GRIGORE T. POPA UNIVERSITY OF
MEDICINE AND PHARMACY IASI

HABILITATION THESIS

ELEMENTS OF NON-INVASIVENESS AND INTERDISCIPLINARITY IN DIGESTIVE DISEASES

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CONTENTS

ABBREVIATION LIST

AI – artificial intelligence
A1G – alpha 1 globulins
A2G – alpha 2 globins
ACC – accuracy
AS – ankylosing spondilitis
5-ASA - 5- Aminosalicylic Acid
AUC – area under curve
AZA – azathioprin
BASDAI - Bath Ankylosing Spondylitis Disease Activity Index
BASFI - Bath Ankylosing Spondylitis Functional Index
BMI – body mass index
CAP - controlled attenuation parameter
CBT - cognitive behavioral therapy
CD – Crohn´s disease
CDAI - Crohn's disease activity Index
CDEIS - Crohn's Disease Endoscopic Index of Severity
CDI – *Clostridioides difficile* infection
CEUS – contrast enhanced ultrasound
CRP – C-reactive protein
CS – corticosteroids
CT – computed tomography
DAA - directly acting antivirals
DEXA - Dual Energy X-rays Absorptiometry
DM – diabetes mellitus
EIM – extraintestinal manifestations
EN – enteral nutrition
ESR – erythrocyte sedimentation rate
FC – fecal calprotectine
FNH – focal nodular hyperplasia
GLP-1 - glucagon-like peptide-1
HCV – hepatic C virus
HCC – hepatocellular carcinoma
HCT – hematocrit
HGB – hemoglobin
IBD – inflammatory bowel disease
IBDQ - inflammatory bowel disease questionnaire
IBS – irritable bowel syndrome
IFX – infliximab
LGB – lower gastrointestinal bleeding

MAFLD – metabolic associated fatty liver disease
MAPE - mean absolute percentage error
MCHC - mean corpuscular hemoglobin concentration
ML – machine learning
MONO – monocytes
MPV - mean platelet volume
MRI – magnetic resonance imaging
MSE - mean square error
NAFLD - non-alcoholic fatty liver disease
NEUT – neutrophils
NN – neural network
NPV – negative predictive value
PCT – plateletcrit
PDW - platelet distribution width
PLCR - platelet large cell ratio
PLT – platelet count
PPV – positive predictive value
PSC – primary sclerosing colangitis
QoL – quality of life
ROC – receiver operator characteristic
RBC – red blood cells
RDW – red cell distribution weight
SE – sensitivity
SEI – Score of endoscopic inflammation
SES-CD - Simple Endoscopic Score for Crohn's Disease
SD – standard deviation
SI – serum iron
SP – specificity
SPEC - specialized educational and psychological counseling
SVR - sustained viral response
TNF - Tumor Necrosis Factor
TP – total proteins
UC – ulcerative colitis
UCDAI - Ulcerative Colitis Disease Activity Index
UCEIS - Ulcerative Colitis Endoscopic Index of Severity
WBC - white blood cells

REZUMAT

Teza de abilitare intitulată "**Elemente de noninvazivitate și interdisciplinaritate în bolile digestive**" reprezintă sinteza activității mele științifice din perioada postdoctorală și până în prezent. Teza descrie principalele direcții de cercetare și rezultatele obținute, constituindu-se ca suport necesar obținerii abilitării de a coordona doctoranzi.

Structura tezei se încadrează în recomandările Consiliului Național de Atestare a Titlurilor, Diplomelor și Certificatelor Universitare (CNATDCU) și cuprinde 3 secțiuni:

- Secțiunea I: Realizări științifice din perioada postdoctorală;
- Secțiunea II: Proiecte viitoare în activitatea științifică;
- Secțiunea III: Referințe bibliografice.

SECȚIUNEA I începe cu o trecere în revistă a activității mele didactice, științifice și profesionale. Sunt descrise reperele devenirii în cariera didactică și profesională, activitatea în comunitatea academică, principalele arii de interes în cadrul specialității. De asemenea, am prezentat principalele realizări științifice în domeniu: 38 lucrări indexate ISI și 19 lucrări Proceeding ISI indexate în Clarivate Analytics Web of Science Core Collection, indice Hirch 10, 287 citări ISI, 65 lucrări în extenso indexate în alte baze de date internaționale, 59 capitole de carte. Am fost membru în 6 proiecte de cercetare – dezvoltare, am prezentat 364 abstracte la manifestări naționale și internaționale și am obținut 18 premii naționale pentru lucrări științifice.

Capitolul 1 se referă la abordarea non-invazivă a bolilor inflamatorii intestinale și cuprinde 2 subdirecții: biomarkeri și inteligența artificială. În domeniul biomarkerilor sunt descrise cercetările legate de valoarea calprotectinei fecale în diagnostic și monitorizare, precum și corelarea biomarkerilor cu activitatea endoscopică a bolii inflamatorii intestinale. Inteligența artificială, bazată pe rețele neuronale, are multiple aplicații în medicina modernă, de la creșterea acurateții de diagnostic, la estimarea prognosticului sau a răspunsului la tratament. Lucrările la care am colaborat au demonstrat rolul inteligenței artificiale în aprecierea activității endoscopice și histologice a bolilor inflamatorii intestinale, precum și estimarea răspunsului la tratamentul cu anticorpi anti-factor de necroză tumorală alfa.

În **capitolul al 2-lea** sunt prezentate elemente de abordare interdisciplinară în bolile inflamatorii intestinale. Am analizat principalele manifestări extraintestinale și epidemiologia acestora în nord-estul României. Una din lucrările principale se referă la modificările microbiotei intestinale la pacienții care asociază boală inflamatorie intestinală și spondilită ankilozantă. O altă subdirecție importantă este reprezentată de studiile privind modificările calității vieții la pacienții cu colită ulcerativă și/sau boală Crohn. Pornind de la un grant câștigat prin competiție la Universitatea de Medicină și Farmacie "Grigore T Popa" Iași, în colaborare cu catedra de psihologie a Universității "Alexandru Ioan Cuza", am realizat un studiu de urmărire a calității vieții pacienților cu boli inflamatorii intestinale în urma ședințelor de educație și consiliere psihologică. Variate alte aspecte de abordare interdisciplinară – de la sarcină și infecția cu *Clostridioides difficile,* până la asocierea dintre infecția SARS CoV2 și boala inflamatorie intestinală - au fost publicate de-a lungul anilor.

Capitolul al 3-lea se referă la ecografia cu substanță de contrast – ca explorare non-invazivă a leziunilor focale hepatice. Studiul "Contrast-enhanced ultrasound (CEUS) for the evaluation of focal liver lesions - a prospective multicenter study of its usefulness in clinical practice", publicat în Ultraschall Medizine (factor de impact 4,924) în 2014 a obținut Premiul de cercetare al Unității Executive pentru Finanțarea Învățământului Superior, a Cercetării, Dezvoltării și Inovării (UEFISCD) și are 60 citări Clarivate Analytics Web of Science. Alte

două lucrări referitoare la diferențierea nodulilor benigni – maligni și hiperplazia nodulară focală au fost publicate în reviste cu factor de impact.

Capitolul al 4 - lea cuprinde noțiuni de abordare interdisciplinară între bolile digestive și diabet, nutriție și boli metabolice. Acest capitol are, la rândul său, mai multe subdirecții: cercetări legate de nutriție (în sindromul de intestin iritabil, boli inflamatorii intestinale); asocierea dintre diabetul zaharat și tulburările motorii ale tractului digestiv; sindromul metabolic și tratamentul antiviral oral în hepatita cronică virală C.

A DOUA SECȚIUNE prezintă noile direcții de cercetare. Experiența și realizările din perioada post-doctorală îmi permit să formulez obiective și strategii de dezvoltare pe plan didactic, profesional și științific. În domeniul cercetării științifice îmi propun continuarea studiilor privind bolile inflamatorii intestinale; voi căuta noi markeri de evoluție și prognostic, voi aprofunda relația complexă cu factorii metabolici și tromboembolici, voi folosi ultrasonografia ca tehnică non-invazivă de management. Îmi doresc să realizez studii complexe în domeniul ultrasonografiei, în care să utilizez toate tehnicile derivate din această metodă, de la elastometrie la ecografia cu substanță cu contrast. O altă direcție extrem de actuală și provocatoare este reprezentată de ficatul gras metabolic – cea mai frecventă patologie hepatică în prezent, cu multiple necunoscute privind patogenia și tratamentul.

SECȚIUNEA a 3-a cuprinde referințele bibliografice pe care le-am utilizat pentru această teză și pentru articolele incluse.

Îmi doresc ca această teză de abilitare, în care am prezentat realizările profesionale din perioada post-doctorală, să se constituie ca argument în dobândirea dreptului de îndrumare a doctoranzilor și ca suport al realizărilor științifice viitoare.

ABSTRACT

The habilitation thesis entitled "**Elements of non-invasiveness and interdisciplinarity in digestive diseases**" is a synthesis of my scientific activity from the postdoctoral period to present day. The thesis describes the main research directions and the results obtained while also supporting me in acquiring the right to coordinate doctoral students.

The structure of the thesis is in line with the recommendations of the National Council for Attestation of University Degrees, Diplomas and Certificates (CNATDCU) and includes 3 sections:

- Section I: Scientific achievements in the postdoctoral period;
- Section II: Future scientific projects;
- Section III: References.

SECTION I begins with a review of my academic, scientific, and professional activity. I have described the landmarks of development in my didactic and professional career, the activity in the academic community and the main areas of interest within the specialty. I have also presented my main scientific achievements in the field: 38 ISI indexed papers and 19 ISI Proceeding papers indexed in Clarivate Analytics Web of Science Core Collection, Hirch index 10, 287 ISI citations, 65 extenso papers indexed in other international databases, 59 book chapters. I was a member of 6 research and development projects, I presented 364 abstracts at national and international meetings and I won 18 awards for scientific papers.

Chapter 1 covers the non-invasive approach in inflammatory bowel disease and includes 2 sub-directions: biomarkers and artificial intelligence. In the field of biomarkers, I describe the research related to the value of fecal calprotectin in diagnosis and monitoring, as well as the correlation of biomarkers with the endoscopic activity of inflammatory bowel disease. Artificial intelligence, based on neural networks and deep learning, has multiple applications in modern medicine, from increasing the accuracy of diagnosis, to estimating the prognosis or response to treatment. Our work demonstrated the role of artificial intelligence in assessing the endoscopic and histological activity of inflammatory bowel disease, as well as the response to anti-tumor necrosis factor antibodies treatment**.**

Chapter 2 presents elements of interdisciplinary approach in inflammatory bowel diseases. It includes a review of extraintestinal manifestations and their epidemiology in northeastern Romania. One of the main research areas is the change in the intestinal microbiota in patients who associate inflammatory bowel disease and ankylosing spondylitis. Another important field is the research of the quality-of-life changes in patients with ulcerative colitis and/or Crohn's disease. Starting from a grant won through competition at "Grigore T Popa" University of Medicine and Pharmacy Iasi, in collaboration with the Department of Psychology of the "Alexandru Ioan Cuza" University, we conducted a study to monitor the quality-of-life for patients, following educational and psychological counseling sessions. Various other aspects of interdisciplinary approach have been published over the years: from pregnancy and *Clostridioides difficile* infection to the association between SARS CoV2 infection and inflammatory bowel disease.

Chapter 3 deals with contrast-enhanced ultrasound – as a non-invasive exploration technique of focal liver lesions. The study "Contrast-enhanced ultrasound (CEUS) for the evaluation of focal liver lesions – a prospective multicenter study of its usefulness in clinical practice", published in Ultraschall Medizine (Impact Factor 4.24) in 2014 won the Executive Unit for Funding Higher Education, Research, Development and Innovation Research Award and has 60 citations Clarivate Analytics Web of Science Core Collection publications. Two

other papers on the differentiation of benign / malignant nodules and focal nodular hyperplasia have been published in journals with impact factor.

