria of the American Society of Gastrointestinal Endoscopy (ASGE) (Chandrasekhara et al.,2017). Definition of post-ERCP pancreatitis was done after the revised Atlanta classification criteria (Banks et al., 2013). (ASGE) (Chandrasekhara et al., 2017).

The post-ERCP infections consisted of cholangitis, cholecystitis, liver abscess and symptomatic bacteremia episodes including the suspected duodenoscope-transmitted multidrug resistant infections. Post-procedural mortality was recorded.

*Statistical Analysis* and calculations were made with the SPSS version 25

We searched for possible predictors of each post-ERCP adverse event and of the cumulative complications rate. In addition to the papillary morphology, univariate analysis was also conducted for other procedure-related variables. Univariate analysis and multiple regression of predictors were performed after the logistic regression model. Odds ratio (OR) and Wald test were calculated. Marked effects of tests were considered significant at $p < 0.05$.

All patients gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Research Ethics Committee of the Grigore T. Popa University of Medicine and Pharmacy of Iasi.

## Results

A total of 403 consecutive patients were referred for inclusion in the study. After applying inclusion and exclusion criteria, a total of 322 patients met the criteria and participated in the prospective study. Due to patient selection and model design, classification of the papilla into either regular morphology or one of the anatomical variants was possible in all cases. Distribution of the papilla types is shown in Table 3. VII. As expected, a regular papilla was the most frequently encountered endoscopic feature, accounting for 52.1% of cases, followed by the Type 2 papilla found in 18.9% of cases.

**Table 3.VII.** Distribution of the different endoscopic aspects of the papilla.

| Papilla | N% |
|---|---|
| Regular | 168 (52.1%) |
| Type 1 | 36 (11.1%) |
| Type 2 | 61 (18.9%) |
| Type 3 | 32 (9.9%) |
| Type 4 | 25 (7.7%) |

The impact of papillary morphology on cannulation and the overall post-ERCP adverse events rates have been assessed. Subsequently, data regarding the predictive power of the papillary morphology on complication rates is shown in a multivariate regression and compared to that of other procedure-related variables. The overall post-ERCP 60 day survival rate was 95.4% in patients without post-ERCP adverse events, and 82.6% for patients where adverse events occurred.

*Impact of Papillary Morphology on Cannulation*

The overall frequency of difficult cannulation, regardless of the papilla types, was 34.4% (95%CI: 0.550–0.683). Within the different anatomical variations there were non-homogenous rates of difficult cannulation, as shown in Table 3.VIII. Nevertheless, the Type 1 small and retracted papillae have been significantly more difficult to cannulate (66.7%, r = 0.282, $p$ = 0.00358) when compared to both regular papillae and other anatomical variations. As shown in Figure 3.14., there was a significant predictive power of the papillary aspect on the rate of difficult cannulation (AUC = 0591, 95%CI: 0.526–0.655, $p$ = 0.008). Protruding and hooded papillae tended to be more difficult to cannulate (46.9% and 40%, r = 0.282), while regular papillae were the least associated with a difficult cannulation (25%, r = 0.282).

Given the overall significance and predictive power of papillary morphology on the rates of difficult cannulation, the next step was to assess the impact of different papilla types on the number of cannulation attempts and the total time for cannulation. As shown in Figure 3.15, the patients with Type 1 small and/or retracted papillae needed significantly more cannulation

attempts when compared to those with a regular papilla (mean value of 5.5 attempts, $p$ = 0.00108). However, the other anatomical variations did not seem to require more cannulation attempts than a regular papilla.

On the other hand, difficult cannulation rates were more clearly delineated by the time required until successful cannulation among the different types of papilla. Thus, as shown in Figure 3.16, Type 1 papillae needed significantly more time (mean value of 5.6 min) until successful deep cannulation was achieved compared to both regular papillae (mean value 3.7 min, $p$ = 0.00181), and the other was achieved compared to both regular papillae (mean value 3.7 min, p = 0.00181), and the other anatomical variations: Type 2 (mean value 3,8 min, $p$ = 0.0014), Type 3 (mean value 3.8 min, $p$ = 0.0008) anatomical variations: Type 2 (mean value 3,8 min, p = 0.0014), Type 3 (mean value 3.8 min, p = 0.0008) or Type 4 (mean value 4.1 min, $p$ = 0.0022).

**Table 3.VIII.** Difficult cannulation among different papilla types.

| **Papilla** | **Cannulation** | | | | **Total** | **95% CI** | |
|---|---|---|---|---|---|---|---|
| | **Standard** | | **Difficult** | | | **Yates Chi-Square = 24.96** | |
| Regular | 126 | 75% | 42 | 25% | 168 | | |
| Type 1 | 12 | 33.3% | 24 | 66.7% | 36 | | |
| Type 2 | 41 | 67.2% | 20 | 32.8% | 61 | r = 0.282 | p = 0.00358 |
| Type 3 | 17 | 53.1% | 15 | 46.9% | 32 | | |
| Type 4 | 15 | 60% | 10 | 40% | 25 | | |

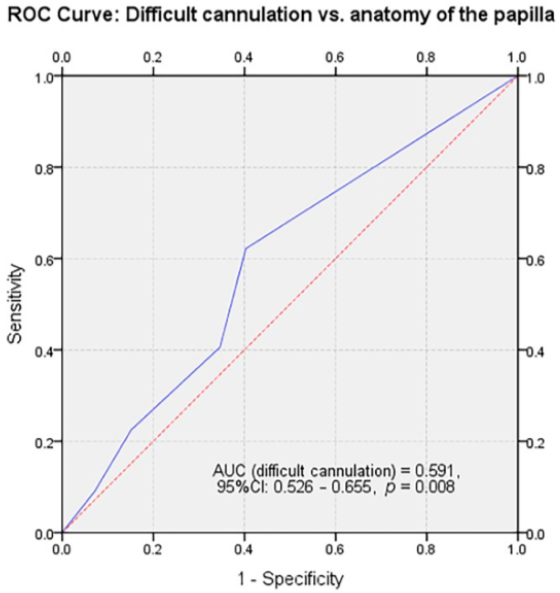


**Fig. 3. 14.** Receiver operating characteristic (ROC) curve for evaluating the predictive power of different papilla types on the rate of difficult canulation.

The overall rates of failed deep cannulation reached 9% within the analysed cohort with non-significant differences between the papilla types (r = 0.0247, $p$ = 0.9096). Knowing that difficult cannulation is an individual risk factor for the post-ERCP adverse events rates, and that it has a significant correlation with the papilla's appearance, our next approach was to evaluate whether the papillary morphology is associated with higher rates of post-procedural complications.

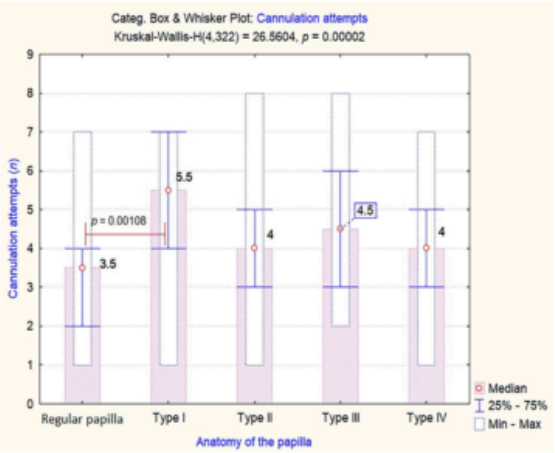


**Fig. 3.15.** Number of cannulation atempts among the different endoscopic aspect of the papilla. Levene Test of Homogeneity of Variances: F= 1.3557, $p$ = 0..249188..

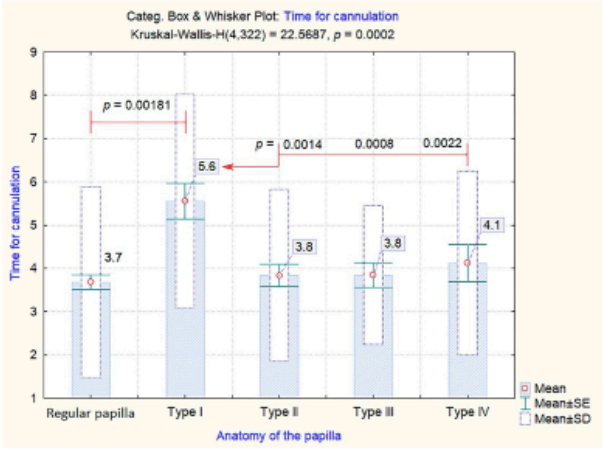


**Fig. 3.16**. Time for successful cannulation among the different endoscopic aspects of the papilla Levene Test of Homogeneity of Variances: F=0.603396, p=0.660458.

*Impact of Papillary Morphology on the Overall Post-ERCP Adverse Events Rate. Univariate Analysis*

As shown in Table 3.IX, the appearance of the papilla major was significantly associated in univariate analysis to both the overall post-ERCP adverse events rates ($p$ = 0.006) and the post-ERCP pancreatitis (PEP) rates ($p$ = 0.01). No significant correlation was found for post-ERCP bleeding and infections. Perforations were scarce in the studied cohort, making statistical analysis impossible. Patients with Type 4 large or hooded papillae had overall significantly higher post-ERCP adverse events rates (44% compared to 16.6% in patients with a regular papilla, 95%CI). The same was observed for PEP rates (28% in patients with Type 4 papillae, versus 10% in patients with a regular papilla, 95%CI). The frequency of PEP within the cohort regardless of the papilla type was 8.68%. In cases with difficult regular cannulation, the PEP rate increased significantly to 17.8% compared to only 4.91% when cannulation was not difficult (r = 0.614, $p$ = 0.00007).

**Table 3.IX.** Univariate analysis showing correlations between the different endoscopic appearances of the papilla and post-Endoscopic retrograde cholangiopancreatography (ERCP) adverse events rates.

| **Papilla (*n*= 322)** | **Overall Post-ERCP Adverse Events** | | **Test†** | **p-Value * (95% CI)** |
|---|---|---|---|---|
| | **Absent (*n* = 262)** | **Present (*n* = 60)** | | |
| Regular papilla | 140 (83.33%) | 28 (16.67%) | | |
| Type 1 | 26 (72.22%) | 10 (27.78%) | | |
| Type 2 | 53(86.89%) | 8(13.11%) | 16.087 | 0.0066 * |
| Type 3 | 29 (90.63%) | 3 (938%) | | |
| Type 4 | 14 (56%) | 11 (44%) | | |
| | Post-ERCP pancreatitis | | | |
| | Absent (*n* = 289) | Prcsent (*n* = 33) | | |
| Regular papilla | 150 (89.29%) | 18 (10.71%) | | |
| Type 1 | 31 (86.11%) | 5(13.89%) | | |
| Type 2 | 59 (96.72%) | 2 (328%) | 13.275 | 0.01001 * |
| Type 3 | 31 (96.88%) | 1 (3.13%) | | |
| Type 4 | 18(72%) | 7 (28%) | | |
| | Post-ERCP bleeding | | | |
| | Absent (*n* = 312) | Present (*n* = 10) | | |
| Regular papilla | 163 (97.02%) | 5 (2.98%) | | |
| Type 1 | 36(100%) | 0 (0%) | | |

| | | | | |
|---|---|---|---|---|
| Type 2 | 58 (95.08%) | 3 (4.92%) | 2.9099 | 0.5730 |
| Type 3 | 31 (96.88%) | 1 (3.13%) | | |
| Type 4 | 24 (96%) | 1 (4%) | | |
| | Post-ERCP infections | | | |
| | Absent ($n$ = 299) | Present ($n$ = 23) | | |
| Regular Papilla | 160 (95.24%) | 8 (4.76%) | | |
| Type 1 | 29 (80.56%) | 7(19.44%) | | |
| Type 2 | 57 (93.44 %) | 4 (6.56%) | 9.145977 | 0.05756 |
| Type 3 | 31 (96.88%) | 1 (3.13%) | | |
| Type 4 | 22 (88%) | 3(12%) | | |

Variables: $n$ (%). ŧ Pearson Chi-square test; * Marked effects are significant at $p < 0.05$.

In this context, we aimed to assess the need to use alternative papillotomy techniques and stenting the pancreatic duct depending on the type of papilla.

Although not statistically significant, patients with large papillae (Type 3 and 4) seem to require more frequently access papillotomy such as freehand precut or fistulotomy (r = 0.377, $p$ = 0.014) compared to the patients with smaller types of papilla (regular and Type 1).

It also appears that patients with Type 4 papillae required more frequent pancreatic prophylactic stenting, compared to that of patients with regular papillae, although we did not obtain statistically significant differences ($p$ = 0.754). The lack of statistically significant data is due to a low number of patients with pancreatic stents.