Chapter 4 covers notions of interdisciplinary approach between digestive diseases and diabetes, nutrition and metabolic diseases. This chapter also has several distinct areas: research on nutrition (in irritable bowel syndrome, inflammatory bowel diseases), the association between diabetes and gastric emptying and between metabolic syndrome and oral antiviral treatment in chronic viral hepatitis C.

SECTION II presents new directions of research. The experience and achievements from the post-doctoral period allow me to formulate objectives and development strategies on a didactic, professional and scientific level. In the field of scientific research, I propose to continue my studies on inflammatory bowel diseases; I will look for new markers of evolution and prognosis, I will look deeper into the complex relationship with metabolic and thromboembolic factors, I will use ultrasonography as a non-invasive technique. I want to carry out complex studies in the field of ultrasonography, in which to use all the techniques derived from this method, from elastometry to contrast-enhanced ultrasonography. Another very current and challenging direction is the metabolic fatty liver – the most common liver disease today, with multiple unknowns regarding its pathogenesis and treatment.

SECTION III contains the bibliographic references used for this thesis and for the included articles.

I want this habilitation thesis, in which I presented the professional achievements of the post-doctoral period, to be an argument in acquiring the right to coordinate doctoral students and as a support for future scientific achievements.

SECTION I

SCIENTIFIC ACHIEVEMENTS OVER THE POSTDOCTORAL PERIOD

OVERVIEW OF PERSONAL, PROFESSIONAL, ACADEMIC AND SCIENTIFIC CONTRIBUTIONS

I feel privileged that my work encompasses two of the most noble and rewarding professions: doctor and teacher. The opportunity to bring hope and relief and also to be able to contribute to the formation of those who will bring even more hope and relief in the future is more than a profession, it's a way of life that requires dedication, responsibility, hard work and sacrifices, but also brings great satisfaction. The scientific research activity comes to round up my professional career bringing rigour, recognition and the prospect of continuous development.

I shall briefly describe the 3 main axes defining the medical profession in the university environment: medical practice, academic activity and scientific research while trying to outline the main points of reference in my postdoctoral formation and evolution – as a foundation for future development. In real life, the three axes intertwine to define the profile of a doctor in academia.

PROFESSIONAL ACHIEVEMENTS

In 1987 I graduated „Petru Rareş" high school in Piatra Neamt, in the mathematics-physics section. Between 1988-1994, I attended the „Grigore T. Popa" University of Medicine and Pharmacy in Iaşi, majoring in general medicine and graduating with a score of 9,87 out of 10 (Licence Diploma no. 118/ 27.07.1995). After successfully passing the resident exam & contest, from 1994 to 1999, I worked as a gastroenterology resident in Second Medical Clinic – Gastroenterology in the Clinical Emergency Hospital "Sf. Spiridon" Iași (subsequently Institute of Gastroenterology and Hepatology). I qualified as a specialist physician in 1999 (Order of the Health Ministry no. 900/15.12.1999) and then as a senior physician of gastroenterology în 2004 (Order of the Health Ministry no.1067/25.08.2004). In 2003 I also passed the exam for the general ultrasonography competence, in 2009 the exam for the diagnostic digestive endoscopy competence and in 2019 I acquired the therapeutic digestive endoscopy competence.

I chose a very generous speciality – gastroenterology – that combines harmoniously clinical activity with advanced invasive explorations sometimes bordering surgery. It's an extremely dynamic and challenging domain where you have every chance to continuously evolve as a practitioner. I was lucky enough to work from the beginning in one of the elite gastroenterology teams in Romania under the guidance of true personalities in the field. Along my 25 years of experience I've gone through all the stages of the medical career: specialist physician, senior psychian, acquiring the competencies in general ultrasonography and diagnostic, and therapeutic digestive endoscopy. I've covered thousands of on-call hours and permanently tried to be a good clinician and to enhance my skills in the areas of diagnostic and therapy. As I wish to contribute to the formation of medical personnel able to provide high quality services, I do my best to determine my students and residents to develop and employ

logical thinking, medical reasoning, hierarchical explorations, optimal choice of treatment and a holistic view of the pacient.

Along the years, I've completed several courses in order to advance my scientific knowledge. I have participated every year in postgraduate courses organized at european and world conferences under the auspices of scientific societies: United European Gastroenterology Week, European Association for the Study of the Liver, European Crohn's and Colitis Organization, Digestive Disease Week, American Association for Study of the Liver Diseases, Journées Francophones d'Hépato-gastroentérologie et d'Oncologie Digestive.

In 2001 I have participated in a 3-week training course in the Netherlands, at the hospitals in Zwolle and Arnhem. In 2003 I received a scholarship which consisted of an internship at the „Georges Pompidou" Hospital in Paris, under the guidance of Professors Philippe Ruszniewsky and Mathieu Allez. These internships have opened up new horizons and opportunities for me. I gained experience and consolidated my knowledge in the field of inflammatory bowel disease and endoscopic examination.

Another constant professional area of focus was ultrasonography. I have regularly participated in theoretical courses and hands-on sessions organized annually during the United European Gastroenterology Week. Aware of thecontribution of new ultrasonographic techniques in the modern management of liver diseases, I took two advanced training courses: Elastometry internship by Hitachi – Craiova, 2007 and Intensive Contrast-Enahnced Ultrasound Course, 2010, Timisoara. Aquiring the knowledge about these techniques enabled me to further apply and develop them in the University Center of Iasi, with benefits for patient care, teaching and research.

I consider that any trainer has the obligation to be first and foremost a good practitioner, able, through expertise and professionalism, to be a model for students and residents.

ACADEMIC ACTIVITY

I took my first steps in academia in 1988 as an University Assistant in Medical Semiology at "Grigore T. Popa" University of Medicine and Pharmacy – position obtained through contest. From 2002 I was an Assistant Professor and from 2009 to 2019 a Lecturer at the Medical Semiology – Gastroenterology discipline. From 2019 I am an Associate Professor in the same department. In 2003 I graduated a course in Psychopedagogy provided by the Teachers' Training Department of „Alexandru Ioan Cuza" University from Iasi.

In 2008 I completed my doctoral studies – under the guidance of Professor Carol Stanciu – with the thesis entitled „Foreign bodies in the upper digestive tract" and I was awarded a „cum laude" PhD in medicine (Order of the Ministry of Education and Research no. 5837/04.11.2008).

In parallel with my work as a clinician, my academic career also evolved over more than 20 years through all stages from University Assistant to Associate Professor. This covers practical internships and courses in Medical Semiology and Gastroenterology in both Romanian and English. Following direction from "Grigore T. Popa" University of Medicine and Pharmacy, I've also taught at the Physician Assistant College in Botoşani and Iaşi. From the very first years I collaborated with Professor Cijevschi in the optional course for students of "Commented Clinical Cases" and, later, in the "Abdominal Ultrasound in Clinical Practice". I am currently the holder of the second course, which is enjoying a real success, with a large number of participants annually and an extremely positive feedback.

My teaching work load was at the maximum level allowed and I've also worked supplementary hours when needed. I tutored more than 20 licence theses, workshops and papers (some even award-winning) for student congresses.

Another area of my academic activity regards the medical residents in gastroenterology and also in related fields. To them, I provide guidance for their daily duties, courses and

internships. Besides their clinical activity I also covered their scientific development giving lectures and courses, tutoring them for their own presentations and coordinating their scientific research work.

From the very beginning of my academic work, I've been actively involved in the producing of teaching materials: diagrams, illustrations, clinical cases, tests for partial and final evaluations. I've been a contributor to the revised manuals of Medical Semiology in both Romanian and English under the coordination of Prof. Carol Stanciu. Together with Prof. Cristina Cijevschi, we authored a manual of gastroenterology („Notions of gastroenterology and hepatology for students") for 5thyear students. I was a coauthor of „Course Notes", fourth edition, under the coordination of Prof. Anca Trifan and of „Notions of gastroenterology and hepatology", under the patronage of the Romanian Society of Gastroenterology and Hepatology – a medical treatise for gastroenterology residents and specialists.

My participation in the project „The Integrative Module for the Study of theLiver" of the "Grigore T. Popa" University of Medicine and Pharmacy in Iași was a very interesting experience as it proposed a new teaching approach, of transversal type, from anatomy to a disease treatment. I was proud to co-author the chapters on digestive pathology in the revised edition of Medical Therapy, under the coordination of Prof. Gabriel Ungureanu and Prof. Adrian Covic, a book that I studied from as a student.

I have taken part as a lecturer to the courses of the Summer School of Gastroenterology – a programme for continuous medical education for residents and for specialists in areas related to gastroenterology. I have also been a lecturer for over 10 years in the postgraduate course for obtaining competence in general ultrasonography and I have participated – as a lecturer – in numerous courses, presentations, workshops organized by the College of Physicians or other scientific societies. The course „Optimize Speaker Training Meeting" 22-23 June 2018, Berlin, has improved my teaching abilities.

I am an active member of the academic community, being part of numerous commissions for license, residency, admission, evaluation for positions in academia or the medical network, specialist and consultant qualifications, ultrasonography competence. Every year, I took part in the admission, license and residency commissions in our university. The participation, as a member, in the doctoral admissions or doctoral tutorial commissions provided me with a good experience for becoming a PhD coordinator.

SCIENTIFIC RESEARCH

Scientific research is a sine qua non component of any successful academic career. I believe that along my 25 years of activity, I have conducted extensive and diverse research work, resulting in:

- 38 articlespublished in journals indexed by ISI Thomson Reuters with impact factor, 20 of these as the main author;
- Hirch Index 10;
- 287 citations in the ISI Thomson Reuters system;
- 84 articles published in extenso in journals proceeding ISI or other international database;
- Author or coauthor to 59 book chapters;
- 364 papers presented as posters, over 100 presentations at national and international congresses;
- Participating as a member to 6 research projects, 3 of which, international;
- Winner of 16 prizes at national scientific events and 2 research prizes.

I am a member of the board of the Romanian Club for Crohn and Colitis and member by invitation of the board of the Romanian Society of Ultrasound in Medicine and Biology. I

am also a member of the Romanian Gastroenterology and Hepatology Society, Society of Physicians and Naturalists Iași, European Association of Study of the Liver, European Crohn's and Colitis Organization.

I am a member of the scientific committee of numerous national and international events and I have often been invited to the medical conferences as a guestspeaker. In recent years I also became a peer-reviewer for many journals indexed by ISI (World Journal of Gastroenterology, World Journal of Gastrointestinal Oncology, PlosOne, Turkish Journal of Gastroenterology, Scandinavian Journal of Gastroenterology, PhytotherapyResearch).

My first articles were published in The Journal for Continuous Medical Education – Gastroenterology, where I also covered the role of editor assistant. It was a journal recognised by the National Council for Scientific Research in Higher Education but without indexation in international databases; however, the experience of those early years had a significant contribution to my formation, helping me acquire the foundation skills for documenting with accuracy and precision and for evaluating a scientific paper.

I began my research work during my doctoral studies on the topic „Foreign bodies in the upper digestive tract" under the guidance of Professor Carol Stanciu. This resulted in publishing 1 ISI article, 2 other international database articles and 3 book chapters.

While keeping with the focus area of my team, under the coordination of Professor Cristina Cijevschi Prelipcean, I expanded the research in the inflamatory bowel disease (IBD) domain. I held several conferences, courses and lectures. I published book chapters, extensive papers, posters of different aspects of epidemiology, clinical and biological picture, complications and treatment of IBD. Furthermore, I took part as a member in a grant financed by UMF Iaşi to study the role of psychological counselling in improving the quality-of-life of these patients. I was also the regional coordinator for a grant of the Romanian Club for Crohn and Colitis studying the epidemiology of inflamatory diseases in the general population. Many of the papers published in this domain were presented at national symposiums of inflamatory diseases and gastroenterology. Most of the work was possible due to the existence of a National Register of hospitalized patients (IBD PROSPECT) where Iasi university center has almost 1/3 of the total number of patients enrolled. I further mention the main research topics in the field of IBD:

- Epidemiology: IBD epidemiology in nort-eastern part of Romania, aging and IBD, epidemiology of extraintestinal manifestations;
- Pathogenesis: intestinal microbiota;
- Diagnosis: laboratorybiomarkers, fecal calprotectin, artificial intelligence as a non-invasive tool;
- Complications: dysplasia and colorectal cancer, anemia, osteoporosis, *Clostridioides difficile* infection;
- Treatment: biologic therapy, nutrition, surgery;
- Factors influencing quality of life in IBD.

The team I am part of is nationally recognized as having a high scientific expertise in the field of IBD, many of the works in this field winning awards.

Another main area of research was abdominal ultrasound. I lectured many times at the national ultrasound congresses, I published an article in The Romanian Ultrasound Journal (currently indexed by ISI) and I took part in the multi-center studies about the role of contrast-enhanced ultrasound in hepatic pathology evaluation. The results of these studies were published in Ultraschall Medizine (this work being recognised by winningthe 2014 Executive Unit for Funding Higher Education, Research, Development and Innovation Research Award) and also in the Journal of Gastrointestinal and Liver Diseases and in the Medical Ultrasound.

It was probably not by chance that I was always interested in a multidisciplinary approach, therefore carrying out multiple research works about the link between the digestive

pathology and that of diabetes, nutrition and methabolic diseases. As a result, I published several papers – in summary and extended form – addressing the various facets of this interface: diabetic gastroparesis, hepatic steatosis, metabolic syndrome. In the same domain, I also focused on the nutritional aspects linked to digestive diseases, lecturing at the congresses of The Romanian Nutrition Society, publishing extensive papers and posters at international events and contributing to two nutritional guides for digestive diseases.

These main research topics will be practically detailed in the habilitation thesis. However, my research activity is much more complex, covering many other research topics.

Fuelled by the enthusiasm of an innovative treatment and given the large number of patients with chronic viral hepatitis C in my care, I published my findings from the experience in the oral antiviral treatment of hepatitis C. This resulted in the publication of two ISI articles as a principal author („Role of PNPLA3 in the assessment and monitoring of hepatic steatosis and fibrosis in patients with chronic hepatitis C infection who achieved a sustained virologic response" was published in Medicina, 2021 – IF 2.43 and „Severe hyponatremia after direct-acting antiviral treatment in a patient with virus C compensated liver cirrhosis and kidney transplant" was published in Journal of Gastrointestinal and Liver Diseases in 2018 – IF 2.063). I have also participated in two other multicenter studies whose results have been published in impact factor journals: „Efficacy and safety of paritaprevir/ritonavir, ombitasvir, and dasabuvir with ribavirin for the treatment of HCV genotype 1b compensated cirrhosis in patients aged 70 years or older", Medicine, 2017 and „Effectiveness of 8- and 12-week treatment with ombitasvir/ paritaprevir/ritonavir and dasabuvir in treatment-naïve HCV patients in a real-life setting in Romania: the AMETHYST Study", Journal of Gastrointestinal and Liver Diseases, 2021. Many other aspects (predictive role of alphafetoprotein, portal hypertension significance, hepatocarcinoma risk, side effects, compensated and decompensated liver cirrhosis) of direct acting antivirals in chronic hepatitis C have been studied and presented at national and international meetings.

Another area of interest is the pathology of the upper and lower digestive tract. I have published in ISI journals articles related to the eradication of *Helicobacter pylori* („*Lactobacillus reuteri* – an alternative in the first-line of *Helicobacter pylori* eradication", Farmacia, 2019), duodenal ulcer („Forrest III duodenal ulcers during treatment with adalimumab for psoriazis", Journal of Dermatological Treatment, 2019), irritable bowel syndrome („A comparison using standardized measures for patients with irritable bowel syndrome: Trust in the gastroenterologist and reliance on the internet", Neurogastroenterology and Motility, 2021), colorectal cancer („E-cadherin expression in primary colorectal cancer and metastatic lymph nodes", Romanian Journal of Morphology and Embryology, 2016). I am a member of two grants (won through competition) in the field of digestive oncology. The first research project referred to „Nanoparticle-enabled Terahertz molecular imaging as advanced early-stage diagnostic of gastric neoplasia" and resulted in the publication of 1 ISI paper, 1 other international database paper and many abstracts. The second project in which I took part referred to „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 (HOPE)". The extra knowledge acquired during this project enabled me to subsequently graduate the course „Training guide for advanced high-specialized intervention in oncogenetics" and also to participate in the elaboration of the manual „How oncogenetics predicts&educates".

Complications of liver cirrhosis have been another area of research that has resulted in 1 ISI article („Diagnosis of minimal hepatic encephalopathy in a tertiary care center from eastern Romania: validation of thepsychometric hepatic encephalopathy score (PHES)", Metabolic Brain Diseases, 2016), as well as other abstracts, articles and book chapters addressing a variety of topics, from osteoporosis to liver cancer.

In the field of pancreatic diseases I published two ISI articles as the main author: „Pancreatico-Pleural Fistula – from Diagnosis to Management. A Case Report", Journal of Gastrointestinal and Liver Disease, 2018 and „Extrapancreatic necrosis volume: A new tool in acute pancreatitis severity assessment?" World Journal of Clinical Cases, 2021.

The scientific merits of my research team have been broadly and continuously recognised, as proven by the 18 awards won along the years:

1. **Mihai C**, Dranga M, Pintilie I, Nedelciuc O, CijevschiPrelipceanC**.** Single focal liver lesion in chronic viral hepatitis – has CEUS any contribution?*Romanian Journal of Hepatology* 2010, vol 6, nr. 3, suppl 1: 95. **Prize in the posters section, National Congress of Hepatology 2010;**
2. Lăcătuşu CM, Graur M., **Mihai C**, Dranga M, Popescu R, Cijevschi-Prelipcean C, Mihai B.Variation of metabolic parameters in patients with chronic viral and / or metabolic hepatitis. *Acta Diabetologica Română* 2013, Vol. 39, PS 33. **Second prize – National Congress of Diabetes Mellitus 2013**.
3. Nedelciuc O, Badea M, Dranga M, Blaj A, Cucos A, Ungureanu I, CijevschiPrelipcean C,**Mihai C**. Health-related quality-of-life in Romanian patients with Crohn's disease. **Third prize – The 6th National Symposium on Inflammatory Bowel Diseases 2014;**
4. Sporea I, Badea R, Popescu A, Spârchez Z, Sirli RL, Dănilă M, Săndulescu L, Bota S, Calescu DP, Nedelcu D, Brisc C, Ciobâca L, Gheorghe L, Socaciu M, Martie A, Ioaniţescu S, Tamas A, Streba CT, Iordache M, Simionov I, Jinga M, Anghel A, CijevschiPrelipcean C, **Mihai C**, Stanciu SM, Stoicescu D, Dumitru E, Pietrareanu C, Bartos D, Manzat Saplacan R, Pârvulescu I, Vădan R, Smira G, Tuţă L, Săftoiu A. Contrast-enhanced ultrasound (CEUS) for the evaluation of focal liver lesions – a prospective multicenter study of its usefulness in clinical practice.*Ultraschall Med* 2014; 35(3):259-66. **Research Award of Executive Unit for Funding Higher Education, Research, Development and Innovation, 2014**;
5. Popa IV, **Mihai C**, Dranga M, Badea M, Popa RC, Didita A, Gavrilescu O, Cijevschi-Prelipcean C**.** Biological therapy and anemia evolution in inflammatory bowel disease**. First prize** – **8thNational Symposium on Inflammatory Bowel Diseases, 2016**;
6. Popa RC, Gavrilescu O, Badea M, Dranga M, **Mihai C**,CijevschiPrelipcean C.Neutrophyl-lymphocyte ratio as a predictive marker of Crohn's disease activity. **Mention** – **8thNational Symposium on Inflammatory Bowel Diseases, 2016**;
7. **Mihai C**, Mihai B, Cardoneanu A, Dranga M, Gavrilescu O, Drug V, CijevschiPrelipcean C. First line *Helicobacter pylori* eradication in dyspeptic patients. **Prize in the posters section** – **Meeting of the Romanian Society of Neurogastroenterology with ROME IV Regional Central – East European Meeting, 2017;**
8. Gavrilescu O, Dranga M, Ungureanu I, Popa R, **Mihai C**, CijevschiPrelipcean C. Anemia and quality-of-life in inflammatory bowel disease. J Gastrointest Liver Dis 2017; 26 (3): PP145. **Third prizeof the Romanian Society of Gastroenterology and Hepatology, The XXXVIIth National Congress of Gastroenterology, Hepatology and Digestive Endoscopy, 2017;**
9. Cucos A, Ungureanu A, Nedelciuc O, **Mihai C**, CijevschiPrelipcean C. The correlation between circulating periostin levels, the metabolic syndrome, and nonalcoholic fatty liver disease among obese patients. **Third prizeof the Romanian Society of Gastroenterology and Hepatology, The XXXVIIth National Congress of Gastroenterology, Hepatology and Digestive Endoscopy, 2017;**
10. Trifan A, Stanciu C, Gheorghe L, Iacob S,Curescu M,CijevschiPrelipcean C, Stefanescu G,Girleanu I, Chiriac S,**Mihai C**,Brisc C, Goldis A, Sporea I,Miftode E,Bataga S, Rogoveanu I, Preda C, Caruntu AF, Singeap AM. Efficacy and safety of

paritaprevir/ritonavir, ombitasvir, and dasabuvir with ribavirin for the treatment of HCV genotype 1b compensated cirrhosis in patients aged 70 years or older. *Medicine*2017; 96(50):e9271 – **Research Award of Executive Unit for Funding Higher Education, Research, Development and Innovation, 2017**;

11. Dorobăț AG, Gavrilescu O, Dranga M, Popa I, Gavril I, Popa R, Savin A, Chiosa AM, **Mihai C**, CijevschiPrelipcean C.Clinical significance of C-reactive protein levels in predicting responsiveness to iron therapy in patients with inflammatory bowel disease and iron deficiency anaemia. P**rizeof the Romanian Society of Gastroenterology and Hepatology, The XXXVIIIth National Congress of Gastroenterology, Hepatology and Digestive Endoscopy, 2018;**
12. Dorobăț AG, Dranga M, Popa R, Popa I, Bejinariu I, Cardoneanu A, Toader E, CijevschiPrelipcean C, **Mihai C**.Can the neutrophil-to-lymphocyte and platelet-to lymphocyte ratios predict disease activity in patients with ulcerative colitis? **Second** P**rizeof the Romanian Society of Gastroenterology and Hepatology, The XXXVIIIth National Congress of Gastroenterology, Hepatology and Digestive Endoscopy, 2018;**
13. Cardoneanu A, CijevschiPrelipcean C, Rezus E, Dranga M, Popa I, Popa R, Bejenariu I, **Mihai C.**The articular involvement in IBD can modulate the composition of intestinal microbiota?**First** P**rizeof the Romanian Society of Gastroenterology and Hepatology, The XXXVIIIth National Congress of Gastroenterology, Hepatology and Digestive Endoscopy, 2018;**
14. Dranga M, Gavrilescu O, CijevschiPrelipcean C, **Mihai C**. Anemia in patients with inflammatory bowel disease. **Second prize** – **11thNational Symposium on Inflammatory Bowel Diseases, 2019**;
15. Gavrilescu O, Dranga M, Gavril I, Popa I, CijevschiPrelipcean C, **Mihai C**. Quality of life and iron replacement in inflammatory bowel disease. **Third prize** – **11thNational Symposium on Inflammatory Bowel Diseases, 2019**;
16. Leustean AM, Lupascu A, Betisor E, Andronic A, Popa R, Dranga M, **Mihai C**, CijevschiPrelipcean C. Bone mineral density in patients with inflammatory bowel disease. **Third prize** – **11thNational Symposium on Inflammatory Bowel Diseases, 2019**;
17. Dobru D, Cijevschi C, Dumitru E, Mateescu B, Goldis A, Negreanu L, Fratila O, **Mihai C**, Singeap AM, Pantilie D. Understandingthe Impact of UlcerativeColitisandits Associated DiseaseBurden on Patients (ICONIC Study) – Data of Studyfrom Romanian Patients. **Special Prize** – **12th National Symposion on InflammatoryBowelDiseases, 3rd Franco-Romanian Meeting, 2020**;
18. Gavrilescu O, Dranga M, Gavril I, Popa I, Andronic A, Cijevchi Prelipcean C, **Mihai C**. Quality of lifeand fecal calprotectin in inflammatoryboweldiseases. **Special Prize** – **12th National Symposion on InflammatoryBowelDiseases, 3rd Franco-Romanian Meeting, 2020.**

All these accomplishments in scientific research can become a foundation for taking the established avenues further and can also serve as starting points for new research.