As detailed above, in the univariate analysis we obtained a significant correlation between Type 4 papillae and the risk of side effects in general and with PEP in particular. Given such a statistically significant correlation in univariate analysis, we proceeded with the multiple regression that would conform and increase reliability of the results.-

*Multiple Regression*

In order to achieve a reliable multiple regression, besides papillary morphology, we also included other predictive procedure-related parameters that were significantly correlated with the post-ERCP adverse event rates following univariate analysis. Table 3.X summarizes the multivariate correlations between procedure-related variables and the post - ERCP adverse events.

**Table 3.X.** Multiple regression. Identification of procedure-related risk factors for post-ERCP adverse events.

| **Multiple Regression** | **SE** | **Wald Test** | **P** | **Odd Ratio** | **95% CI** | |
|---|---|---|---|---|---|---|
| | | | | | **Lower** | **Upper** |
| **Overall post-ERCP adverse events** | | | | | | |
| Papillary morphology (ref.: regular papilla) | | 7.324 | 0.198 | | | |
| Type l | 0.515 | 0.985 | 0.321 | 0.600 | 0.219 | 1.646 |
| Type 2 | 0.546 | 1.001 | 0317 | 0.579 | 0.199 | 1.688 |
| Type 3 | 0.458 | 2.982 | 0.084 | 0.453 | 0.185 | 1.113 |
| Type 4 | 0.507 | 0.552 | 0.458 | 1.457 | 0.540 | 3.932 |
| Constant | 0.430 | 2.270 | 0.132 | 0.523 | | |
| Duodenal diverticulum (ref.: absent diverticulum) | | 1.487 | 0.685 | | | |
| Type l | 0.272 | 0.715 | 0.999 | 0.231 | 0.142 | 0.528. |
| Type 2 | 0.464 | 0.684 | 0.408 | 1.468 | 0.591 | 3.642 |
| Type 3 | 0.080 | 0.716 | 0.397 | 0.401 | 0.048 | 3.331 |

| Multiple Regression | SE | Wald Test | P | Odd Ratio | 95% CI | |
|---|---|---|---|---|---|---|
| Difficult ca nnulation | 0.299 | 11.370 | 0.001 • | 2.744 | 1.526 | 4.933 |
| Alternative access papillotomy (ref.: standard bibary sphincterotomy) | | 2.472 | 0.480 | | | |
| Needle-knife freehand precut | 0.473 | 0.543 | 0.461 | 1.417 | 0.561 | 3.581 |
| Needle-knife fistulotomy | 0.079 | 0.370 | 0.543 | 1.929 | 0.233 | 15.994 |
| Transpancreatic biliary sphincterotomy | 0.834 | 1.303 | 0.254 | 0.386 | 0.075 | 1.979 |
| Altered biliary anatomy (ref.: normal an atomy) | | 0.286 | 0.593 | 0.817 | 0.389 | 1.716 |
| Bile duct stones (ref.: absence of stones) | 0.375 | 3.107 | 0.078 | 0.516 | 0.247 | 1.077 |
| **Post-ERCP pancreatitis** | | | | | | |
| Papillary anatomy (ref.: normal papilla) | | 15.453 | 0.009 * | | | |
| Type l | 0.921 | 1.939 | 0.164 | 3.605 | 0.593 | 21.924 |
| Type 2 | 0.283 | 0.034 | 0.854 | 0.789 | 0.064 | 9.762 |
| Type 3 | 0.055 | 0.107 | 0.744 | 0.708 | 0.089 | 5.603 |
| Type 4 | 0.889 | 7.901 | 0.005 * | 12.176 | 2.131 | 69.567 |
| Difficult ca nnulation | 0.438 | 5.421 | 0.020* | 2.775 | 1.175 | 6.551 |
| Alternative access papillotomy (ref.: standard | | 7.804 | 0.050 * | | | |
| biliary sphincterotomy) | | | | | | |
| Needle-knife freehand precut | 0.598 | 7.610 | 0.006 * | 5.203 | 1.612 | 16.795 |
| Needle-knife fistulotomy | 0.934 | o.ooo | 0.999 | 0.000 | 0.000 | 0.001 |
| Transpancreatic biliary sphincterotomy | 0.130 | MOO | 0.995 | 1.007 | o.i io | 9.219 |
| Constant | 0.744 | 29.358 | 0.000 | 0.018 | | |
| **Post-ERCP bleeding** | | | | | | |
| Difficult cannulation | 0.402 | 13.012 | <0.001 * | 4.270 | 1.940 | 5.397 |
| Pancreatic duct cannulation | 0.417 | 0.687 | 0.407 | 1.413 | 0.624 | 3.200 |
| Papillotomy (ref.: no papillotomy) | | 4.577 | 0.03 1* | | | |
| Complete biliary sphincterotomy | 0.665 | 3.319 | 0.068 | 3.356 | 0.912 | 12.346 |
| incomplete papillotomy | 0.645 | 4.573 | 0.032 * | 3.976 | 1.122 | 4.086 |
| Brush cytology: malignant | 0.735 | 6.580 | 0.010* | 6.592 | 1.560 | 7.845 |
| Balloon sphincteroplasty | 0.630 | 4.096 | 0.043 * | 0.279 | 0.181 | 0.961 |
| Constant | 0.635 | 38.612 | <0.001 | 0.019 | | |
| **Post-ERCP infections** | | | | | | |
| Papillotomy (ref.: no papillotomy) | | 4.336 | 0.114 | | | |
| Complete biliary sphincterotomy | 0.448 | 3.645 | 0.036* | 0.425 | 0.177 | 0.823 |
| Incomplete papillotomy | 0.528 | 2.626 | 0.010* | 0.428 | 0.151 | 0.896 |
| Indication: bile duct stones (ref.: stenoses) | 0.475 | 0.781 | 0.377 | 0.657 | 0.259 | 1.667 |
| Biliary stent insertion (ref.: absence of stents) | 0.406 | 4.956 | 0.026* | 2.467 | 1.114 | 5.463 |
| Constant | 0.337 | 28.388 | 0.000 | 0.166 | | |

* Marked effects are significant at $p < 0.05$. Cl—confidence interval, SE—standard error.

**Discussion**

Most of the experienced endoscopists can differentiate among several characteristics of the papilla but, to date, the impact of such endoscopic findings on ERCP outcomes and adverse events rates have not been clearly defined. The main contribution within the literature of the last decade comes from a team of researchers from Scandinavia that published the first interobserver- and intraobserver-validated classification of the papillary morphology (Haraldsson et al., 2017). The same team has recently published an original research on the role of the different types of papilla in bile duct cannulation, proving that papillary morphology affects bile duct cannulation (Haraldsson et al., 2019).

Despite its multicenter validation and systematic description of morphology for the four different types of papilla, the classification scheme was described as limited, on one hand due to the non-inclusion of some other relatively frequent papilla types and, on the other hand, because of the lack of evidence regarding the risk that such anatomical variations pose on the post-ERCP occurrence of adverse events (Adler, 2019). Therefore, the expanded systematic arrangement in our study aimed to cover some other papillary aspects like the small and retracted papillae or the large, hooded papillae with multiple folds over the orifice. Furthermore, our study went a step further and evaluated not only the impact on cannulation, but also that on ERCP outcomes and adverse events.

As in the previous multicenter studies, regular papillae are the ones most frequently encountered also in our cohort (Haraldsson et al., 2017; Haraldsson et al., 2019). In what the impact of anatomy on cannulation is concerned, the study demonstrated that Type 1 small and/or retracted papillae are more frequently difficult to cannulate compared to the regular ones, and the results are consistent with those of the recent multicenter study from Haraldsson et al. (Haraldsson et al., 2019)

The endoscopic appearance of the papilla was a risk factor for both PEP and overall post-ERCP risk for adverse events in univariate analysis. Moreover, it was proven by the AUROC analysis to be predictive for difficult cannulation. Such correlation has not been confirmed by previous studies (Halttunen et al., 2014; Haraldsson et al., 2019). Given the fact that in the present study difficult cannulation has been itself proven an individual and predictive risk factor for PEP, post-procedural bleeding and for the overall post-ERCP risk for adverse events in multivariate analysis, the morphology of major papilla should play at least an indirect role. The higher rates of PEP associated with difficult cannulation are by now well described and thoroughly documented by both prospective studies, meta-analyses (Elmunzer, 2017 Tse et al., 2017; Lee et al., 2020) and guideline publications (Testoni et al., 2016; Freeman et al., 1996; Chandrasekhara et al., 2017; Dumonceau et al., 2014; Dumonceau et al., 2020). The rates of failed cannulation and PEP in the study were consistent with those cited in the current guidelines (Freeman et al., 1996; Chandrasekhara et al., 2017; Dumonceau et al., 2020).

Interestingly, Type 4 papillae were individual and predictive risk factors for PEP, contrary to previous expert opinion that only small and/or retracted papillae could add on such risk (Horiuchi et al., 2007, Matsushita et al., 2008). Nevertheless, Haraldsson et al. found that the protruding or pendulous papillae are often more difficult to cannulate (Haraldsson et al., 2019), thus demonstrating indirectly the possible correlation.

**Conclusions**

The present study described an expanded classification of the endoscopic appearance of the major papilla covering most of the papillary morphologies. The various types of papilla have been significantly correlated to different rates of difficult cannulation, small and/or retracted papillae being more frequently difficult to cannulate. After thorough prospective monitoring, it has been shown that the anatomy of the papilla may pose a different risk on the overall post-ERCP adverse events rates, and subsequently, large and folded papillae can be regarded to as

independent and predictive risk factors for PEP as such patients may be prone to inadvertent pancreatic duct manipulation and pancreatic stenting.

*Originality and applicability of the results in medical practice*

Only limited research that focused mainly on the impact of papillary morphology on deep cannulation could be identified in literature (Matsushita et al.,2008; Haraldsson et al., 2017; Haraldsson et al., 2019). One of the main reasons for such paucity within the literature is that native papillae have such variable appearances that a commonly accepted classification does not yet exist (Adler, 2019). Therefore, the expanded systematic arrangement in our study aimed to cover some other papillary aspects like the small and retracted papillae or the large, hooded papillae with multiple folds over the orifice. Furthermore, our study went a step further and evaluated not only the impact on cannulation, but also that on ERCP outcomes and adverse events

The results obtained from our study showed that not only small and retracted papillae represent anatomical conditions associated with the presence of PEP but also Type 4 papillae were individual and predictive risk factors for PEP.

To our knowledge there is no similar study confirming such assumption.

Recognizing the type of papilla and related to it, assessing the difficulty of cannulation can help with decision making and implementation of early rescue techniques during the procedure, thus limiting the number of attempts at cannulation and thus the risk of adverse effects after ERCP.

*Limitations of the study*

Nevertheless, by covering supplementary endoscopic characteristics of the papilla, we needed to consider the limitation of using a non-validated classification.

Another limitation of the study lies in the fact that the success rate and postinterventional evolution related to the endoscopist experience were not evaluated but the present study was not designed to investigate the influence of endoscopists' experience on successful cannulation of the various types of papilla and subsequent ERCP outcomes.

*Future directions*

Further dedicated studies on training in ERCP that would consider the impact of duodenal and papillary anatomy on cannulation and outcomes are clearly required.

The results of our study on the correlation between papilla anatomy, cannulation success rate and subsequent evolution should be further validated by multicenter prospective trials.

### *3.2.2.2. Assessing pancreatic toxicity of the contrast substance after Endoscopic Retrograde Cholangiopancreatography*

The second aspect related to the interventional technique that has been little studied and that has been the subject of our research is represented by the potential toxicity of the contrast substance used on the pancreas.

Even if in theory the intraductal presence of contrast agent could cause local toxicity and pancreatitis (Pezzilli et al., 2002), guideline studies suggest that no clinical risk has been identified to be specifically linked to such contrast media (CM) (ASGE,, 2005). However, there is high suspicion in the literature about the influence that CM osmolality has on the risk of post-ERCP pancreatitis. High osmolality contrast media (HOCM) are approximately five to eight times the osmolality of serum. Low osmolality contrast media (LOCM) are less than three times the osmolality of human serum and preferred for intravascular and intrathecal administration.