Chapter 1.
NONIVASIVE APPROACH IN INFLAMMATORY BOWEL DISEASES

1.1. INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic condition with undulating evolution, in which periods of activity alternate with periods of remission. With an increasing incidence and prevalence, their pathogenesis is incompletely elucidated to date. Genetic and environmental factors in conjunction with the intestinal microbiota cause an abnormal immune response and the onset of inflammation in the intestine. The two main conditions in the IBD spectrum are ulcerative colitis (UC) and Crohn's disease (CD). The positive diagnosis implies a corroboration of the clinical, endoscopic, histopathological, biological, radiological elements, but, in 10% of cases, elements from UC overlap with those from CD, the disease being called "indeterminate colitis". Research in recent years has brought important advances in understanding the etiopathogenesis, diagnosis and treatment of IBD, the practical approach of the patient being a continuous challenge.

In the 21st century, IBD has become globally prevalent (Ng et al, 2018). Over the past two decades, UC and CD have emerged as debilitating chronic conditions associated with complications, surgery, and high hospitalization costs. There is a growing body of evidence indicating that the incidence and prevalence rates of IBD are expanding worldwide, with IBD currently affecting over 2.2 million people in Europe and over 1.5 million in the United States (Burisch, Munkholm, 2015). IBD currently affects the most active segment of the population, that is, usually those aged 20–30 years for CD and those aged 30–40 years for UC.

IBD is the field with the widest scientific and professional expertise. Personal research conducted to date has included many and varied aspects of IBD, from notions of epidemiology and etiopathogenesis to treatment. I have published 15 ISI articles (of which 10 as main author), 8 ISI Proceeding articles and 18 other international database articles.

Next I will refer to the studies that are part of the theme of the habilitation thesis, namely non-invasive exploration and interdisciplinary approach in IBD.Non-invasive exploration of IBD focuses on two major sub-directions: biomarkers and artificial intelligence.

1.2. BIOMARKERS IN INFLAMMATORY BOWEL DISEASE

1.2.1. State of the art

Both the diagnosis and the monitoring of patients with IBD involve repeated colonoscopic examination - an invasive method, difficult to accept, expensive for the medical system. As a result, there have been scientific concerns for finding non-invasive markers capable of establishing the positive diagnosis and especially to assess the response to treatment, prediction of relapses, personalized approach in IBD patients. Given the increasing incidence and prevalence of IBD globally, as well as the SARS CoV 2 pandemic, non-invasive methods are becoming an urgent necessity.

The best known and most studied non-invasive marker in the diagnosis and monitoring of patients with IBD is fecal calprotectin (FC). It has been shown to be effective in differentiating irritable bowel syndrome (IBS) from IBD, in assessing endoscopic and

histological activity, predicting relapses, and monitoring the therapeutic response (Khaki-Khatibi et al, 2020).

Calprotectin is an antimicrobial protein secreted by neutrophils. Elevated serum values have been reported in many conditions, from sepsis to aneurysmal subarachnoid hemorrhage, from rheumatoid arthritis to neoplasms (Khaki-Khatibi et al, 2020). Although there are studies on the role of serum or salivary calprotectin in IBD (Azramezani et al, 2019), the most valuable and studied marker remains FC. It stays stable at room temperature for 4-7 days. It can be determined quantitatively, semi-quantitatively or qualitatively. Rapid tests, although with lower sensitivity and specificity compared to laboratory determinations, have the advantage of being cheap, easily accessible, with immediate results. The first FC studies were performed in the 1990s to differentiate IBD from IBS, given the common symptoms of the two conditions (diarrhea, abdominal pain). Although there are a multitude of studies that have shown significantly increased FC values in patients with IBD compared to IBS or healthy volunteers, due to their heterogeneity, cut-off values, sensitivity and specificity are not clearly established (Freeman et al, 2019). A recent meta-analysis estimates a sensitivity of 88% (95% CI, 80-93%), specificity of 72% (95% CI, 59-82%), and area under curve (AUC) 0.89, with no significant differences between cut-off values of 50 μg / g or 100 μg / g feces (An et al, 2019). In addition, there are a number of other conditions besides IBD that can increase FC values: colorectal cancer, colonic diverticulosis, celiac disease, infectious enteritis, use of non-steroidal anti-inflammatory drugs, or proton pump inhibitors (Walsh et al, 2019).

Another challenging area is the non-invasive estimation of endoscopic activity using FC. A 10-year meta-analysis concerning endoscopic healing in IBD revealed odds ratio 11.2 for diagnostic FC and the area under the summary receiver operating characteristic curve 0.829 in CD and the diagnostic odds ratio 14.48, with the the area under the summary receiver operating characteristic curve of 0.858 for UC (Bromke et al, 2021). However, the very high variability of cut-off values, individual variability, the influence of other conditions or drugs, limit the use of FC in clinical practice.

Although there is no IBD-specific serum marker, a number of accessible, inexpensive, and non-invasive inflammatory markers are used to assess disease activity: C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), platelets, albumin, hemoglobin. Although there are many studies that correlate the values of these markers with endoscopic activity, the data are still contradictory and none of the serum markers appear to be superior to FC (Rodrigues et al, 2020). Things are even more difficult in CD, where the inflammation is transmural and discontinuous, often beyond the possibility of an endoscopic approach. A lot of new biomarkers (genetics, metabolomics, proteomics, gut microbiota biomarkers) have been studied, but they are not yet ready for clinical practice (Chen et al, 2020).

In the following I will refer to the main research that the working group I belong to has in the use of biomarkers in IBD (3 ISI indexed articles of which 2 as main author, 3 ISI Proceeding articles and 3 other international database articles).

1. **Mihai C**, Cijevschi Prelipcean C, Dranga M, Gavrilescu O, Cardoneanu A, Lacatusu C, Mihai BM. Correlations between inflammatory biomarkers and activity in inflammatory bowel diseases. *Revista de Chimie* 2018; 69 (3):710-713. IF=1.412
2. Dranga M, **Mihai C**, Gavrilescu O, Cardoneanu A, Floria M, Mihai B, Prelipcean, Cijevschi C. The Role of Combining Biochemical Markers in Assessing the Endoscopic Activity in Ulcerative Colitis. *Revista de Chimie* 2018; 69 (5):1268-1271. IF=1.412
3. Dranga M, **Mihai C**, Drug V, Dumitrescu G, Cijevschi Prelipcean C. Can We Recommend a Rapid Test in Assessing Activity in Ulcerative Colitis? *Turk J Gastroenterol*2016;27(2):149-55. IF=0.966
4. Dranga M, **Mihai C**, Mihai BM, Gavrilescu O, Prelipcean Cijevschi C. Fecal calprotectine –

can we differentiate IBD from IBS?Proceedings. Meeting of the Romanian Society of Neurogastroenterology with Rome IV Regional Central – East European Meeting, Neurogastro 2017: 82-86.
5. Popa IV, **Mihai C**, Dranga M, Gavrilescu O, Cardoneanu A, Cijevschi Prelipcean C. Beta 2 - Microglobulin and Inflammatory Bowel Disease. Proceedings XXXVIth National Congress of Gastroenterology, Hepatology and Digestive Endoscopy, Cluj Napoca, 2016. Editor: Dan Dumitrascu. FILO diritto editure. www.gastro2016.medical-congresses.ro: 351-355.
6. Dranga M, **Mihai C**, Drug V, Mihai B, Nedelciuc O, Badea M, Cijevschi Prelipcean C. Organic or Functional by Biomarkers. Proceeding of 49th Annual Scientific Meeting of the European Society of Clinical Investigation, 27-30 MAY 2015, Cluj Napoca, Romania: 153-158.
7. Otea AG, Popa RC, Popa IV, Bejinariu I, Cardoneanu A, Gavrilescu O, Dranga M, Cijevschi Prelipcean C, **Mihai C**. Clinical significance of C-reactive protein levels in predicting responsiveness to iron therapy in patients with inflammatory bowel disease and iron deficiency anemia. The Medical Surgical Journal 2019; 123(1): 70-76.
8. Gavrilescu O, Dranga M, Soponaru C, Mihalcea LN, Cijevschi Prelipcean C, **Mihai C**. Quality of life and fecal calprotectin in inflammatory bowel diseases.The Medical-Surgical Journal 2018; 122(2): 283-288.
9. Dranga M, Dumitrescu G, Badea M, Blaj A, **Mihai C**, Cijevschi Prelipcean C. The semi – quantitative calprotectin rapid test – is it useful in inflammatory bowel disease? The Medical-Surgical Journal 2012; 116(3): 761-765.

1.2.2. Fecal calprotectin in the management of IBD patients

1.2.2.1. *The role of semiquantitative determination of fecal calprotectin in assessing endoscopic activity in ulcerative colitis*

Background & Aim. In recent years, the therapeutic goals of IBD have evolved beyond simply inducing and maintaining clinical remission to achieve mucosal healing (endoscopic healing) and even histological healing, in order to change the course of the disease and increase the quality of lifeof patients.This involves repeated colonoscopic examinations, which is difficult for the patient to accept, with high costs for the medical system. In this context the aim of our study was to determine the usefulness of the semi-quantitative rapid test Cal Detect ® (Sofar; Florence, Italy) for the determination of FC in predicting endoscopic activity in UC. At the same time, the role of other common biomarkers was evaluated: CRP, ESR, platelet count, hemoglobin, quantifying the role of each, compared to FC, in the evaluation of endoscopic activity (Dranga et al, 2016).

Material and method. The prospective study included 103 patients with UC evaluated in the Institute of Gastroenterology and Hepatology Iași over a period of 2 years. Each patient had a worksheet that included demographic data, physical examination, usual biological parameters, colonoscopic and histopathological examination. For the assessment of endoscopic activity we used the endoscopic Mayo score, considering remission a score of 0 or 1 and endoscopic activity a score of 2 or 3. Fecal calprotectin was determined by rapid semiquantitative test. Sampling was performed according to the manufacturer's instructions. The results were read after 2-3 minutes. The first band occured for a calprotectin concentration of under15 μg/g and indicates the absence of inflammation. The second band appeared at a calprotectin concentration ranging between 15 μg/g and 60 μg/g in the presence of mild inflammation. The appearance of the third band indicated a calprotectin value higher than 60 μg/g, and a high degree of mucosal inflammation. The patients with comorbidities that could influence the studied parameters (malignancies, infections, chronic and acute liver diseases,

kidney diseases, and other autoimmune diseases) were excluded from the study.The study was approved by the Local Ethics Committee, and statistical data processing was performed using the Statistical Package for the Social Sciences (SPSS; IBM, New York, USA) 18.0 with nominal significance defined as p <0.05.

Results. The patients' age ranged from 20 to 75 years, with a mean of 46.32±14.0 years. The gender distribution showed a preponderance of male cases (68.9%), with a sex ratio M/F of 2.2 / 1. In terms of disease activity, 48 patients were in endoscopic remission. Our study showed a positive, statistically significant correlation between FC value and endoscopic Mayo score (Fig. 1.1).