Nevertheless, there are also some procedure-related risk factors for CM related post-ERCP pancreatitis – the volume of CM injected leading to parenchymal acinarization and high pressure CM injection (Loperfido et al., 1998). Especially high pressure pancreartic duct CM injection with subsequent prolonged acinar filling due to ductal occlusion and/or repeated ductal cannulations are associated with a higher risk of post-ERCP pancreatitis (Goebel et al.,

2000). A relatively recent clinical report enforced that CM related post-ERCP pancreatitis is a complication resulting from a combination of pressure and subsequent contrast exposure of the pancreas (Frank, Adler, 2006), this hypothesis being afterwards confirmed by recent experimental studies.
Based on these aspects, we aimed to conduct a pilot study to evaluate the pancreatic toxicity of iopamidol.
The interventional procedure (ERCP) involves, after visualizing the papilla by duodenoscopy, the cannulation of Vater's ampoule, an aspect that was detailed earlier. (Chutkan et al., 2006). Afterwards, water-soluble iodine-based contrast media (CM) is injected through a transampullary catheter into the biliary and pancreatic ducts (Kimmey et al., 1996). One of the most widely used CM in Romania is *iopamidol* which is an iodinated nonionic monomer and low-osmolar CM Iopamidol is an organic compound used as water soluble radiographic contrast medium, and acts by blocking x-rays as they pass through the body, thereby allowing body structures not containing iodine to be visualized. Trade names of iopamidol include Iopamiro®, Isovue®, Iopamiron®, Solutrast®, or Niopam®.
Iopamidol is used not only in ERCP or other endoscopic contrast-enhanced procedures. Firstly, the substance was designed to be used as a CM for angiography, excretory urography or myelography. To date, iopamidol is most widely used as intravascular CM for computed tomography. Most of the literature findings and guidelines recall, knowledge regarding CM efficacy, safety and side effects derives in the vast majority of cases from their intravenous use (ASGE, 2005). Subsequently, many studies assessed potential side effects of iopamidol, especially regarding allergic reactions and kidney toxicity, as iopamidol is administered mainly intravenously and excretion is almost entirely urinary.
Systemic concentrations and pancreatic toxicity of iopamidol after intrabiliary and intrapancreatic administration during ERCP were not thoroughly studied.

***Experimental part***
Findings on four patients with post-ERCP pancreatitis were assessed in order to evaluate potential toxicity of iopamidol. All cases were patients previously diagnosed with multiple choledocholithiasis and gallbladder stones, without clinically or biologically manifest pancreatitis. ERCP with endoscopic sphincterotomy and stone removal was primary elective indication in all patients and procedures were performed by two experienced endoscopists in the Institute of Gastroenterology and Hepatology of Iasi, Romania, between January and March, 2016. Deep sedation and bowel movements control were achieved. In all cases, intra-procedural cholangiography followed by endoscopic sphincterotomy and stone extraction was performed. Selection of cases for the toxicity study was made according to the fact that all four patients needed a second ERCP for repeated extraction of bile duct stones (after 48 to 72h). Moreover, in all four cases, first ERCP was a difficult procedure due to multitude of bile duct stones, repeated cannulation of primary bile duct and time span of procedure. Most importantly, in all four cases, during the first ERCP large amounts of iopamidol were infused (60, 40, 40, 50 mL iopamidol 100%). In contrast, second ERCP was characterized by usage of diluted iopamidol 50% with amounts not exceeding 40mL of iopamidol solution 50% infused.
Dynamics of lipase and C-reactive protein (CRP) between the two procedures (6h and 24h) were studied and pancreatic ERCP-guided brush cytology was obtained during the second procedure.

**Results**
All patients showed ascendant dynamics of both lipase levels and CRP up to more than 180 U/L lipase and 22 mg/dL CRP. In all patients, both lipase and CRP levels were characterized

by prolonged and continuous elevation towards 24h. Pain started mildly after the procedure and needed analgesics control 12 h after the procedure.

Cytology of the main pancreatic duct was obtained with techniques similar to those used with brush cytology of the bile duct using wire-guided brush is used to collect cytologic material from the pancreatic duct. Cytology characteristics of ductal epithelium were studied: cytoplasm, nuclei and architectural features. This is the less invasive method for obtaining pancreatic cells in a clinical experimental study. All patients showed necrotic background cells, cellular debris, and saponification necrosis with various degrees of reactive changes in the ductal epithelia No patient developed severe macroscopic necrotic pancreatitis after ERCP and both lipase elevation and CRP levels reached back normal levels 4 to 7 days after the procedure. Even if pancreatic duct brush cytology is itself characterized by a high risk of post-procedure pancreatitis, interestingly, no patient developed sustained pancreatitis after second ERCP. Furthermore, two possible explanations could coexist: firstly the possible additional cause of pancreatitis may have been persistent bile duct stones after first ERCP and endoscopic sphincterotomy altogether with large amounts of CM infusion; secondly, pancreatic duct brush instrumentation may have helped the clearance of Wirsung duct, in this way helping pancreatitic and toxic phenomena heal. Valuable recent experimental findings by Jin et al., 2015, on pancreatic exposure to radiocontrast agents suggests that even brief exposure to low concentrations of CM induces aberrant cytosolic $Ca^{2+}$ signals selectively in mouse and human pancreatic acinar cells by activation of $Ca^{2+}$ dependent phosphatase calcineurin linked in vivo to pancreatic nuclear factor k-light-chain-enhancer of B cells (NF-kB) activation and pancreatitis (Jin et al., 2015). Past studies have shown that acidic contrast is linked to mediation of severity of pancreatitis in rats (Noble et al., 2008). Experimental in vitro and ex vivo studies results are consistent with the clinical findings on pancreatic duct brush cytology and are attested by enzymatic and inflammatory response dynamics of the patients included. On the other hand, no study assessed the role of CMs viscosity in pancreatic toxicity, as temperature may strongly affect this parameter, to that extent that CMs may be warmed before procedures. Nevertheless, in what post-ERCP pancreatic toxicity is concerned, such practices should be considered as just speculations, as no study to date assessed such positive effect.

Pancreatitis is considered to debut inside acinar cells, one of the earliest critical factors in most experimental models being induction of large amplitude and sustained $Ca^{2+}$ signaling (Petersen, Sutton, 2006; Husain et al., 2012). Principal regulator of inflammation in pancreatitis was found to be the transcription factor NF- kB, which can subsequently induce inflammatory genes IL6, Spi2a, transforming growth factor-β and IL1β (Zhang et al., 2019, Neuhofer et al., 2013). It was subsequently confirmed that presence of CMs induces $Ca^{2+}$ signaling and calcineurin activation in the primary acinar cells causing also increase in NF-kB luciferase directly proportional with CMs concentration and time of exposure (Jin et al., 2015). Recent research may also suggest that CMs might contain a triglyceride lipid emulsion with potential liberation into toxic nonesterified fatty acids when exposed to pancreatic lipases during ERCP (Durgampudi et al., 2014; Navina et al., 2011).

*Metanalysis findings*

There is one single metanalysis in the literature assessing the possible role of CMs as a contributing factor for post-ERCP pancreatitis. This role seems dependent on the osmolality of the CM, HOCM supposedly being associated with a higher incidence of post-ERCP pancreatitis or at least elevation of pancreatic enzymes. In this respect, as the findings of this metanalysis show, one randomized crossover study and 5 randomized control trials have suggested a benefit from using LOCM, while 11 other studies have shown no difference between HOCM and LOCM as to what post-ERCP pancreatitis is concerned (George et al., 2004). However, such discrepancies could be explained by the lack of unitary definition

standards for post-ERCP pancreatitis. Therefore, for the moment we should refrain from stressing differences between HOCM and LOCM even if LOCM would be desirable choice.

**Discussions**

Post-ERCP pancreatitis was proven to result from a combination of pancreatic ductal pressure and CMs exposure, as each additively worsens disease outcome (Jin et al., 2015). These findings are confirmed by clinical data showing that insertion of pancreatic duct stents that would lower intraductal pressure on the first hand, and injecting only the minimul necesarry amount of CM during ERCP on the other hand – both may work as prophylaxis measures for post-ERCP pancreatitis (Fazel et al., 2003; Sofuni et al., 2007). At a cellular level, the toxicity process was explained by $Ca^{2+}$ signalling emerging 1-2 minutes CM exposure, with mimiked high amplitude peak-plateau preceding acinar cell injury and pancreatitis (ASGE, 2005; Petersen, Sutton, 2006, Pandol, 2010). The debate on whether HOCM or LOCM should be electively used for minimizing post-ERCP pancreatitis incidence is still going. Studies suggesting the superiority of LOCM (iopamidol being includes in this cathegory) were based mainly on the incidence of asymptomatic elevation of pancreatic enzymes – biochemical pancreatitis. (ASGE, 2003).

**Conclusions**

Iopamidol is a relatively old and well known LOCM. Despite high costs comparative to HOCM, Iopamidol is largely available in Romania and is frequently and routinely used as CM in ERCP. Post-ERCP pancreatitis development status is an important quality indicator of ERCP. Moreover, when it is dependent on the specific type of CM used, once more careful selection of radio-contrast substances should be performed. As it was seen in CMs studies, data from large randomized controlled trials or metanalyses are frequently lacking and only singular trials are available. Literature analysis shows that iopamidol should be a safe and efficient CM for ERCP especially when used strictly in minimal necessary amounts and concentrations. Independently on the iodinated CM used, the amount of iodine injected was shown to be direct proportional to the amplitude of potential CM-related side effects.

*Originality and applicability of the results in medical practice*

To date, no local clinical trial to evaluate iopamidol safety and efficacy was performed.

In patients considered at high risk for CM-related reactions, prophylactic premedication and prior substitution of LOCM may be an option at least based on theoretical considerations.

*Limitations of the study*

Main downsides on potentially statistically significant studies involving CMs reside in the small number of patients included in studies leading to lack of direct delimitations between various causes on pancreatic damage occuring post-ERCP in the study groups. However, when objective findings even on such series of cases are sustained by statistically significand animal and in vitro studies, a higher degree level of confidence of the results is achieved.

*Future directions*

Based on the reports in the literature and the results of our experimental study, it would be interesting to evaluate the safety of iopamidol use in relation to the ERCP indication.

*

In most cases, ERCP is a safe procedure, without post-procedural complications. However, as we mentioned at the beginning of the chapter, there are situations where these may occur and one of the most common complications is acute pancreatitis (PEP).

Despite the increasingly performing equipment used in ERCP and the wider experience of endoscopists, the incidence of PEP has remained relatively constant (Masci et al., 2001).

In this context, it is understandable the permanent interest in the study of physio-pathological mechanisms and risk factors, in order to identify methods of preventing acute pancreatitis. (Sethi et al.,2014)
Concerning the prophylaxis of post-ERCP acute pancreatitis, it seems that pancreatic stent placement has the highest efficiency in preventing this event in high risk patients and consequently, this method is recommended by some guidelines (Dumonceau et al., 2014; Tenner et al., 2013). All things considered, pancreatic duct stenting is a difficult, costly maneuver and is not routinely indicated to all patients undergoing ERCP. Nevertheless, it requires special training and expertise as it can be even harmfull in untrained hands. (Dumonceau et al., 2014).
Considering the involved pathophysiology, there are many studies evaluating the efficacy of various agents (NSAIDs, octreotide, antioxidants), in various dosage forms, with oral, intrarectal or parenteral administration routes, before or after the procedure (Leerhøy, Nordholm_Carstensen, 2014; Wong, Tsai, 2014; Sotoudehmanesh et al., 2007; Ding et al., 2012; Murray et al., 2003)
These studies have reported conflicting results. Although there are many studies proving that intrarectal administration of NSAIDs before or after ERCP is efficient in preventing post-ERCP pancreatitis (Murray et al., 2003; Sotoudehmanesh et al. , 2007 ) and as a consequence the International guidelines do recommend NSAIDs as a primary prophylaxis measure, a series of more recent studies tend to contradict this previous findings (Rainio et al., 2017; Levenick et al., 2016). Moreover, although the role of oxidative stress in the pathophysiology of this preocess has been clearly stated, the efficacy of various antioxidant agents in preventing post-ERCP pancreatitis has not been confirmed (Maziar et al., 2015; Fuentes-Orozco et al., 2015).
Considering this background, we aimed to perform two studies: one that analyzed the incidence of PEP and the presence of risk factors in a group of patients with indication for ERCP and a second comparative study that evaluated the effectiveness of three prophylactic approaches aiming to reduce the risk of PEP, using pharmacologic agents with different mechanisms of action (NSAIDs and/or N-acetylcysteine -NAC) in three different regimens.
Based on these premises, we initiated two studies (Pavel et al., 2018; Pavel et al., 2019) which aimed to evaluate the post-ERCP progression of patients. In first study the patients received standard pharmacological prophylaxis with indomethacin, regardless the presence of risk factors, in the second one received three pharmacological combination therapies.
Patients from both studies were monitored to assess the evolution from the perspective of acute pancreatitis as a complication of interventional endoscopy.