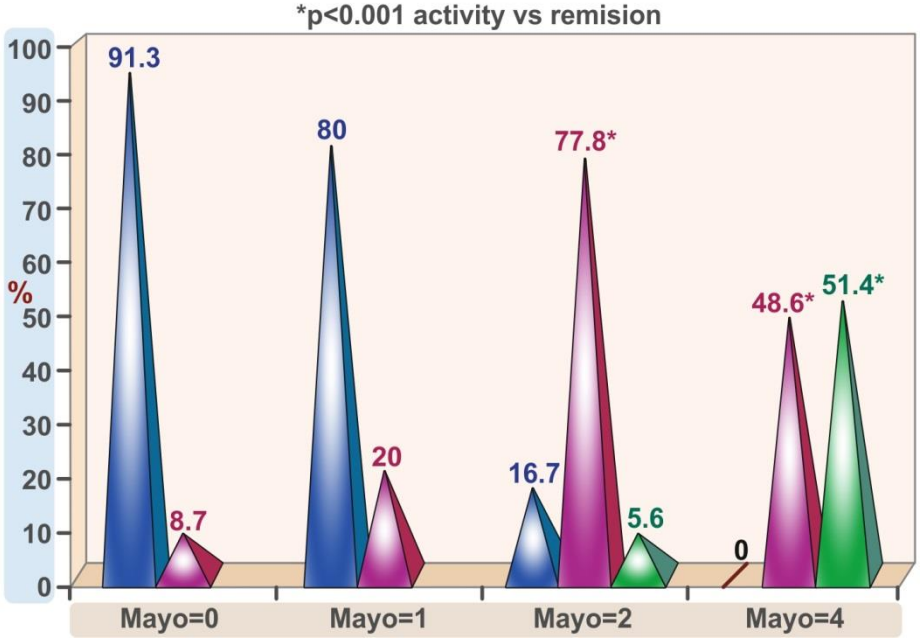


Fig. 1.1. Correlation between level of fecal calprotectin and Mayo endoscopic score in ulcerative colitis patients.

At a cut-off of 15 µg / g, compared to the other parameters studied, FC had the highest sensitivity and accuracy in discriminating endoscopically active disease compared to endoscopic remission (Table 1.1, Fig. 1.2).

Table 1.1. Sensitivities, specificities, and predictive values for fecal calprotectin, CRP, ESR, and hemoglobine for active endoscopic disease in UC

Marker	PPV (%)	NPV (%)	Sensitivity (%)	Specificity (%)	Accuracy	P
Calprotectin	95.7	46.8	98.0	76.7	90.9	0.001
CRP	95.7	69.8	69.4	52.0	602	0.001
ESR	95.7	502	61.1	52.0	59.7	0.001
Hb	513	56.0	50.0	53.0	45.9	0.001
CRP&ESR	745	76.9	84.4	55.0	672	0.001
CRP&ESR&Hb	913	50.0	80.8	71.4	76.1	0.007
Calprotectin & CRP	89.1	77.8	953	643	79B	0.001
Calprotectin &CRP&ESR	952	77.8	952	77B	865	0.001
Calprotectin & CRP&ESR&Hb	952	77.8	952	77B	865	0.001

CRP c-reactive protein; ESR: erythrocyte sedimentation rate; Hb: hemoglobin,
PPV: posrtive predictive value; NPV. negative predictive value; UG ulcerative colitis.

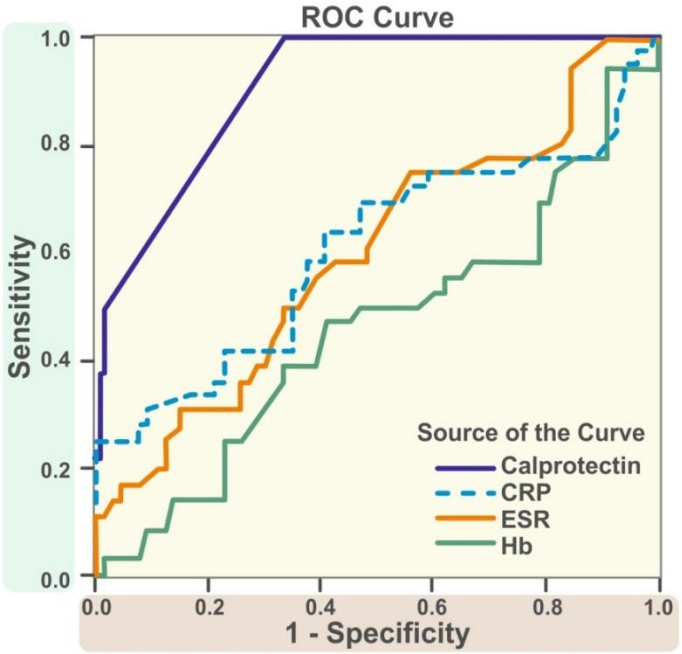


Fig.1.2. The ROC curve analysis on the abilities of calprotectin, CRP, ESR, and Hb to make a difference between active UC and inactive UC. UC: ulcerative colitis; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; Hb: hemoglobin

Discussions. Although at the time of publication of the study there was data in the literature about FC - as a surrogate marker of endoscopic activity(Lin et al, 2014), the merit of our study was to demonstrate the usefulness of a fast semi-quantitative test, cheaper and with immediate results. In our study, we used the cut-off of 15 µg/g, at which the manufacturer estimates the presence of acute inflammation. At this cut-off value, the sensitivity and specificity of calprotectin found in our study were 98.0% and 76.7%, respectively. The ROC curve analysis revealed significantly higher values compared to the rest of the parameters analyzed: CRP, ESR, and hemoglobin. The Cal Detect® (SOFAR; Florence, Italy) test's effectiveness in discriminating between actively and inactively endoscopic disease, assessed by the area under the ROC curve, was 0.909, which confirms its viability for the noninvasive assessment of patients diagnosed with UC. The combined use of calprotectin and CRP leads to an increase in sensitivity compared to the individual use of CRP at 95.3%; however, it drops compared to the individual use of FC, due to the CRP low sensitivity. Although the global sensitivity was very high, the number of false-positive results remained high, with a still low specificity of 63.4%. Sensitivity remained the same when we added the third inflammatory marker studied, ESR. Moreover, we noticed an increase in specificity to 77.8%, which constitutes an advantage for patients, due to the decrease in the number of unnecessary colonoscopies. Adding Hb to the model did not bring any modification in sensitivity/specificity.

Conclusions. The FC semi-quantitative rapid test proved to be a good predictor test for differentiating between the endoscopic active and inactive disease. Besides this, it is easy to use, replicable, and inexpensive and may be useful in monitoring the UC activity. The other markers analyzed in the study (CRP, ESR, hemoglobin) did not prove their effectiveness when used individually. The individual use of FC presents the highest sensitivity in determining the endoscopic activity. Nevertheless, in monitoring patients, the combined determination of the three inflammatory markers studied (CRP, ESR, and calprotectin) is more useful in reducing the number of false-positive values and, implicitly, the unnecessary colonoscopies, or treatment change.

1.2.2.2 .*Fecal Calprotectin – can differentiate inflammatory bowel disease from irritable bowel syndrome?*

Background&Aim. IBS and IBD (especially CD) may have common clinical manifestations, which may imply a difficult differential diagnosis. Moreover, the two conditions may overlap, which makes the therapeutic approach difficult (for example, the persistence of diarrhea or abdominal pain due to an IBS overlaped over CD, may mistakenly increase the immunosuppressive therapy of the underlying disease). Because, as mentioned earlier, colonoscopy is an invasive, difficult-to-accept by the patient, expensive method, the research has focused on finding non-invasive markers that can differentiate organic from functional damage.Within this context, the present study aimed to analyze the role of FC in differentiating between IBD and IBS (Dranga et al, 2017).

Material and method. This study was a prospective one, conducted over a year (2016), and included 2 groups of patients: 58 patients with IBD - CD were compared with 46 patients with IBS. For all patients, we determined the following parameters: haemoglobin, hematocrit, red cell indices, leucocytes, platelets, ESR, fibrinogen, CRP, total protein, serum albumin. Colonoscopy was used for certify the diagnosis of IBD and to exclude other conditions in patients with IBS. The FC was determined through the semiquantitative rapid test prior to colonoscopy, as we have described in the prior study (1.2.2.1).

Results.The two groups were matched in order of gender, age and area of origin so there were no significant differences between the two groups. The patients in the IBS group were predominantly female, while the IBD group was predominantly male (61.7% vs. 58.3%; p=0.068). The mean age was higher in the IBS group, compared to the age recorded in the IBD group (49.22 vs. 1.23 years; p=0.053). The abdominal pain was recorded in approximately two thirds of the patients in both groups. 86% of IBD patients and 65.9% of IBS patients had diarrhoea.

Analysing the biological parameters, the significant differences between the two groupswere found for: serum albumin, inflammatory markers (ESR, CRP) and FC (Table 1.2).

Table 1.2. Average values of the laboratory markers compared on the study samples

Biological marker	IBS group (n=46) average±SD (extreme)	CD group (n=58) average±SD (extreme)	P values for FANOVA test
Serum albumin (g/1)	47.64±5.19 (30.66-58.40)	37.87±8.72 (19-54.9)	**0.001**
ESR (1 mm/h)	3.75±2.65(1-14)	20.26±18 84 (1-89)	**0.001**
CRP (mgdl)	0.59±0.46(0.04-1.62)	3.0±5.78 (0.04-25.61)	**0.001**
Calprotectin, n (%) TI T2 T3	 45 (95.7%) 1 (4.3%) -	 36 (66.0%) 12(20.8%) 8 (13.2%)	**0.001**

The analysis of the Receiver Operating Characteristic (ROC) curve pointed to a sensitivity of 54.38% and a specificity of 50.56% for albumin. ESR sensitivity was 54.87% and ESR specificity was 55.12%, while the values for CRP were 73.77% in sensitivity and 64.87%

specificity, when distinguishing between IBS and IBD. The best values for sensitivity and specificity were those recorded for FC, namely 96.54%, and 95.83% (Table 1.3, Fig. 1.3).

Table 1.3. Balance sensitivity/specificity for serum albumin, ESR, CRP and FC in patients with CD

Biological marker	VPP (%)	VPN (%)	Sensitivity (%)	Specificity (%)	Accuracy	P
Albumin	66.80	40.84	53.38	50.56	52.47	0.001
ESR	95.74	50.19	55.17	54.42	55.00	0.001
CRP	95.74	69.81	74.27	63.67	69.32	0.001
Calprotectin	95.74	46.79	86,54	95.83	96.19	0.001

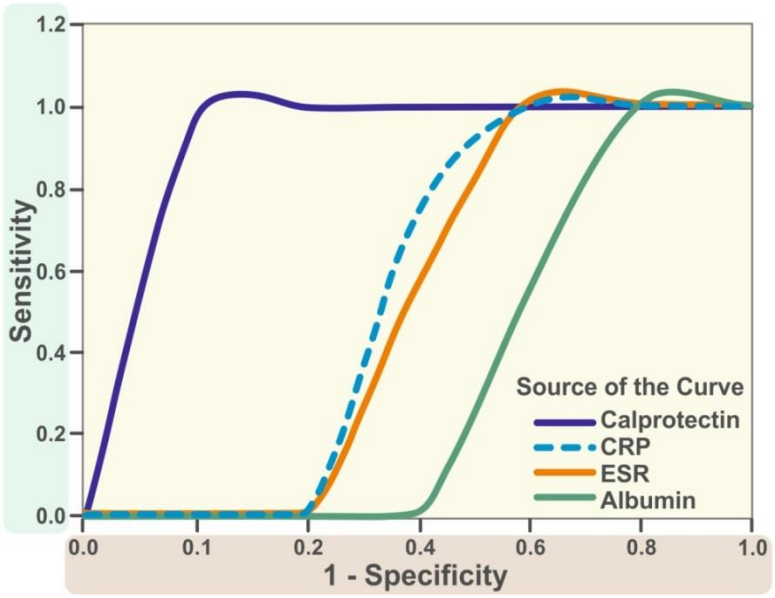


Fig. 1.3. ROC curve for serum albumin, ESR, CRP and FC levels in patients with CD

Discussions. At the time of publication of our study, there was evidence in the literature on the value of FC in differentiating IBD from IBS. A meta-analysis of 30 prospective studies has demonstrated that FC sensitivity was 95% and the specificity was 91%, with an ROC under the curve of 0.95 for a cut-off value of 100 µg/g (von Roon et al, 2007). Menees´s meta-analysis showed that CRP and calprotectin of ≤0.5 mg/dl or 40 µg/g, respectively, essentially excludes IBD in patients with IBS symptoms (Menees et al, 2015).All of these studies are based on the Elisa method for the determination of FC. The merit of our study was the use of a semi-quantitative test, accessible, with immediate results, which can be used in the outpatient setting.

Conclusions. The immunochromatographic rapid test for FC turned to be a rapid, easy to conduct test and well-performing in differential diagnose between IBS and CD. Its use in clinical practice may reduce the number of colonoscopies.

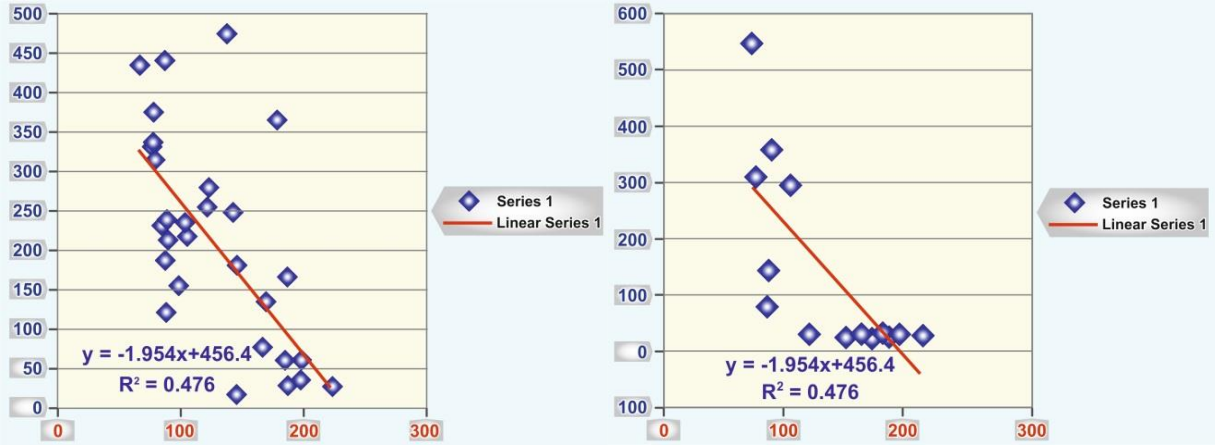
1.2.2.3. *Quality of life and fecal calprotectin in inflammatory bowel diseases*

***Background&Aim*.**Numerous studies have shown the value of FC - as a non-invasive marker of IBD activity (Sipponen, Kolho, 2015). On the other hand, the quality of life (QoL) of

the patient is a complex concept, in which the aspect of physical health plays a decisive role. Our study aimed to correlate FC - as a marker of endoscopic activity, with QoL scores, assessed by means of the IBDQ-32 questionnaire, one of the most widely used tools in assessing QoL in patients with IBD.

Material and methods.The prospective study included 60 patients with IBD, 19 with CD and 41 with UC. Fecal calprotectin was determined by the Elisa method. Ulcerative Colitis Disease Activity Index (UCDAI) and Crohn's Disease Activity Index (CDAI) scores were used to assess disease activity. Quality of life was evaluated using the IBDQ-32 questionnaire.The questionnaire contains 32 questions grouped into four fields: bowel symptoms (diarrhea, abdominal pain, rectal bleeding, and urgency), systemic symptoms (fatigue, sleep disorders), emotional functions (depression, irritability, anger) and social functions (absenteeism, affected social status, sexual activity). The answers were marked on a scale from 1 (the worst) to 7 (the best). The total score ranged between 32 and 224. The lowest the score, the most affected the QoL.

Results.There was correlations between disease activity and QoL scores. Also, the value of FC was significantly statistically correlated with QoL in IBD, both in UC and CD (Fig. 1.4 a, b); however, the QoL correlation with disease activity scores (UCDAI and CDAI) was stronger compared to the correlation with FC (Gavrilescu et al, 2018).



a b

Fig. 1.4. Correlations between QoL assessed by means of IBDQ-32 and the endoscopic activity of UC (a) and CD (b) assessed by means of FC

Discussions. In our study, IBDQ-32 values ranged from 67 to 207, with a mean value of 137.42. In a previous study by our team (Gavrilescu et al, 2015), performed on a group of 254 patients (187 with UC and 67 with CD), the IBDQ value was 134.74 ± 32.52 (66-200) for UC and 138.82 ± 31.84 (70-200) for CD, correlating with the activity of the disease expressed by UCDAI and CDAI scores (clinical remission improves QoL).Of the 4 subscales of the IBDQ score, the scores highlighting the emotional and social component were significantly more reduced in the patients with UC, while the scores for the bowel symptoms were significantly more reduced in the patients with CD. It has been proven in the literature that the severe activity of the disease influences negatively the QoL, both in the patients with CD and in those with UC (Zhou et al, 2010; Romberg-Camps et al, 2010; Casellas et al, 2005). The merit of our study is demonstrating the link between FC and QoL, even if, predictably, clinical symptoms influence QoL to a greater extent. However, the study is an argument for using mucosal healing as a therapeutic target, beyond simple clinical remission.

Conclusions. In our study, the activity of the disease had a negative impacton the QoL in the patients with IBD. We found significantnegativecorrelations between the activity of the disease evaluated by UCDAI and CDAI and the QoL both in UC and in CD. Also, the endoscopic activity evaluated by FC correlates with the QoL.

1.2.3. Correlations between inflammatory biomarkers and activity scores in inflammatory bowel disease

Background & Aim. The assessment of IBD is challenging and complex. In current clinical practice the most commonly used severity scores are the CDAI (Crohn's disease activity Index) for CD and the UCDAI - Ulcerative Colitis Disease Activity Index for UC. Although their use and validity has been established over time, there is evidence that these well established scoring systems based especially on clinical symptoms do not correlate with inflammation or mucosal healing. Inflammatory biomarkers provide information regarding the activity of the disease and are widely accepted because of their non-invasitivity. In IBD there is no single best marker of disease activity. The most commonly used markers are the acute phase reactants: CRP, ESR, fibrinogen, ferritin, platelets and albumin. These are accessible, cheap, non-invasive, but have a reduced sensitivity and specificity. As I stated in previous studies, FC demonstrated its ability to differentiate IBD from IBS, as well as assess disease activity, facilitates prognosis, predicts mucosal healing, response to therapy, need of surgery (Khaki-Khatibi et al, 2020). Our study aimed to correlate IBD disease activity as determined by the CDAI and Mayo scores with inflammatory biomarkers in patients admitted to a tertiary referral centre in North-Eastern Romania.

Material and Methods. A prospective study was performed in 196 (48 with CD and 148 with UC) IBD inpatients at the Institute of Gastroenterology and Hepatology in Iasi, Romania, in a two years period (Mihai et al, 2018). All patients had a confirmed diagnosis of IBD. The following inflammatory parameters were analysed: platelets, ESR, fibrinogen, CRP, ferritin, albumin. FC was assayed using a immunochromatography semiquantitative method (CalDetect, Sofar) and categorised as follows: T1 < 15 µg/g, T2: 15-60 µg/g, T3> 60µg/g. The severity of the disease flare in CD was determined according to the CDAI score, whilst for UC using the UCDAI scoring system. The correlation of laboratory findings with disease severity was performed using all the episodes of disease flare requiring inpatient admission.

Results. In CD patients disease severity had a statistically significant correlation with platelets, ferritin, fibrinogen, CRP, FC but not with ESR and albumin (Table 1.4). The mean CRP was 7.11 mg/dL in patients with severe disease compared to 0.61mg/dL in those with remission. Similarly, the fibrinogen was 5.35 g/L in patients with severe disease compared to 3.5 g/L in those with remission. Regarding FC 86% of patients with a severe flare had a raised FC, being similar in T2 and T3; in contrast, 75% of patients in remission had a low FC level (T1).

In UC patients there were a statistically significant correlation with platelets, fibrinogen, ferritin but not with ESR, CRP and albumin (Table 1.5). The strongest correlation was found between disease severity and FC. Thus, 93.1% of patients in remission had a reduced FC level (T1), whilst 95.1% of those with severe forms of the disease had a T3.

Discussions. In CD patients, CRP is considered a powerful serum marker of inflammatory activity, which has been confirmed not only by our study, but also the existing literature (Vermeire et al, 2006).Moreover than that, many authors consider CRP not only an indicator of inflammation but an independent biomarker predicting response to biologic therapy, low Infliximab levels, risk of relapse (Hibi et al, 2014). Despite this, there were patients with a normal CRP and increased disease activity, as well as patients with a raised

CRP and inactive disease, consistent with other published studies (Jones et al, 2008). We founded a positive correlation between FC and CDAI score, although almost a third of patients in clinical remission had significantly raised FC levels (T2 and T3), which suggest the possible ongoing presence of intestinal inflammation even in those in remission. Data from the literature on the correlation of FC with CDAI score and mucosal healing in CD are still contradictory (D'Haens et al, 2018; D'Incà et al, 2008).

Table 1.4. Correlations between inflammatory markers and CDAI in CD patients

Parameters	CDAI < 150	CDAI 150-450	CDAI > 450	P
Platelets (10^3/µL)	271.413(±46 194)	345 666(±135.297)	417.857(±116 869)	0.005
ESR (mm/1h)	15.24(±11 34)	35 22(±31 09)	30 00(±20.44)	0.059
Fibrinogen (g/L)	350.48(±45.35)	444.50(±101.97)	535(±64 81)	0.000
CRP (mg/dl)	0.61(±1)	4.22(±5.86)	7.10 (±10.16)	0.000
Ferritin(ng/ml)	84 44(±61.25)	113 87(±199 99)	921.31(±1858 52)	0.029
Albumin (g/dl)	24 83 (±22.62)	27 93(±20.57)	16 77(±15 84)	0.06
Faecal calprotectin T1 T2 T3	 21 5 3	 11 19 4	 1 3 3	0.001

Table 1.5. Correlations between inflammatory markers and UCDAI in UC patients

Parameter	MAYO 0 -2	MAYO 3 -6	MAYO 7 -10	MAYO > 10	P
Platelets (10^3/µL)	229.821(±85 857)	295.947(±117.002|	293.241(±84.101)	387 500(±163 562)	0.000
ESR (mm/1h)	19.00(±14.85)	16.71(±16.66)	2867(±23.41)	34 74(±32 89)	0 06
Fibnnogen (g/L)	350 00(±57 66)	351.19(±63 32)	412.42(±90.43)	406.18(±106.16)	0.014
CRP (mg/dl)	1.00(±1.97)	2 78(±8 81)	3.13(±5 89)	7 65(±18.04)	0056
Ferritin(ng/ml)	62.66(±71.23)	65.60(±66.06)	91.52(±82.14)	172 85(±127 98)	0.011
Albumin (g/dl)	31.85 (±29.76)	35.80 (±30.45)	31.84(±29.47)	34.96(±28.04)	0.70
Fecal calprotectin T1 T2 T3	 27 1 1	 9 18 12	 7 30 23	 0 2 39	0.000

The inflammatory markers in UC patients had mean values similar to those in CD. Fibrinogen, ferritin and platelets had a statistically significant correlation with disease severity, the strongest association being with thrombocytosis. Although the mean CRP level was greater in patients with UC than those with CD, it did not correlate with disease severity. This proves that CRP is a useful marker of disease activity in CD, but less so in UC – this has also been mentioned by other sources (Ricanek et al, 2011). A possible explanation would be the lower levels of IL-6 in UC compared to CD, as well as the transmural inflammation seen in CD but

not in UC (where inflammation occurs at a mucosal level) (Vermeire et al, 2006). Similar to other studies, we found that there was a stronger correlation between FC and disease activity in UC than in CD.