### *3.2.2.3. The evolution of clinico-biological profile of patients undergoing prophilaxis for post-ERCP pancreatitis*

The first study was a prospective one and was conducted in Institute of Gastroenterology and Hepatology - Iași between January-July 2017 (Pavel et al., 2018).
It was a preliminary study in which we aimed to analyze the risk factors for the occurrence of PEP in patients with choledochal lithiasis, who required interventional endoscopy (ERCP).

**Materials and methods**

*Patients*

All 108 patients were over 18 years old, with biliary stones, and were proposed for inclusion in the study, due to the indication of performing an ERCP.
Exclusion criteria were the presence of acute pancreatitis or other acute inflammatory diseases at the moment of admission, contraindications for NSAIDs, recent episodes of upper gastrointestinal bleeding (less than 1 month), pregnancy and patient refusal.

After the initial evaluation, 98 patients were enrolled in the study that met the inclusion criteria and signed informed consent.

*Research protocol*

After signing the informed consent, each patient included in the study was evaluated before the intervention, to identify the risk factors through anamnesis, clinical examination, laboratory tests (complete blood count, pancreatic serum and urinary enzymes hepatic and renal tests) and imaging: ultrasound and MRCP, according to the protocols in force for biliary pathology.

ERCP was performed following the standard protocol, after evaluation and anesthesia physician's opinion. Pharyngeal anesthesia was administered with 2% lidocaine spray and sedation with midazolam, ketamine and propofol. The equipment and materials used for the ERCP included a TJF 160 series (Olympus, Tokyo, Japan), standard sphincterotomas - Olympus and Boston Scientific (Marlborough, MA, United States), Visiglide hydrophilic guide wires (Olympus), and catheter extraction balloon (Boston Scientific and Wilson-Cook Medical Inc. Bloomington, IN, United States) and / or Dormia basket from Olympus. Contrast agent was water-soluble iopamidol.

All patients were continuously monitored during the intervention, assisted by the anesthesiologist, and parameters related to the procedure (number of cannulations, amount of contrast substance, length of procedure) were recorded in the database.

All patients received 100 mg of indomethacin intrarectal, immediately after ERCP, for the prophylaxis of post-ERCP acute pancreatitis, according to the European guidelines, and administered Ringer's lactate solution (1.5 mL/Kg /h) during the procedure and 8 hours post ERCP. After the procedure, at 6 and 24 hours, as well as in case of algic post-ERCP symptomatology, the clinical parameters (presence of abdominal pain, vomiting, fever, transit disorders) and some of the parameters (cells blood count, C-reactive protein, pancreatic enzymes) were evaluated. In addition, all adverse effects related to the procedure or medication administered, duration of hospitalization was recorded.

The diagnosis of PEP was set if the patient reported post-procedural abdominal pain or increased preexisting pain while pancreatic enzymes increased by at least three times the normal. Pancreatic amylase elevation of at least three times the normal value in the absence of symptomatology was classified as asymptomatic increased amylase.

Monitoring was discontinued 24 hours after ERCP if no special events were reported or after the event had been resolved if it occurred within the first 24 hours post-ERCP. In case of a complicated evolution, the patients were re-evaluated 30 days after discharge.

Data obtained from patient evaluation at all stages of monitoring (pre and post procedural) were set in a database.

Statistical analysis was conducted using SPSS 20.0 version.

The study protocol and the patient consent form were approved by the Ethics committee of Grigore T Popa University of Medicine and Pharmacy and Sf Spiridon Clinical Emergency Hospital, Iasi, Romania.

**Results**

The study group consisted of 98 with an average age of 63.56 ± 14.09 years old without statistically significant differences between genders.

*Study of clinical and biological data at study inclusion*

Among the clinical data recorded in the initial evaluation, the most common symptom was the pain in the right hypochondrium, identified in 67% of cases, followed by jaundice (53% of patients).

Related to the biological data recorded in the initial assessment of patients, cholestasis and hepatic cytolysis were the main changes recorded, both identified in 83.7% of patients.

*Study of the clinical and biological parameters evolution at 6 hours and 24 hours post ERCP*

The PEP diagnosis was recorded based on the clinical examination associated with biological samples (pancreatic enzymes and parameters of inflammatory syndrome- leukocytes, C-reactive protein) monitored at 6 and 24 hours post-ERCP.
From the 98 patients enrolled in the study, 8 patients (8.16%) developed acute pancreatitis manifested clinically after the interventional procedure. It should be noted that in all cases the onset was early, less than 6 hours after the intervention, with no cases of late onset. From the patients who developed acute pancreatitis after ERCP, 6 patients were mild (75%) and 2 moderate forms. There were no severe pancreatitis and all cases had a favorable progression. No adverse effects related to study medication (digestive bleeding, rectal irritation, or allergy) have been reported.
The comparative study of pancreatic enzymes (amylase and lipase) and C-reactive protein at the two monitoring periods (6 and 24 hours post-ERCP) is synthetically represented in Table 3.XI. At 6 hours post ERCP, amylase increases of more than 3 times the normal value in 18.4% of patients, while in 24 hours only 8.2% of patients had values compatible with the diagnosis of asymptomatic hyperamylasemia. Lipase values had a similar dynamics to amylase.
Also, the inflammatory status assessed by C-reactiv protein determination has undergone significant variations during the follow-up: 51% of patients experienced C-reactiv protein increase, more than half of them with moderate / marked elevated values. It should be noted that the inflammatory status rate was maintained in the same proportions at the 6 and 24 hours respectively.

**Table 3.XI.** Dynamics of clinical and biological factors at 6 and 24 hours

| | **At 6 h** | **At 24 h** |
|---|---|---|
| **Amylasemia** | | |
| < 3 x N | 81.6% | 91.8% |
| > 3 x N | 18.4% | 8.2% |
| **Lipase** | | |
| < 3 x N | 69.4% | 91.8% |
| > 3 x N | 30.6% | 8.2% |
| **CRP** | | |
| normal | 49.0% | 49.0% |
| minor increase | 20.4% | 20.4% |
| moderate increase | 26.5% | 22.4% |
| important increase | 4.1% | 8.2% |
| **Clinical-biological status at 6 h** | | |
| Absence of PEP criteria | 91.8% | 91.8% |
| PEP | 8.2% | 8.2% |

*Study of risk factors for PEP development*
Another objective of the research was to determine whether there is a correlation between the occurrence of acute pancreatitis and the presence of risk factors associated with the development of PEP in the study group.
We assessed the risk of pancreatitis in the presence and absence of risk factors. Five distinct risk factors (RF), ranging from RF 1 to RF 5, have been studied and have the following meaning:
- RF 1: female gender,
- RF 2: young age,
- RF 3: normal bilirubin,
- RF 4: excessive contrast substance,
- RF 5: history of PEP

77.6% of patients enrolled in the study had at least one risk factor for PEP. We studied the presence of the risk factors and we identified: female sex (45% of cases), age under 60 years

(36.7%), normal bilirubin (20.4%), excess of contrast substance (12.2%) and acute pancreatitis post-ERCP in the past (4.1%).
All patients who developed PEP were females. As a result, for 18.2% of the patients who developed the complication, female gender was a risk factor. The value is statistically significant, especially since no male patient presented the complication.
Since no cases of post-ERCP pancreatitis have been reported in patients under the age of 60, we cannot claim that young age has been a risk factor for the patients in our study group.
Also the normal serum level of bilirubin before endoscopic intervention was not related to the risk of developing the complication. Among the patients who developed acute pancreatitis, 4 of them had normal serum bilirubin and the other 4 had hyperbilirubinemia. As a result, this did not significantly correlate with the occurrence of acute pancreatitis.
Excessive contrast substance administration correlated with the occurrence of acute early pancreatitis (in the first 6 hours): 66.7% of patients with excess contrast were diagnosed with acute pancreatitis, while none of the patients without excess of contrast substance developed the complication. Acute pancreatitis did not statistically correlate with the presence of other episodes of pancreatitis (RF5) in the past. None of the patients with a history of pancreatitis developed acute pancreatitis, whereas among those without a history of pancreatitis, 8.5% of the patients presented the complication. OR risk analysis has shown that the only significant risk factor for pancreatitis is the use of an increased amount of contrast (table 3.XII)

**Table 3. XII.** Chi-square test values to compare the presence of PEP
Depending on the presence of risk factors

| Clinical status | Chi-square | p | OR |
|---|---|---|---|
| **RF pres. / abs.** | 1.261 | 0.261 | - |
| **RF 1 pres. / abs** female gender. | 5.345 | 0.021 | - |
| **RF 2 pres. / abs**. young age | 2.529 | 0.112 | - |
| **RF 3 pres. / abs.** normal bilirubin | 2.348 | 0.125 | 4.625 |
| **RF 4 pres. / abs**. excessive contrast subst | 31.215 | 0.000 | - |
| RF 5 pres. / abs. **history of PEP** | 0.185 | 0.667 | - |

## Discussions

In our study, the incidence of PEP was 8.16%. The results are similar to those reported in other studies: Cheng et al reported an incidence of 2-9% in the general population and 15% in high-risk patients.(Cheng et al., 2006)
The patients in our group were not selected based on the presence of risk factors and they all received prophylaxis with indomethacin, according to international guidelines. These aspects may explain differences compared to other studies. (Yang et al., 2017)
It should be emphasized that the clinical and biological evolution of the cases that were diagnosed with PEP and asymptomatic hyperamylasemia was favorable, with the improvement of the symptomatology and biological parameters in all patients, with no severe forms or progression to worsening or death. These favorable results may have been obtained because all patients received NSAIDs prophylaxis regardless of the presence or absence of risk factors. According to the literature, prophylaxis with indomethacin is effective, reducing the incidence of acute pancreatitis and improving the clinical and biological evolution of post-ERCP cases of pancreatitis. (Yang et al., 2017; Liu et al., 2018; Sheikh et al., 2014)
Another objective was to study whether the factors recognized in the literature as associated with the occurrence of acute post-ERCP pancreatitis (female sex, age under 60 years, normal

serum bilirubin, excess contrast substance and personal history of pancreatitis) are associated in our group with the occurrence of acute pancreatitis. (Freeman et al., 2001). Risk factor analysis demonstrated that although all of the factors mentioned were present in varying proportions in the patients from the study group, only female gender and excess contrast were correlated with the occurrence of pancreatitis, OR risk assessment demonstrating that the latter influenced the most the risk of PEP.
These results, discordant compared to other published studies, can be explained by the fact that the number of patients included in the study (and implicitly those who developed PEP) was small.

**Conclusions**
The incidence of acute post-ERCP pancreatitis in our study was similar to the one reported in the literature. No severe pancreatitis was recorded, and the progression of cases with pancreatic complications was rapidly favorable. Out of the risk factors associated with the occurrence of acute post-ERCP pancreatitis, in the patients in our study, only female sex and excess contrast substance correlated with PEP.

***3.2.2.4. Comparative evaluation of standard prophylaxis versus other pharmacological regimens for the prevention of post-ERCP pancreatitis***
The second study aimed to comparatively evaluate the efficacy of three therapeutic regimens administered for prophylactic purposes to patients with an indication for ERCP (Pavel et al., 2019). Efficacy was assessed by analyzing the incidence of PEP and asymptomatic hyperamylasemia as well as by monitoring the short- and medium-term evolution of patients who developed PEP.

**Material and methods**
*Patients*
The second study was performed between April 2017- July 2018. The study design was that of a prospective, single blind randomized trial comparing and dinamically evaluating the efficacy of three pharmacological combination therapies (Indomethacin in various doses with or without an extra dose of N-acetylcysteine), aiming to prevent acute pancreatitis in patients with choledocholithiasis and indication for undergoing ERCP.
Inclusion criteria consisted in: diagnosis of choledocholithiasis and indication for ERCP procedures, age 18 years and older, willingness to participate in the study and ability to sign the informed consent.
The exclusion criteria were presence of acute pancreatitis or other inflammatory diseases at admission, pregnancy, contraindication for NSAID administration, recent episode of upper digestive bleeding (less than one month), hypersensibility to antioxidants, necessity of a prophylactic pancreatic stent insertion or patients' disapproval to take part in the study.
After initial evaluation, 186 patients were enrolled in the study, after fulfilling the inclusion criteria and signing the informed consent form. They were divided into three groups, subjected to simple randomization: 98 patients comprised the control group, who underwent prophilaxis according to the European Guideline, consisting of intrarectal administration of 100 mg Indomethacin after ERCP, while 32 patients constituted the *group A*, who were administered 600 mg NAC 15 minutes prior to ERCP and per rectum administration of 50 mg Indomethacin prior and after completion of ERCP and *group B* which consisted of 56 patients, who were administered 50 mg Indomethacin per rectum both prior and after ERCP.
*Study protocol*
After obtaining the written informed consent, each patient included in the study was evaluated for identifying precise indication for ERCP. ERCP was performed following the standard

protocol, described in the previous study. No pancreatic stents were used with prophylactic purpose per study protocol as insertion of pancreatic stents was an exclusion criteria because of additional study biases. Monitoring during and after the procedure was done in the same way as in the previous study. Number of cannulation attempts, time for cannulation, amount of contrast agent used, duration of procedure, and number of involuntary pancreatic guidewire passages) were registered in the database. Moreover, hospitalization time and all procedure- or medication- related adverse events were recorded.
Several clinical (abdominal pain, nausea, emesis, fever, change of bowel habit) and biological parameters (complete blood count, C reactive protein, serum pancreatic enzymes) have been recorded at both 6 and 24 hours following the procedure.
Diagnosis of PEP was established after the criteria described by Cotton et al. (Cotton et al., 2009) as in the previous study. An asymptomatic increase of serum pancreatic enzymes above upper normal value was interpreted as asymptomatic hyperamylasemia.
The collected data from all monitoring stages were introduced in an electronic database.
Statistical analysis was conducted using SPSS 20.0 version.
The protocol and the patient consent form were approved by the Ethics committee of Grigore T Popa University of Medicine and Pharmacy and St Spiridon Clinical Emergency Hospital, Iasi, Romania.