Conclusions. Our study demonstrated that is a good correlation between serologic inflammatory markers (platelets, fibrinogen and ferritin, not ESR and albumin) and severity of IBD. CRP is a good marker in CD but not in UC. FC is the best inflammatory biomarker which correlates with activity both in UC and CD. Further studies are needed in order to find the ideal biomarker (non-invasive, cheap, with good sensitivity and specificity) to estimate severity, disease course and response to treatment among individualized patients with IBD.

1.2.4. The role of combining biochemical markers in assessing the endoscopic activity in ulcerative colitis

Background & Aim. Following the study presented above (Mihai et al, 2018), we tried to combine serum markers with FC to find a non-invasive biological score correlated with endoscopic activity in patients with UC.

Material and methods. A prospective study was conducted on 114 patients with UC (Dranga et al, 2018). There were included patients with confirmed diagnosis of UC, both in periods of activity and in remission, which had colonoscopy. Before colonoscopic examination, venous blood was taken to assess laboratory parameters: platelets, ESR, fibrinogen, CRP, seric albumin. FC was measured by Elisa method. For the endoscopic activity evaluation, we used Mayo endoscopic score, considering Mayo score 0 or 1 remission and > 1 activity. Statistical analysis was performed in SPSS 18.0, with nominal significance defined as $p<0.05$. Continuous variables were described using ANOVA test. Relations between laboratory markers and Mayo score were reported by Pearson correlation coefficients. ROC curves were analyzed to assess the optimal cut-off values of markers. Sensitivity, specificity, positive predictive value and negative predictive value were calculated in 95% confidence intervals for this cut-off value. The multiple linear regression analysis was used for the score.

Results. The analysis of serological inflammatory parameters demonstrated significant statistical correlations between CRP, ESR, fibrinogen and endoscopic activity. On the contrary, platelets and albumin did not correlate with endoscopic activity.The strongest statistical correlation was recorded between FC and Mayo endoscopic score ($p=0.001$). For each parameter we analyzed positive predictive value, negative predictive value, sensitivity, specificity, accuracy at the following cut-off values: ESR 15 mm/1h, CRP 5 mg/L, fibrinogen 340.5 mg/dl, FC 200 μg/g. We found that FC had the best predictability for endoscopic activity (area under the ROC curve 96.9%), followed by PCR (62.2%), ESR (59.7%) and fibrinogen (59.3%) (Table 1.6). The model of multiple correlations shows inter-correlations between parameters: FC, CRP, ESR and fibrinogen, being significant from a statistical point of view, facilitated the elaboration of predictability score for endoscopic inflammation.

Score of endoscopic inflammation (SEI) (Mayo > 1) = 1 (ESR > 15 mm/1h) x 0.305+ 1 (fibrinogen > 340.5 mg/dl) x 0.309 +1 (CRP > 5 mg/dl) +1 (FC>200 μg/g).

The SEI calculated on the basis of higher cut-off value of markers: ESR, fibrinogen, CRP and FC of our patients proved a good predictability (86.41%) of Mayo activity with 85.08% accuracy. The ROC curve analysis revealed that the sensitivity and specificity of calculated SEI and FC are superior to ESR, fibrinogen and CRP but without significant differences between FC and calculated SEI (Fig.1.5).

Table 1.6. Predictive values, sensitivities, specificities and accuracy for CRP, ESR, fibrinogen, FC and calculated score of endoscopic inflammation for active endoscopic disease in UC

Marker	PPV (%)	NPV (%)	Sensitivity (%)	Specificity (%)	Accuracy	P
CRP	63.7	76.2	62.5	62.06	62.2	0.089
ESR	57.1	34.8	62.1	51.0	597	0.105
Fibrinogen	62.5	56.5	64.0	50.0	59,3	0050
FC	73	91.1	96.05	83.78	96 9	0.001
Calculated score	76.3	71.05	86.41	81.81	85.08	0.001

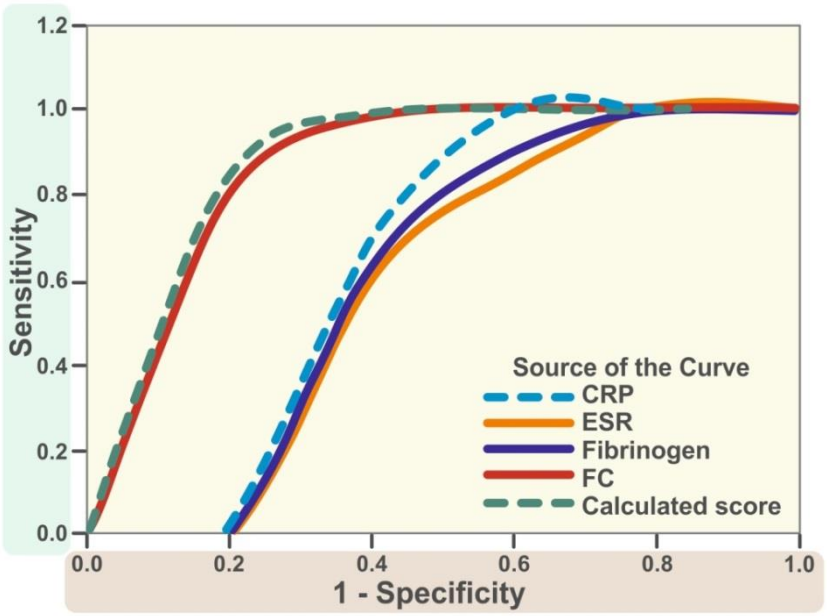


Fig. 1.5. The ROC curve analysis on the abilities of CRP, ESR, fibrinogen, FC and calculated score to make a difference between endoscopic active UC and inactive UC.

Discussions. Our study, similar to the previous ones, showed significant correlations between serological inflammatory markers (fibrinogen, CRP, ESR, not platelets and albumin) and endoscopic activity. FC level over 200 µg/g showed the greatest sensitivity (96.05%) and specificity (83.78%). Nevertheless, 27% of cases, although they had levels of calprotectin under the established cut-off value had endoscopic activity. 8.9 % of patients, although with high levels of FC, were in endoscopic remission. The ROC curve analysis reveals significantly higher values compared to the rest of the parameters analyzed. The efficacy of endoscopic discrimination between endoscopic active and inactive UC assessed by the area under the ROC curve was 0.969. Our data are in agreement with numerous studies in literature which demonstrates the positive correlation of FC with the endoscopic activity of UC, although there are variable cut-off values (Lin et al, 2014). We used the linear regressive method to see if there were correlations between the noninvasive biological markers, trying to find a score with a better diagnosis accuracy for Mayo score. From the literature of the domain, we have found that, up to now, there is no score made up exclusively of quantifiable biological markers, for

estimating endoscopic activity. Interesting, adding the clinical activity index to the FC, Yonn et al. showed higher AUC (0.980) for estimating endoscopic activity in UC (Yoon et al, 2014). Our score, which uses cut-off values of fibrinogen, ESR, CRP, FC, has a positive predictive value, 3.3% greater than that of FC. In spite of this, the score values occurred under the sensitivity and specificity values of FC, because of low sensitivity and specificity of serological inflammatory markers.

Conclusions. It was the first attempt to make a score made up exclusively of accessible biological markers, used in current clinical practice up to now. The obtained score, although had the greatest positive predictive value in comparison with each of the studied biomarkers, was inferior to FC regarding sensitivity, specificity and accuracy. Further studies are required, which should identify a non invasive biomarker (or that combination of biomarkers), with high sensitivity and specificity, with reduced variability, replicable, that should be accessible for a proper evaluation of endoscopic activity in UC.

The main points regarding researches in the field of non-invasive biomarkers in IBD patients are presented in Table 1.7.

Table 1. 7. Main points in IBD biomarkers researches

SCIENTIFIC/CLINICALRELEVANCE
• The immunochromatographic semiquantitative rapid test for FC turned to be a rapid, easy to conduct and well-performing test in IBD patients
• FC is a good marker for endoscopic activity assessment in ulcerative colitis patients
• FC can differentiate CD from IBS patients with a very good accuracy
• FC correlates with quality of life, both in UC and CD patients
• It is a good correlation between serologic inflammatory markers (platelets, fibrinogen and ferritin, not ESR and albumin) and severity of IBD. CRP is a good marker in CD but not in UC. FC is the best inflammatory biomarker which correlates with activity both in UC and CD
• Our score of endoscopic inflammation, made up exclusively of accessible biological markers, had the greatest positive predictive value in comparison with each of the studied biomarkers, but was inferior to FC regarding sensitivity, specificity and accuracy.

1.3. THE ROLE OF ARTIFICIAL INTELLIGENCE IN THE EVALUATION OF IBD PATIENTS

1.3.1. State of the art

Artificial intelligence (AI) has increasingly found its place in modern medicine, with multiple applications in various fields of activity, trying to improve the accuracy of diagnosis, to estimate the prognosis and response to treatment (Le Berre et al, 2020). Artificial neural networks are inspired by the neuroanatomy of the human brain, consisting of interconnected units, similar to neural synapses. Each network has an input layer (which includes demographic, biological, imaging data, etc.), an output layer (the task to be performed by the network as a binary with 2 variables – example: present/absent) and one or more hidden layers(Popa IV et al, 2017).

In a first stage, the training of the artificial neural network takes place, which involved the introduction of data and the "training" of the network, so that, when new data are introduced, the desired output is obtained. The development of increasingly complex neural networks has led to the concept of "deep learning" (LeCun et al, 2015). Most models use two rows of data: one for learning and the other for testing and validation. Learning can be supervised or unsupervised(Gubatan et al, 2021). Supervised learning involves a predefined output; the model learns to analyze the data entered in relation to the established output; after the association has been learned, the model will be able to analyze newly introduced data. In unsupervised learning, the computer learns to associate introduced data unrelated to a predefined event, which allows the identification of unknown predictors (Gubatanet al, 2021).

AI has been used, with remarkable results, in multiple fields of gastroenterology:

- Analysis of malignant and premalignant lesions in the digestive tract (polyps, Barret's esophagus, esophageal cancer, gastric cancer, colorectal cancer); most used endoscopic images as input and obtained a diagnostic accuracy of over 80% (Wang et al, 2019; Liu et al, 2016);
- Assessment of the prognosis and response to treatment in digestive neoplasms (Lee et al, 2018; Peng et al, 2016);
- Diagnosis of patients with celiac disease (Hujoel et al, 2018);
- Detection of digestive hemorrhage in images obtained through the video capsule or assessment of the risk of hemorrhagic recurrence(Hassan, Haque, 2015; Wonget al, 2019);
- Detection of pancreatic carcinoma based on serum markers or echoendoscopy(Yang et al, 2014; Săftoiu et al, 2015);
- Assessment of the severity and prognosis of acute pancreatitis(Hong et al, 2013);
- Identification of patients with choledocholithiasis who need endoscopic therapy to extract stones(Jovanovic et al, 2014);
- Diagnosis of non-alcoholic fatty liver disease (Yip et al, 2017);
- Assessment of hepatic fibrosis in chronic viral hepatitis (Wang et al, 2019; Wei et al, 2018);
- Prognostic factors of patients with liver cirrhosis(Konerman et al, 2017);
- Evaluation of the quality in endoscopy (cecal intubation, following guidelines) (Sinonquel et al, 2021).