**Results**

*Study Groups description*

211 patients have been evaluated during the study, of which 186 patients fulfilled the inclusion criteria (102 women - 54.8% and 84 males - 45.2%). The patients have been randomized to one of the following 3 arms:

a) *Control group* - 98 patients (52.7%) receiving 100 mg indomethacin suppository immediately post ERCP,
b) *Group A* - 32 patients (17.2%) receiving NAC 600 mg before performing ERCP and indomethacin suppository 50 mg before and after performing ERCP
c) *Group B* - 56 patients (30.1%) receiving indometacin suppository 50 mg before and 50 mg after ERCP.

Median age of all included patients was 64.32 ± 14.91 years, without statistically significant differences between the 3 groups. Also there was similar number of male and female patients (t Test t = 1.056, p = 0.294, NS)
There have been evaluated the known risk factors involved in PEP occurence: sex, age, bilirubin, amount of contrast medium/agent used, number of cannulations and history of post-ERCP acute pancreatitis (PEP). The evaluation of each group showed no statistically significant differences between the distribution of the analized risk factors. (Table 3.XIII).

**Table 3.XIII.** Pearson's chi-squared test
to compare the risk factors occurrence in the 3 study arms

| PEP | Pearson Chi-square | df | P |
|---|---|---|---|
| **Existent risk factors** | 0.627 | 2 | 0.731, NS |
| *Female sex* | 5.651 | 2 | 0.059, NS |
| *Young age* | 3.030 | 2 | 0.220, NS |
| *Normal bilirubin* | 5.446 | 2 | 0.066, NS |
| *Excess contrast agent* | 2.409 | 2 | 0.300, NS |
| *No. of cannulations* | 0.645 | 2 | 0.724, NS |
| *PEP* | 1.835 | 2 | 0.399, NS |

After evaluating and comparing the demography and risk factors for acute pancreatitis in the 3 study groups it was concluded there are no statistically significant differences between the 3

pools of patients regarding the factors that might influence the features studied below (PEP occurence, specific laboratory results or the responsiveness to treatment).
As a consequence the selected study groups are similar thus creating the premises of a correct evaluation, with valid comparisons between the study arms.

*Comparative analysis of the evolution of clinical features and laboratory results at 6 and 24 hours post-ERCP in the 3 study groups*
The efficacy of the 3 studied regimens was evaluated by comparing the clinical evolution and the laboratory results in the 3 study groups.
PEP was diagnosed based on clinical exam and the laboratory tests; the considered laboratory tests have been pancreatic enzymes and inflammatory syndrome markers (white blood cells-WBC, C-reactive protein-CRP), measured at 6 and respectively 24 hours post-ERCP. For the evaluation of the statistical significance the Pearson's chi-squared test was used.
Of the 186 patients included in our study 18 patients developped clinically significant acute pancreatitis after the procedure. The percentage was similar in the 3 study groups: 8 patients from the control group (8.16%), 4 patients from *Study Group A* (12.5%), 6 patients in *Study Group B* (10.71%), the differences showed no statistical significance ($\chi^2$=2.793, p = 0.247). Of note, in most of the cases (16 patients) the debut was precocious, under 6 hours post ERCP and only in 2 cases the debut was late, both those cases belonged to *Study Group B*. Of the acute pancreatitis cases post-ERCP 14 patients were mild cases (77.77%) and 4 were moderate cases. No severe cases have been recorded and in all cases the evolution was favorable.
There have been no reported adverse events related to study medication (digestive haemorrhage, rectal irritation or allergies).
The comparative evaluation of pancreatic enzymes and CRP at the 2 considered time points (6 and 24 hours post-ERCP) is briefly represented in tables 3.XIV and 3.XV.

**Table 3.XIV.** Pearson's chi-squared test
to compare between the 3 study arms clinical and laboratory factors at 6 hours

| | **Control Arm** | **Study Group A** | **Study Group B** | **Chi-square** | **p** |
|---|---|---|---|---|---|
| **Amylasemia at 6 hours** | | | | 4.288 | 0.368 |
| < 3 x ULN | 81.6% | 100.0% | 85.7% | | |
| > 3 x ULN | 18.4% | 0.0% | 14.3% | | |
| **Lipasemia at 6 hours** | | | | 10.046 | 0.040 |
| < 3 x ULN | 69.4% | 68.7% | 71.4% | | |
| > 3 x ULN | 30.6% | 31.3% | 28.6% | | |
| **CRP at 6 hours** | | | | 12.165 | 0.058 |
| Normal | 49.0% | 56.2% | 57.1% | | |
| Mild increase | 20.4% | 12.5% | 35.7% | | |
| Moderate increase | 26.5% | 31.2% | 0.0% | | |
| Severe increase | 4.1% | 0.0% | 7.1% | | |
| **Clinico-biologic Status at 6 hours** | | | | 0.397 | 0.820 |
| PEP Absent | 91.8% | 87.5% | 92.9% | | |
| PEP | 8.2% | 12.5% | 7.1% | | |

In the *Control Group* at 6 hours post-ERCP there have been identified 3 fold ULN raises of amylasemia for 18.4% of patients, while at 24 hours only 8.2% of patients exhibited values that might be recorded as asymptomatic hyperamylasemia.

In *Study Group A*, although at 6 hours post-ERCP 25% of patients exhibited raised amylasemia and at 24 hours there were 12.5% patients with amylasemia over normal limit there have been no cases of 3 fold ULN increase either at 6 hours nor at 24 hours post-ERCP.
In *Study Group B* at 6 hours post-ERCP the percentage of patients exhibiting asymptomatic hyperamylasemia was 14.3%, with no statistically significant differences compared to *Study Group A* and the *Control Group* (p=0.368), while at 24 hours the percentage of these patients raised at 28.6%, significantly larger compared to the other study arms (p=0.019), an unfavorable evolution of this criterion.
Lipasemia values showed a similar evolution as amylasemia, with significant improvement in the *Control Group* and the *Study Group A* (Tables 3XIV and 3XV).
In the *Control Group* at 6 hours post-ERCP have been registered 30.6% patients with a 3 fold ULN increased lipasemia, but at 24 hours this percentage decreased at 8.2%.
In *Study Group A* at 6 hours 31.3% patients presented with a 3 fold upper limit of normal (ULN) increased lipasemia and at 24 hours only 12.5% percentage.
In *Study Group B* the percentage of patients with a 3 fold ULN increased lipasemia remained unchanged at both measurements (28.6%) with a statistically significant difference compared to the other two study arms, at 6 hours (p=0.040) and at 24 hours (p=0.029) post-ERCP.
Inflammatory status, evaluated by CRP values suffered significant variations during these evaluations but significant diffreneces between the study arms have been noticed only at 24 hours post-ERCP (p=0.046) (table 3.XIV and 3.XV).

**Table 3.XV.** Pearson's chi-squared test
to compare between the 3 study arms clinical and laboratory factors at 24 hours

| | Control Arm | Study Group A | Study Group B | Chi-square | p |
|---|---|---|---|---|---|
| **Amylasemia at 6 hours** | | | | 11.835 | 0.019 |
| < 3 x ULN | 91.8% | 100.0% | 71.4% | | |
| > 3 x ULN | 8.2% | 0.0% | 28.6% | | |
| **Lipasemia at 24 hours** | | | | 9.889 | 0.029 |
| < 3 x ULN | 91.8% | 87.5% | 71.4% | | |
| > 3 x ULN | 8.2% | 12.5% | 28.6% | | |
| **CRP at 24 hours** | | | | 12.824 | 0.046 |
| Normal | 49.0% | 43.8% | 64.3% | | |
| Mild increase | 20.4% | 0.0% | 7.1% | | |
| Moderate increase | 22.4% | 56.2% | 21.4% | | |
| Severe increase | 8.2% | 0.0% | 7.1% | | |
| **Clinico-biologic status at 24 hours** | | | | 2.793 | 0.247 |
| PEP Absent | 91.8% | 87.5% | 78.6% | | |
| PEP | 8.2% | 12.5% | 21.4% | | |

## Discussions

The incidence of PEP varies in different studies according to the included patients risk level, mild or medium (Rustagi et al., 2015; Chandrasekhar et al., 2017; Cotton et al., 1991; Cooper, Slivka, 2007; Inamdar et al., 2017).
In our study the post-ERCP acute pancreatitis incidence was 9.67% over all included patients and considering there was no inclusion selection risk related, the global incidence was similar with previous reported data (Rustagi et al., 2015; Chandrasekhar et al., 2017).
Decreased post-ERCP acute pancreatitis occurence can be achieved by a thorough patient selection either by choosing the therapeutic use of this procedure to prevail (Yang et al., 2017; Kochar et al., 2015) or by using special ERCP techniques, especially in high-risk patients.

On the other hand, as previously mentioned, there has been some pharmacological research for PEP prophylaxis, most of the studied molecules specifically interfering with different steps of the inflammatory cascade. The most convincing results appeared to be those related to intrarectal administered NSAIDs (Ding et al., 2012; Mazaki et al., 2014; Choudhary et al., 2011; Elmunzer et al., 2012).
The efficacy was studied NSAID type related (indomethacin or diclofenac), dose related (50mg indomethacin versus 100mg indomethacin), route of administration related (rectal, oral or iv), time of administration (before or after ERCP) and compared to placebo. Several meta analyses showed a risk reduction related to moderate or severe PEP occurence and a similar efficiency of rectal route of administration immediately before or after ERCP (Rustagi et al., 2015; Choudhary et al., 2011; Sethi et al., 2014).
The most cited study belongs to Elmunzer and it reported the efficacy of 100 mg indomethacin suppository administered intrarectal immediately after ERCP (Mazaki, et al. 2014).
Although most studies report favorable results, a recent prospective, double-blind, placebo-controlled study showed no benefit in indomethacin administration for PEP prophylaxis (Levenick et al., 2016) or is protective against PEP in high-risk patients but not average-risk patients. (Puig et al., 2014) .
Also a recent study published in by Yang C claims the efficiency of NSAIDs prophylaxis for post-ERCP acute pancreatitis but it is related to the moment of administration, before or after the procedure (Luo et al., 2016).
On the other hand there are recent papers published suggesting the efficient use of other drug classes (nitroglycerin, antioxidants, somatostatin, antibiotics) that might be useful in PEP prophylaxis (Kubiliun et al., 2015; Akshintala et al., 2013; Gooshe et al., 2015; Xingkang et al., 2018).
Considering these hypotesis our study evaluated the efficacy of 2 regimens with indomethacin: one with the administration of indomethacin suppository, before and after ERCP and the other one with the administration of NAC (antioxidant) associated with indomethacin before and after, both compared with the standard regimen recommended by European Guidelines (indomethacin suppository immediately after ERCP).
Analizing in our study arms the efficacy of these regimens correlated with the incidence and the seriousness of PEP we have found no superiority of any regimen used (indomethacin suppository administered before and after ERCP and indomethacin associated with NAC) compared to the regimen using indomethacin monotherapy after ERCP ($\chi^2$=2.793, p = 0.247).
At the same time, analyzing the serum pancreatic enzymes (amylasemia and lipasemia) we have found the mixed regimen with superior efficacy. The criteria evaluating the inflammatory syndrome had a similar evolution as the pancreatic enzymes, showing a significant improvement.
Similar results have been reported in recent studies or meta-analysis (Gooshe et al., 2015; Fuentes-Orozco et al., 2015).

**Conclusions:**
The obtained results demonstrate that both split dose administration of Indomethacin (50mg pre- and post-ERCP) and combined administration of 600 mg NAC before the procedure with per rectum administration of 100 mg Indomethacin post-ERCP have similar efficacy in preventing post-ERCP pancreatitis as compared to the standard, guideline-recommended regimen (per rectum administration of 100mg Indomethacin post-ERCP).
However, our study shows that the post-ERCP biological response, expressed by the number of patients developing asymptomatic hyperamylazuria and pronounced inflammatory syndrome, was attenuated in the study group receiving the combination therapy (NSAIDs associated with NAC), with a faster improvement of the biological response.

*Originality and applicability of the results in medical practice*
The particularity of our study is that it evaluates two pharmacological therapeutic approaches (one using both NSAIDs and NAC and the other one including the usual dose, but with split administration of NSAID) and compares these regimens with the standard therapy, recommended by the European guidelines and not to placebo.
*Limitations of the study*
The main limitation of our study lies in the relatively small sample size. Also, the fact that the type of therapeutic regimen administered did not take into account the risk factors, could be considered a limitation of the research.
*Future directions*
Considering these findings, further research in the field (eventually, through multicentric studies, enrolling high number of patients and modulating the antioxidant dose) could lead to developing more an efficient prophylactic pharmacological approach, with a satisfactory safety profile and tenable costs.

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## 3.3. SUMMARY OF THE RESULTS OBTAINED ON TOPICS DERIVED FROM THE RESEARCH DIRECTION

**Results obtained and novelty elements**
The conclusions of the research carried out in this direction can be summarized and presented in an integrated way as follows:
***- ERCP-associated infections***
Our studies have shown that the impact of routine procedural use and reprocessing on the scope possibly makes it susceptible to bacterial contamination and MDRO biofilm formation due to difficult reprocessing of altered surfaces.

- Regular usage of duodenoscopes lead to surface damages. The occurred degradation by erosion or the chemical interactions influence the surface's susceptibility.
- External alterations increase progressively from the distal to the proximal sample and causing the most damage to the elevator sample. Despite this fact, the coating surface was proven to still be efficient against bacterial adhesion. However, the elevator harbours remnant possibly organic material suggestive for biofilm formation, in spite of the reprocessing and long-term quarantine.
- PAW reprocessing is characterized by a significant decrease of bacterial populations, doubled by no surface and composition damage of the duodenoscope polymer resin. Therefore, PAW could be considered as a new and effective alternative method of disinfection for duodenoscope reprocessing, to be used after current-standard manual cleaning.

***- Post ERCP-Pancreatitis (PEP)***
Our studies on the risk factors for PEP and the prophylaxis of this complication have shown:

- regarding the type of papilla: the small / retracted variant is more frequently difficult to cannulate, while large and folded papillae can be considered as independent and predictive risk factors for PEP.
- regarding the iodine-based contrast media, literature analysis shows that iopamidol should be a safe and efficient CM for ERCP. However, both the literature and our experimental study showed that the amount and the concentration of iodine contrast agent injected are directly proportional to the amplitude of potential CM-related side effects.

- out of the risk factors associated with the occurrence of acute post-ERCP pancreatitis, in the patients in our study, only female sex and excess contrast substance correlated with PEP
- regarding the pharmacological regimens studied for PEP prophylaxis our studies have demonstrated that both split dose administration of Indomethacin (50mg pre- and post-ERCP) and combined administration of 600 mg NAC before the procedure with per rectum administration of 100mg Indomethacin post-ERCP, have similar efficacy in preventing post-ERCP pancreatitis as compared to the standard, guideline-recommended regimen. However, the post-ERCP biological response was attenuated in the study group receiving the combination therapy (NSAIDs associated with NAC), with a faster improvement of the biological response.

**Dissemination of results and scientific visibility**

The results obtained from our research conducted on topics derived from therapeutic endoscopy (retrograde endoscopic cholangio-pancreatography) have been published in journals with international circulation. As I mentioned in the introduction to the third chapter, I was the author or co-author of a number of 9 articles published in extenso (5 in ISI rated journals with impact factor, one article in an ISI proceeding volume and two indexed articles in international databases). Table 3. XVI. presents the list of scientific articles published *in extenso* that addressed topics related to interventional endoscopy, especially ERCP, as well as the number of citations illustrating the IF (impact factor) that these studies have had in the specialty literature according to ISI (international scientific indexing).

**Table 3. XVI:** Scientific papers published *in extenso* in specialized journals

| Nr crt | Titlu/ autori/revistă/ IF | Citari WOS | Citări Google Scholar |
|---|---|---|---|
| **Scientific papers published *in extenso* in ISI-ranked journals** | | | |
| 1. | PRELIMINARY STUDY ON EROSION OF POLYMER COATINGS OF DUODENOSCOPES. Balan GG , Pavel L , Sandu AV, **Stefanescu G***, Trifan AV *Materiale plastice* Volume: 53 Issue: 4 Pages: 791-795 Published: DEC 2016 **IF=0,778** | 12 | 3 |
| 2. | ANATOMY OF MAJOR DUODENAL PAPILLA INFLUENCES ERCP OUTCOMES AND COMPLICATION RATES: A SINGLE CENTER PROSPECTIVE STUDY. Balan GG, Arya M, Catinean A, Sandru V *, Moscalu M, Constantinescu G, Trifan A, **Stefanescu G***, Sfarti CV . *J. Clin. Med.* 2020, *9*, 1637; doi:10.3390/jcm9061637, **IF - 4.242** | 1 | 6 |
| 3. | SPLIT-DOSE OR HYBRID NONSTEROIDAL ANTI-INFLAMMATORY DRUGS AND N-ACETYLCYSTEINE THERAPY FOR PREVENTION OF POST-RETROGRADE CHOLANGIOPANCREATOGRAPHY PANCREATITIS. Pavel L, Bălan GG, Nicorescu A, Gîlcă-Blănariu GE, Sfarti CV, Chiriac Ș, Diaconescu S, Drug VL, Bălan Gh, **Ștefănescu G**. *World J Clin Cases* (2018) **IF=1,013** | 1 | 3 |
| 4. | EXPOSURE TO IOPAMIDOL AFTER ENDOSCOPIC RETROGRADE CHOLANGIOPANCREATOGRAPHY. ASSESSING PANCREATIC TOXICITY. Balan GG, Pavel L, Sfarti CV, **Stefanescu G**, Bălan Gh, Trifan A. *Rev.Chim.* (Bucharest), 67; No. 5; 2016 **IF= 1,232** | 5 | |
| 5. | PLASMA-ACTIVATED WATER: A NEW AND EFFECTIVE ALTERNATIVE FOR DUODENOSCOPE REPROCESSING . Balan GG , Rosca, I, Ursu EL, Doroftei F, Bostanaru AC , Hnatiuc E, Nastasa V, Sandru V, **Stefanescu G,** Trifan A, Mares M. *Infection and drug resistance* 2018 (11): 727-733. **IF=3,000** | 31 | 38 |

| | | | |
|---|---|---|---|
| 6. | DUODENOSCOPE-ASSOCIATED INFECTIONS BEYOND THE ELEVATOR CHANNEL: ALTERNATIVE CAUSES FOR DIFFICULT REPROCESSING. Balan GG , Rosca I, Ursu EL, Fifere A, Varganici CD, Doroftei F, Turin-Moleavin IA, Sandru V, Constantinescu G, Timofte D, **Stefanescu G,** Trifan A , Sfarti CV . *MOLECULES* 24 : 12 DOI: 10.3390/molecules24122343 Published: JUN 2 2019 WOS:000473816900149:<br>**IF=3.267** | 5 | 9 |
| **Scientific papers published *in extenso* in ISI proceedings journals:** | | | |
| 7. | THE MULTIMEDIA INFORMED CONSENT: A USEFULL TOOL IN ERCP. Balan GG, **Stefanescu G,** Sfarti CV, Trifan A. E-Health and Bioengineering Conference (EHB), 2017, 466-469 | 1 | 2 |
| **Scientific papers published *in extenso* in BDI journals:** | | | |
| 8. | THE EVOLUTION OF CLINICO-BIOLOGICAL PROFILE OF PATIENTS UNDERGOING PROPHYLAXIS FOR POST-ERCP PANCREATITIS - A PROSPECTIVE STUDY. Pavel L, Balan GG , Timofte O, Balan G , **Stefanescu G.**. *Medical-Surgical Journal-Revista Medico-Chirurgicala* Volume: 123 Issue: 3 Pages: 426-433 Published: 2019: WOS:000489620800012 | | |
| 9. | ORO-PHARYNGEAL SYMPTOMS AFTER ENDOSCOPIC RETROGRADE CHOLANGIOPANCREATOGRAPHY. HOW CONCERNED SHOULD WE BE ?. Bălan GG, Şandru V, **Ştefănescu G**; Trifan A. *International Journal of Medical Dentistry* . Jul-Sep 2017, Vol. 21 Issue 3, p153-157. 5p. | 2 | 2 |
| **Total citations** | | **58** | **63** |
| **Cumulative impact factor / direction** | | **IF=13.532** | |

The visibility of my scientific activity in this research direction is proved by the number of citations (WOS- 58 citations and Google Scholar - 63) as well as by the cumulative impact factor of the journals in which articles were published (global cumulative FI / direction = **IF=13.352**.

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Looking globally at the research conducted in this field, I consider that the studies I participated in had continuity, being articulated and integratively addressed to the identification of factors involved in the main complications associated with ERCP and, respectively, to the identification of prophylaxis measures. Regarding the scientific contribution, the novelty elements brought in particular by the research related to the infections associated with the duodenoscope, should be mentioned. I would also like to emphasize that studies on this topic prove openness to collaboration with teams of researchers from outside the medical field.

# SECTION II

# FORTHCOMING PROJECTS AND DEVELOPMENT IN MY ACADEMIC CAREER

Academic career is a challenge due to the particular situation determined by the variety of roles included: role of expert in his profession, didactic and educational role (as a trainer), institutional and community role (support and opinion leader) and, the last but not the least, the role of researcher.

Consequently, multidisciplinary development is essential for professional performance. Teaching, clinical and research activities need to be continuously improved and updated and new topics of interest are added in order to fulfil the own expectations, the academic community requirements and in order to contribute to the increase in visibility and prestige of the university.

Therefore, my future projects will be presented below in three coordinates: academic, clinical and research activity.

## A. PROJECTS IN CLINICAL MEDICAL PRACTICE

Continuing the tradition of the school in which I have been brought up professionally, I remain convinced of the importance of clinical exam competency in establishing more accurate, early and complete diagnoses and of exploratory investigations specific to each specialty in terms of treatment adequacy and efficacy. To this end, I have set out to increase the percentage of explorations carried out both personally and by the team of which I am part: diagnostic explorations (ultrasound, endoscopic) as well as interventional techniques of moderate complexity.

Throughout my professional education, I have benefited from the guidance of well-established medical personalities and consider it only natural to offer, in turn, from my experience to my younger colleagues. I shall continue to collaborate with younger doctors in order to contribute to their professional development with regard to their clinical reasoning skills as well as training those abilities which will permit them to carry out explorations specific to the gastroenterology specialty.

I have always maintained an open mind in regard to novel therapies for digestive pathology and have been prepared to apply in conditions of pharmacovigilance and utmost deontology, new therapeutic regimens. In this context, I will collaborate honestly with pharmaceutical companies at both the national and international level including in research projects with mutual advantages. I will continue to permanently communicate and expand my relationships with related specialties, with universities and medical institutions both from home and abroad.

In order to ensure permanent, high quality, healthcare services, I consider useful the development of a pilot program in collaboration with specialists in pediatric gastroenterology which would have as its goal the establishment of a regional register and subsequently a national register that would record all adolescents and young adults with chronic digestive pathology (chronic hepatitis, inflammatory bowel disease, celiac disease) such that demographic, clinical, therapeutic and clinical evolution would be included in a unique database with the goal of facilitating both research activities as well as ensuring optimal transfer of the electronic portfolio of each pediatric patient with chronic digestive pathology into the healthcare network dedicated to adult patients.

## B. PROJECTS IN THE ACADEMIC FIELD

For the future, I will endeavor in my actual teaching activity, in which I have persevered for the last 20years – to continually implement elements of progress and stimulating interest of the students and young doctors for medical semiology and gastroenterology. The actual program of professional development of the students will be based on objective parameters: the specific of the specialty, teaching curriculum and its dynamics, the corresponding equilibrium between theoretical concepts and practical competencies, increasing motivation in seminar participation and practical activities through their improved quality and relevance.

I continue to consider necessary that theoretical concepts and practical / interventional skill competencies ought to be reactualized at short intervals (3-4years) so that the progress in instrumentation and techniques might be underscored. As far as the teaching activity per se, I wish to enrich my competencies through continued documentation in the pedagogical process and learning from the teaching manners of my professors and not only them.

I will concentrate on streamlining the methodology of teaching and integrating knowledge in the medical field in general and particularly in gastroenterology. In the current pandemic context, I believe that various online teaching and learning methods must be developed in order to complete the classic teaching activity by the patient's bed. In this regard, I will persevere toward implementation of attractive modern methods such as TBL (team-based learning) and PBL (problem-based learning) as well as practical activities involving the virtual patient in the simulation laboratories.

I will promote development of analytical and synthetic capacities of theoretical knowledge and clinical judgment in current practice of the specialty by encouraging personal initiatives and taking on more responsibilities progressively.

I consider post-graduate teaching to be a component of equal importance in the teaching activity of the staff and together with my professors and experienced colleagues, I will direct my efforts toward improving this aspect as well in the hope of consolidating the conditions and reputation of the teaching centre and the improvement of the clinic in which I carry out my professional activity.

## C. PROJECTS IN THE FIELD OF SCIENTIFIC RESEARCH

From the standpoint of future scientific activity, I intend to achieve significant development and diversification.

Interest areas that I wish to investigate more thoroughly in scientific research include studies regarding biological and psichological modifications in various digestive disorders. In concordance with my experience and interests, the following research directions have materialized:

- *biological studies on pacients* – will involve carrying out biochemical, immunological tests on pacients with chronic digestive pathology (inflammatory bowel disease, chronic fatty liver disease, functional pathology) with the aim of identifying possible biological markers including several types of molecules such as hormonal markers, inflammatory, microelements;
- *studies regarding social, economic and cultural factors* involved in the development of digestive disorders dependent on poverty and at-risk addictive behaviors (e.g. alcohol consumption in adolescents and young adults);
- *studies on the involvement of psychological factors* in the etiopathogenesis of chronic digestive diseases (eg. IBS, IBD)

On the one hand, I will continue my research in inflammatory bowel disease, digestive diseases in adolescents and young adults and biliopancreatic illnesses and, on the other hand, I will broaden my field of interest to include functional pathology and chronic hepatic diseases.

## C.1. Continuing older lines of inquiry

The main areas of research that I have addressed so far and which I have detailed in Section I, have given me scientific satisfaction and, as a result, I intend to continue my research from other perspectives.

***C.1.1. Inflammatory bowel diseases***

In this context, I consider it appropriate to continue research on the elements belonging to the main etiopathogenic links in IBD, with a potential role in modulating the inflammatory process, in order to identify possible corelations between various immunological markers, hormones, trace elements and components of the local microenvironment.

- *Microelements and intestinal dysbiosis*

Also in the field of IBD, I intend to approach another direction of research - the intestinal microbiota and the relationship between intestinal dysbiosis and micronutrient deficiencies.

Dysbiosis is an intensely studied etiopathogenic component in IBD, the link with triggering / progression of IBD being a complex one with a as of yet still unspecified dynamic. From a different standpoint, there are studies that have proven that bioavailability of certain biometals play an essential role in modulating the interaction microbiome-host (Hood and Skaar, 2012). Studies on animal models have revealed the fact that the selenium level contributes to modulation of the intestinal microbiome composition (Kasaikina, et al., 2011). At the same time, studies carried out on murine models have revealed the fact that the intestinal microbiome composition influences selenium level via modified expression of selenoproteins in rats (Hrdina et al, 2009). The hypothesis of competition for the utilization of selenium between the intestinal microbiome and intestinal cells has been raised which would contribute to a worsening selenium deficiency in the host leading to an indirect increase of intestinal vulnerability to development of certain diseases.

With regard to the link between selenium-dysbiosis, some studies have indicated the existence of common immunological pathways involving nuclear factor (NF)-κB and peroxisome proliferator-activated receptor-γ (PPAR-γ), taking into account the fact that commensal bacteria with a potential pathogenic role in IBD, especially in the context of a dysfunction of the intestinal barrier, can influence its activation (Byndloss, et al., 2017).

Regarding the zinc deficiency, starting from its role in altering phagocyte activity with suppression of the antioxidant response and impaired cytokine response (Mohammadi et al 2017) we can hypothesize its potential interactions with the intestinal microbiome and progression of IBD, but available data are scarce. Moreover, there remains the problem of the causal relationship between the deficiency of microelements such as selenium and zinc and IBD activity whether these deficiencies are the cause or effect of intestinal inflammation.

Given the current level of knowledge, it is difficult to state the directionality of influence between microelements and the microbiome.

In consequence, additional studies are necessary to investigate potential correlations between intestinal microbiome, microelement concentrations and triggering / progression IBD activity, and the factors that mediate this relationship. Only in light of clarification of these aspects, can we establish a patient profile which might benefit from microelement supplements or administration of newer generation probiotics involving nanoparticles (Gîlcă-Blanariu, et al, 2018).

Starting from these aspects and taking into consideration our prior results, we will aim to study the interactions between microelements and the microbiome (Gîlcă et al, 2021).

- *hormones – melatonin and cortisol*

Another link in the pathogenic chain of IBD is represented by the hormonal profile.

Besides direct immune-mediated factors, there are a series of hormonal influences with an important role in sleep regulation among which are melatonin and cortisol. Melatonin has

multiple neurohormonal functions, presenting a major influence in regulation of circadian rhythm. The highest levels of this hormone are found in the digestive tract where it also has the role of modulating intestinal motility.
Although evidence supporting the anti-colitic effects of melatonin have been accumulating, it is not clear how melatonin affects the microbiota (Kim, et al 2020).
There are animal studies that showed that stress and sleep deprivation could affect intestinal dysbiosis and increase the colitogenic microbiota, which could contribute to the aggravating digestive disease. These studies showed that melatonin concentrations in feces and colon tissue decreased under stress andsleep deprivation. Melatonin treatment brought recovery of melatonin concentration in colon tissue and modulating dysbiosis of intestinal microbiota (Zhu et al, 2018; Young et al, 2020).
Based on these aspects, there are animal studies that have shown that the administration of melatonin can stop / improve the evolution of patients with IBD (Seoane-Viaño et al, 2019)
Starting from our study regarding sleep disorders in patients with IBD (Gîlcă et al, 2020) and in accordance with the previously proposed research direction, it would be interesting to follow up on melatonin and cortisol, especially since there are recent studies that suggest the involvement of these hormonal factors in the pathogenesis of these diseases.

- *Cognition and Depression in Inflammatory bowel diseases*

In the pursuit of our group to contribute to better understanding of IBD pathogenesis, one of the future directions we are considering for research is the study of cognitive function among IBD patients. The close relationship between IBD and nervous system function has been proposed in some previous studies (Gareau M, 2016), especially considering that the negative impact of neuropsychological diseases on IBD had been highlighted for several time (Casella G et al, 2014).
Considering the importance of the gut-brain-microbiome link, its role has been underlined for various digestive diseases, including IBD (Gareau M, 2016). Moreover, the potential neurological impairment in IBD could be an effect of the proinflammatory state which characterizes this disease, through the production of proinflammatory cytokines. Concurrently, the malnutrition and associated mood disorders can enhance the alteration of cognitive profile in IBD patients (Smith PJ, Blumenthal JA, 2016). All these aspects are of utmost importance for managing IBD, especially considering that there is prior data on the positive influence of psychological therapy on health-related quality of life in IBD patients.
On these grounds, there are clinical implications of identifying whether IBD patients suffer from cognitive and psychological impairment. Currently available data are conflicting (Attree E, et al, 2003; Wells C, et al, 2006; Dancey C, et al, 2009; Golan D, et al. 2016), the study designs variable and the presence of comorbidities compromises the shaping of direct result comparisons and accurate conclusions. The used tools for evaluation of the cognitive and psychomotor function were heterogeneous, therefore further studies using both validated questionnaires and computer-assisted psychodiagnostic instruments are needed.
Moreover, there is also a need for identifying whether cognitive performance differs between UC and CD patients, since the systemic impact of disease might differ between the two disease subtypes. Considering that mood disorders are prevalent among IBD patients (Barberio B et al, 2021) and that they may impact various cognitive domains such as memory, one of the future directions involves focusing on this patient subgroup, aiming to identify the degree of influence depression exerts on cognitive impairment in IBD patients and whether this is dependent on disease activity.

***C.1.2. The digestive pathology of adolescents and young adults***

The second old main direction I intend to continue is related to the digestive pathology of adolescents and young adults.

Transition to adulthood is a complicated process for most cases. This is clearly relevant especially if adolescent and young persons have chronic illnesses and ongoing health needs. The transition process has increasingly been recognised as covering a vulnerable period where unaddressed healthcare needs may have long-term consequences. (Brooks et al. 2017, Marani, 2020)

In the past 15 years, there has been a growing interest in the role of pediatric-to-adult transitional care in improving health outcomes among children and young adults with pediatric-onset chronic conditions. (Coyne et al, 2017)

For specific GI diseases, there are documented variations in prognosis and clinical course when the disease presents in childhood compared with adulthood. There are clear differences in diagnostic and therapeutic IBD management dependent on age at diagnosis. Furthermore, children presenting with IBD have a more extensive disease phenotype and rapid early progression, although there no clear differences reported in the rate of disease complications. (Brooks et al. 2017; Stenke et al, 2019)

- although the clinical course of coeliac disease presenting in childhood is often more severe than that presenting later in life, it is difficult to predict outcomes dependent on age at diagnosis with sparse research in this area. There are no studies that assess the benefit or risk of good versus poor disease control during the transition from paediatric to adult care.
- the use of psychological management for functional GI illness in pediatrics is higher than adult practice. (Brooks et al. 2017, Van Limbergen et al, 2008)
- outside the GI tract, the most common indication for liver transplant in children is for congenital reasons (biliary atresia) compared with acquired liver disease in adulthood.

The Recommendations are that adolescent and young persons with IBD, coeliac disease and chronic liver disease should be involved in formal transition arrangements

In this context, I intend, in collaboration with the team of pediatric gastroenterologists, to initiate a pilot study on the chronic digestive pathology of pediatric patients reaching the age at which it is necessary to transition to the adult health system. As an objective, we aim to lay the foundations of a register of chronic diseases (inflammatory bowel disease, celiac disease, chronic liver disease, etc.) in order to facilitate and ensure a natural transition to adult gastroenterology.

***C.1.3. Interventional endoscopy - ERCP***

As detailed in Chapter 2 of Section I of this thesis, Post - ERCP pancreatitis (PEP) is the most common adverse event of ERCP. PEP may be severe or fatal. Therefore, prophylaxis for PEP is a clinically important issue.

Since prophylactic measures are not always effective, moreover, sometimes lead to adverse events, to safely perform ERCP, it is important to individually estimate the risk of PEP in each patient and identify high-risk patients.

Over the past few decades, risk factors for PEP have been the focus of many studies; however, there are currently only a few reliable methods of predicting PEP in individual patients. (Fujita et al, 2021,Dumonceau et al. 2020). Chandrasekhara et al. 2017;

Three more prediction models have recently been reported. However, these models are not suitable for use in clinical practice because of their low discriminability, complexity, or lack of external validation. (Di Mango, et al, 2013; Zheng et al 2020; Chiba et al 2020)

In this context, we aimed to systematically follow-up the post-intervention evolution in order to be able to develop a practical prediction model for PEP.

## C.2. New Research Directions

As I mentioned before, in addition to continuing the old research directions, I intend to expand my research in new or less approached areas so far, such as - functional pathology and chronic liver disease.

***C.2.1. Non-alcoholic fatty liver disease (NAFLD)***

In recent years, as a result of amazing advances in the prophylaxis and treatment of viral hepatitis B and C, there has been a significant decrease in the incidence and prevalence of virus-induced chronic liver disease. At the same time, there is an alarming increase in cases of Non-alcoholic fatty liver disease (NAFLD), which affects up to a quarter of the world's adult population. Consequently, the burden of NAFLD-related liver complications (non-alcoholic steatohepatitis (NASH), cirrhosis and hepatocellular carcinoma) and the need for life-saving liver transplantation are also expected to increase further in the near future. (Targher,et al, 2021, Mantovani et al, 2020).

I believe that NAFLD is a topical field of research and will remain of interest in the years to come, so it is one of the topics I intend to address.

The first direction of research on this topic that I intend to address is related to the non-invasive assessment of portal hypertension.

- *Non- invasive evaluation of clinically significant portal hypertension in NAFLD*

The progression of liver fibrosis with a direct impact on the degree of portal hypertension severity (PHT) represents a crucial element in the appearance of complications in the case of cirrhotic patients, element which influences the mortality among these patients (de Franchis et al, 2010; Ripoll et al, 2009). Quantification of this process has been performed by calculating the porto-supra- hepatic pressure gradient (Bosch et al, 2009). Due to its highly invasive character, the measurement of the porto-supra-hepatic pressure gradient has become marginal instrument, useful mainly in the research procedures (Thiele and Krag, 2018)

The Baveno VI consensus has implemented transient elastography as a reproducible assessment tool for the risk of occurrence of CSPH, respectively for excluding the need for endoscopic screening of the esophageal varices. The Baveno VII Consensus maintains and details the recommendations of the 6th edition of the Baveno Consensus on the non-invasive assessment of HTP (de Franchis, 2015). Adopting the Baveno VI and VII principles, having as background the development of more and more reliable and reproducible methods of non-invasive evaluation of liver fibrosis, creates perspectives for research in order to identify other feasible methods for the non-invasive quantification of PH and of the risk of variceal bleeding in patients with advanced compensated liver disease. Taking into account that up to now 2D-SWE elastometry has shown considerable interest among clinicians we can refer it as a potentially useful and promising resource in assessing severity of PH (Elkrief et al, 2015; Procopeț et al, 2015; Jansen et al, 2017)

In this regard, the extension of the elastometric evaluation on the splenic parenchyma has also recently been discussed, the splenic fibrosis being potentially correlated with the degree of PH and the risk of bleeding (Colecchia et al, 2015, Singh et al, 2014; Karagiannakis et al, 2019, Tarantino, 2019, EASL. Clinical Practice Guidelines, 2021)

In this context, I intend to study the role of non-invasive methods in the assessment of patients with non-alcoholic fatty liver disease. I propose to identify patients with advanced liver fibrosis by establishing correlations between laboratory tests/ultrasound markers liver and spleen stiffness measured non-invasively by two modern methods: Transient elastometry (Fibroscan) and 2D-SWE.GE elastography. Another objective of the research is to develop an algorithm modelled according to the clinical, elastometric and laboratory tests particularities of patients with NAFLD, to increase the accuracy of fibrosis diagnosis and quantification.

- *Non-alcoholic fatty liver disease (NAFLD) and psychiatric disorders*

NAFLD is part of a complex system of mental and organic diseases with a common pathogenesis between genetic, environmental, and epigenetic factors based on dysregulation of inflammation, redox pathways, and mitochondrial biogenesis. In this regard, more and more evidence is being published about the association between metabolic syndrome. (Targher et al,

2021, Soto-Angona et al, 2020, Labenz et al , 2020) NAFLD and psychiatric disorders share many common pathophysiological pathways that point to common underlying mechanisms.
It is probable that potentially bidirectional associations are also present between NAFLD and mental health comorbidity (Shea et al, 2021)
From a clinical perspective, NAFLD and depression share common risk factors, including diabetes mellitus type 2 and obesity. (Chan et al, 2019) A recent study using the National Health and Nutrition Examination Survey (NHANES) observed an association between depression and NAFLD in the United States. (Kim et al, 2019) However, other studies produced conflicting evidence on the potential relation between NAFLD and depression. (Weinstein et al, 2011; Lee et al, 2013). Anxiety is another frequent psychiatric disorder in the Western world. Only a few studies investigated the potential association between NAFLD, disease severity, and anxiety disorders (Youssef et al, 2013, Elwing et al, 2006)
A recent study also reported cognitive impairment in patients with NAFLD and liver fibrosis, suggesting that parameters that assess fibrosis could be used to predict cognitive impairment and dementia (Weinstein et al, 2019).
Although high prevalence rates of psychopathological disorders have been identified among patients with NAFLD , less work has focused on the potential relationship between NAFLD and mental health. So, the potential bidirectional associations between NAFLD and common mental health disorders that may coexist in patients with NAFLD merit further investigation. In this context, I propose to assess the relationship between NAFLD and various psychopathological disorders (depression, anxiety, cognitive impairment and chronic stress).

- *NAFLD/NASH in young people*

The epidemiology of NAFLD / NASH in young people has changed considerably over the last three decades. The incidence of NAFLD / NASH increased from 19.34 million in 1990 to 29.49 million in 2017 among children and adolescents, with an annual increase of 1.35% (Zhang et al, 2021). Almost all countries showed an upward trend, with the most pronounced increase observed in the developed regions. (Zhang et al, 2021)
At the same time, NASH is the most common aetiology for hepatocellular carcinoma (HCC) and the fastest growing indication for LT among young adults in the United States between the ages of 18 and 40 ( 5% of total LT in this age group). (Paik et al, 2020; Doycheva et al, 2018)
Another very recent study showed that 20% of the 4,000 young adults (mean age 24 years) included in the study had non-alcoholic fatty liver disease (NAFLD). Among them, 2.4% showed signs of liver fibrosis. (Cohen et al., 2021)
Regarding the profile of the adolescent / young adult patient with NAFLD, Ciardullo et al reported that a large proportion of adolescents with NAFLD and fibrosis are in the normal weight category and have ALT levels within normal limits. (Ciardullo et al, 2021)
Regarding this topic, in collaboration with the team of pediatric gastroenterologists, I intend to study the prevalence of NAFLD among adolescents and young adults (aged 15 to 29 years) in our region. We also aim to evaluate the socio-demographic and anthropometric characteristics of the identified patients and to assess the risk factors for the development of NAFLD at a young age.

### *C.2.2. Functional gastrointestinal disorders*

The second area of research that I want to explore in the future is related to the functional pathology of the digestive tract. In fact, this is the direction in which I started my scientific career, considering that my doctoral thesis addressed biliary reflux.
The pathophysiology of IBS is complex, involving multiple mechanisms: food sensitivity, inflammation, infection, visceral hypersensitivity, intestinal dysbiosis, genetic factors but also psychosocial impairment (Aziz et al, 2021)
In this regard, similar to the research conducted in patients with inflammatory bowel disease and presented in the previous section, I intend to expand research on the status of

micronutrients, psychological distress and neurohormones in patients with irritable bowel syndrome.
For these future studies, I intend to resume collaboration with researchers from the Petru Poni Institute, with the team from the Socola Institute of Psychiatry and also to initiate a collaboration with researchers in fundamental fields (physiology, pathophysiology, biochemistry).

- *IBS and Psychosocial Distress*

The brain-gut axis involves a complex bidirectional relationship that connects the emotional and cognitive centres of the nervous system with peripheral enteric functions, such as immune activity, enteric permeability, enteric reflexes, and endocrinological signalling.
Very recently published data report that 75% of patients with IBS have a psychopathological impairment. About 30% of IBS patients suffer from anxiety. About 30% to 50% of patients with IBS suffer from mood disorders. About 15% to 30% of IBS patients also experienced suicidal thoughts. The onset of somatization was also predominant in the psychosocial distress of patients with IBS. (Black and Ford, 2020; Hadjivasilis et al, 2019). At the same time, Midenfjord reported that patients with IBS and psychological distress report not only more frequent gastrointestinal symptoms but also more severe ones (Midenfjord, 2019).
Depression as well as anxiety are forms of behavioural disorder caused by the interaction of several factors, such as the environment, sex, age, and comorbidity with other pre-existing conditions, including IBS. (Mokhtar et al., 2020). Patients with IBS often suffer from significantly higher levels of depression compared to healthy subjects (Lee et al., 2017; Zamani et al., 2019). Similarly, it was found that severe depressive symptoms were associated with a high rate of IBS (Lee et al, 2017).
Another aspect that can be assessed is the association between IBS and cognitive function. The data in the literature so far are quite inconclusive.
According to a more recent systematic review, there was insufficient evidence to show a relationship between IBS and cognitive impairments (Lam et al, 2019).
However, as depression is strongly linked to cognitive deficit, and IBS patients are often depressed, it may be hypothesized that this patient category can suffer from some form of cognitive impairment. In line with this argument, some researchers have noted a reduction in verbal IQ in patients with IBS relative to their personal IQ performance and compared with healthy controls (Lam et al, 2019). Also, Chen et al, found that IBS is associated with an increased risk of dementia, and this effect is obvious only in patients who are ≥50 years old (Chen et al, 2016). However, other authors have failed to find a meaningful association between cognitive function and IBS (Farup and Hestad, 2015). So, further studies are needed to confirm prevalence rates and examine potential mechanisms.
Although discussed for a long time, the data on the causal relationship between psychopathological disorders and gastrointestinal functional pathology are still controversial. Considering the presence of common pathogenic links (oxidative stress, microbiota alteration), it is very possible that these associations are not just accidental.
In this context, I propose to study the prevalence of mental disorders in patients with IBS compared to patients with IBD and subjects without digestive pathology.

- *IBS and hormonal disturbancies (oxytocin, melatonin and ghrelin)*

Related to the previously presented research direction, another proposed topic is the study of the profile of some neurohormones.
The relevance of hormones along the hypothalamic-pituitary gonadal axis and the adrenal hypothalamic-pituitary axis for IBS symptoms remains unclear.
Central ghrelin, levodopa, or morphine may induce visceral antinociception by orexynergic signalling. Orexin induces visceral antinociception through dopamine, cannabinoid, adenosine or oxytocin. (Okumura et al, 2021)

In terms of gastrointestinal function, in addition to its visceral antinociception, orexin acts centrally to stimulate gastrointestinal motility and improve intestinal barrier function. Brain Orexin is also involved in regulation the sleep / awake cycle and antidepressant action. (Okumura ey al, 2021).
On the other hand, observations suggest that oxytocin / oxytocin receptor (OT / OTR) signalling acts as a brake on intestinal motility, decreases the activation of the mucosa of enteric neurons, and promotes enteric neuronal development and / or survival. Oxytocinergic signalling thus appears to play an important role in multiple GI functions that are subject to neuronal regulation. (Welch et al, 2014; Pădurariu et al, 2019).
Given the pattern of distribution of both central and peripheral oxytocin receptors and growing data on oxytocin imbalance in mental disorders, we could speculate that the oxytocin system may be at the core of psychosomatic disorders.
Another neurohormone involved in regulating the motility of the gastrointestinal tract is melatonin.Data from the literature show that melatonin plays important roles in the gastrointestinal tract, having in particular enteroprotective effects (immunomodulatory activity, antioxidant effects, maintenance of gastric prostaglandins and promotion of bicarbonate secretion) (Bubenik, 2008; Mozaffari et al, 2010). Melatonin has also been reported to be involved in regulating intestinal motility (Harlow and Weekley, 1986).
In this context, it is very likely that melatonin affects the pathophysiology of IBS. Therefore, it would be interesting to explore the role of melatonin in IBS. (Wang et al, 2020).
Related to this research direction, I was recently part of an interdisciplinary research group (psychiatrists, gastroenterologists, biologists) that conducted preliminary studies based on the hypothesis that the oxytocin system is disbalanced in irritable bowel syndrome considering also the psychological component of this digestive disorder. (Pădurariu et al., 2019; Hrițcu et al., 2020).
Following the results obtained, a scientific objective in the next period is to deepen research in this field. We would like to study the extent to which changes in other neurohormones (orexin and melatonin) may play a role in pathophysiology in a part of patients with IBS who are frequently accompanied by sleep disorders, depression, and anxiety.

***In conclusion***, my future research activity will focus mainly on the continuation of the study directions in which I have gained experience over the past 22 years, as well as on the initiation of new study and research directions in the field of gastroenterology.
An important objective for me is to identify, stimulate and promote young people with research skills in order to set up research teams as well as to develop collaboration with already established research teams.
I also promote interdisciplinarity in research activity and intend to extend my collaboration with researchers from other clinical domains (psychiatry, pediatrics, surgery, radiology) and preclinical (pharmacology, physiology, physiopathology) within the University of Pharmacy and Medicine "Gr. T. Popa" and intend to continue collaboration with other institutions.
I am interested in collaborating with research teams from CEMEX but also from other institutions (eg., Petru Poni Institute, Psychiatry Institute Socola, St Mary Emergency Hospital, other Romanian universities – for example, "Al. I. Cuza" University or from abroad).
One of my medium-term objectives is to apply as project director or partner (together with researchers from teams with which I have collaborated in ongoing projects) for research grants with European funding.

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I conclude this presentation of my professional and scientific activity so far and of my future projects, with the conviction that granting the habilitation certificate to a professor or researcher represents the highest professional ranking, confirming their academic achievements from a scientific perspective and offering real perspectives for future development.

I would like to emphasize that my entire future activity will be carried out at the highest standards of excellence, interdisciplinarity and ethics, thus contributing to the development of medical education and research in Romania and to the provision of quality health services in my field of expertise.

# SECTION III

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