In IBD, where, as we pointed out in the previous chapter, the need to identify non-invasive markers is imperative, AI has found many areas of applicability: etiopathogenesis - genetic risk factors for IBD (Wei et al, 2013), endoscopic diagnosis (Girgis et al, 2010; Takenaka et al, 2020), detection of dysplasia (Maeda et al, 2020),prognostic factors of

evolution(Waljee et al, 2017), prognostic factors in response to certain therapies (Waljee et al, 2017; Waljee et al, 2018). The need for precision medicine in IBD has led to the accumulation of extensive databases that include clinical elements, genomics, proteomics, transcriptomics, and metagenomics data, endoscopic, radiological and histological images. AI, through its technological advances has increased the capacity of storage, processing, interpretation and analysis of "BIG DATA" (Seyed et al, 2020). A recent review of AI in IBD (Gubatan et al, 2021) identified 58 studies, of which 50 have been published in the last 5 years.

However, there are many limitations to the use and validation of AI models in current clinical practice. The main questions that any clinician should ask themselves regarding studies related to AI, learning machines, neural networks are related to the accuracy and number of introduced variables, the clinical value of the prediction, the methods used for training and testing, performance, repeatability and model reproducibility (Carleton, Thakkar, 2020). Regardless of any prediction, a patient must be diagnosed and treated according to preferences, ethical and legal aspects, the possibility of rapid incorporation of new scientific discoveries (Le Berre et al, 2020). However, AI is already part of modern medicine and many studies has been shown its diagnostic and prognostic value.

Starting from these new data, another direction that we have developed in recent years has been the use of AI in the IBD evaluation. These researches were possible due to IBD Prospect (national register of all IBD patients hospitalized). This electronic register allowed access to a very large number of medical records, which were set up as support learning and training of neural networks. Dr. Iolanda Popa – one of our research team member – has graduated the Faculty of Informatics and has initiated all this studies.

In this field of research I have published 3 ISI articles and 1 other international database article.

1. Popa IV, Burlacu A, Gavrilescu O, Dranga M, Prelipcean Cijevschi C, **Mihai C**. A new approach to predict ulcerative colitis activity through standard clinical-biological parameters using a robust neural network model. *Neural Computing & Applications* 2021: 10.1007/s00521-021-06055-x IF 5.606.
2. Popa IV, Diculescu M, **Mihai C**, Prelipcean Cijevschi C, Burlacu A. Developing a Neural Network Model for a Non-invasive Prediction of Histologic Activity in Inflammatory Bowel Diseases. *Turkish J Gastroenterol* 2021; 32(3): 276-286 IF 1,852
3.Popa IV, Burlacu A, **Mihai C**, Cijevschi Prelipcean C. A Machine Learning Model Accurately Predicts Ulcerative Colitis Activity at One Year in Patients Treated with Anti-Tumour Necrosis Factor α Agents. *Medicina* 2020; 56: 628 IF=1,205
4. Popa IV, Dranga M, Gavrilescu O, Popa RC, Cardoneanu A, Cucuteanu B, CijevschiPrelipcean C, **Mihai C**. Artificial neural networks - a new approach in non-invasive monitoring of inflammatory bowel diseases. *The Medical-Surgical Journal* 2017; 121(4): 695-700.

1.3.2. Artificial neural networks - a new approach in non-invasive monitoring of inflammatory bowel diseases

Background & Aim.The first research on artificial intelligence in IBD was conducted in 2017. As we demonstrated in the chapter on biomarkers, there is currently no non-invasive, accessible, repeatable marker or combination of markers to assess patients with IBD. In recent years, artificial neural networks have been successfully introduced for various clinical contexts. The artificial neural network model has proved to be more accurate and with better

performances than multiple logistic regression (Zheng et al, 2013; Liu et al, 2013; Baxt, 1995; Wang et al, 2010). In this study (Popa IV et al, 2017), we tried to investigate the possibility of establishing a complex neural correlation between the various biological and historical data of the disease and IBD activity.

Matherial and methods. The study group included 100 patients aged 18-80 years, diagnosed with UC (59%) and CD (41%) based on clinical, biological, imaging and histopathological criteria. Study database contains multiple parameters obtained through: anamnesis and disease history considering environmental factors and behaviors (smoker/non-smoker, ethanol consumption, medication, alimentary behavior); physical exam and evaluation of nutritional status (body mass index); laboratory tests; imaging investigations (lower gastrointestinal endoscopy, computed tomography – CT- scan). In this study, we built the neural network using Matlab 7.0. Active parameters for the input layer (i.e. those with the highest estimation power), were determined using statistical methods (t-student and chi² tests). Statistical significance was set to $p = 0.05$. Therefore, input layer contains 25 neurons that are: - clinical: smoker status; disease duration; number of kilograms lost in the last three months; presence of asthenia; clinical data from the scores used in the output layer (UCDAI, Rachmilewitz etc.) - biological: white blood cells count (WBC); hemoglobin (HGB); hematocrit (HCT); platelet count (PLT); red cell distribution weight (RDW); ESR; fibrinogen; cholesterol; iron; ferritin; CRP; protein electrophoresis. The output layer consists of the following scores: Ulcerative Colitis Disease Activity Index (UCDAI); Rachmilewitz; Ulcerative Colitis Endoscopic Index of Severity (UCEIS); Geboes (histological score); Crohn's Disease Endoscopic Index of Severity (CDEIS); Simple Endoscopic Score for Crohn's Disease (SES-CD). The accuracy of the mathematical model was measured in terms of mean square error (MSE) and mean absolute percentage error (MAPE). MSE was calculated based on the desired and estimated value and then the average for all data was determined. It was used as a good fit indicator of the model. MAPE indicates the mean deviation from the desired value and is usually expressed as a percentage. The precision of a model is considered excellent if MAPE value is less than 10%.

Results. In this preliminary study the results of MSE and MPAE for the evaluated scores are presented in Table 1.8.

Table 1.8. Calculated MSE and MAPE values for IBD scores

	MSE	MAPE
UCDAI	0.092	52.34%
Rachmilewitz	0.065	47.15%
UCEIS	0.212	65.37%
GEBOES	0.342	70.23%
CDEIS	0.156	62.48%
SES-CD	0.301	69.52%

Discussions. None of the scores were estimated with excellent or high precision. The Rachmilewitz score was estimated with average accuracy, although with a value close to the medium range upper limit. The lack of significant results is because the network included currently few registered patients. Neural networks need hundreds, up to thousands of records to deliver significant results.

Conclusions. These preliminary results on a small group of patients created the premises for further research on the role of neural networks in estimating endoscopic and histological scores in IBD.

1.3.3. A new approach to predict ulcerative colitis activity through standard clinical-biological parameters using a robust neural network model

Background&Aim. Non-invasive estimation (in the absence of colonoscopy) of endoscopic activity in UC is a challenge for clinical practice, with important prognostic and therapeutic consequences. The present study aimed to create a neural network model capable of predicting the endoscopic activity of patients with UC based on routine clinical-biological parameters, commonly used. The model created was subsequently validated on a group of patients and, in addition, an attempt was made to create a categorical model that would estimate each value of the Mayo score.

Material and methods. An observational retrospective single-center cohort study was conducted on a sample of 386 UC patient records (Popa IV et al, 2021) . Documented clinical parameters were: age, gender, smoking status, number of stools/day and presence of diarrhea, tenesmus, lower gastrointestinal bleeding (LGB), abdominal pain, weight loss, asthenia and pallor. Smoking status was a categorical variable with three possible values: 0—smoker, 1—non-smoker and 2—former smoker. Several stools/day was represented as a continuous variable. The presence of diarrhea, tenesmus, LGB, abdominal pain, weight loss, asthenia and pallor were represented as binary categorical variables (1 indicating presence and 0—absence of referred symptom). Laboratory parameters documented were: red blood cells (RBC), white blood cells (WBC), platelets (PLT), hemoglobin (HGB), hematocrit (HCT), plateletcrit (PCT), platelet distribution width (PDW), mean platelet volume (MPV), platelet large cell ratio (PLCR), neutrophils (NEUT), lymphocytes, monocytes (MONO), C reactive protein (CRP), erythrocyte sedimentation rate/1h (ESR), fibrinogen, serum iron (SI), ferritin, total proteins (TP), albumin, alpha 1 globulins (A1G), alpha 2 globulins, beta 1 globulins, beta 2 globulins, gamma globulins, glucose. According to the endoscopic Mayo score, the colonoscopic findings were represented as a categorical variable with four possible values (from 0 to 3); a patient was considered to have endoscopic remission if the Mayo score was 0 or 1. Initial data (356 patient records) were randomly divided into a training set of 285 records (80%) and a test set of 71 records (20%) such that variables distributions in each set were similar to those in the original dataset. Other 30 patient records from the same medical center were added independently to be used as a validation set. Three multilayered perceptron classifiers were developed based on the training set. The first two classifiers were used to predict whether a UC patient has endoscopic activity or remission based on all 20 parameters chosen by the feature selection method (first classifier) or based only on biological parameters (second classifier). The second classifier was built as it is of interest to construct and evaluate a model based only on objective data. The third classifier was used for the prediction of the Mayo score based on all 20 parameters. The first two classifiers have a binary output, while the third has a categorical output with four possible values (Mayo score from 0 to 3). Area under the receiver operating characteristic curve (AUC), sensitivity (SE), specificity (SP), positive and negative predictive values (PPV and NPV) were determined for each model.

Results. Selected clinical characteristics and laboratory findings for all patient records, and each of the Mayo classes are summarized in Table 1.9 .

Table 1.9. Clinical and biological parameters for all patient records and each Mayo group

	All	Mayo 0	Mayo 1	Mayo 2	Mayo 3
Number of records	386	66	65	189	66
Gender (male:female)	257:129	43:23	38:27	122:67	54:12
Age (years)	44.8 ± 13.9	43.6 ± 12.8	44.6 ± 12.1	44.4 ± 14.4	122 ± 14.9
Number of stools/day	4.9 ± 3.8	1.3 ± 0.8	2.9 ± 2.27	5.6 ± 3.4	8.8 ± 4
LGB	257 (66.6%)	1 (1.5%)	24 (36.9%)	168 (88.9%)	64 (97%)
Diarrhea	265 (68.7%)	6 (9.1%)	24 (36.9%)	169 (89.4%)	64 (97%)
Tenesmus	184 (47.7%)	0	12 (18.5%)	121 (64%)	51 (77.3%)
Abdominal pain	226 (58.5%)	10 (15.2%)	23 (35.4%)	140 (74.1%)	53 (80.3%)
Weighl loss	118 (38.6%)	2 (3%)	7 (10.8%)	70 (37%)	39 (59.1%)
Asthenia	210 (54.4%)	7 (10.6%)	23 (35.4%)	133 (70.4%)	47 (71.2%)
Palier	97 (25.1)	1 (1.5%)	8 (12.3%)	59 (31.2%)	27 (40.9%)
WBC *10^3/μL	8.2 ± 3.2	6.6 ± 1.6	7.4 ± 2.3	8.4 ± 3.4	10.1 ± 3.8
HGB g/dL	13.3 ± 2.1	14.3 ± 1.5	13.7 ± 1.6	13 .2 ± 2	12.4 ± 2.6
RBC *100^3/μL	4.7 ± 0.5	4.9 ± 0.4	4.8 ± 0.4	4.7 ± 05	4.4 ± 0.7
PLT *10^3/μL	311.2 ±111.6	252 ±52.1	269.2±95.7	318.1 ±87.2	392.3± 167.1
MONO *10^3/μL	0.68 ± 0.34	0.52 ±0.15	0.56 ± 0.17	0.68 ± 0.32	1 ± 0.45
PDW fl	12.3 ± 2.2	13.1 ± 1.6	13.2 ±2.9	12.1 ± 2	11.7 ± 1.9
ESR mm/lh	15.83 ± 19 6	5.3 ± 5.7	9.) ± 11.6	16.3 ± 15.4	32.9 ± 32
Rbnnogen mg/dl	388.1 ±83	338.1 ±74.2	359.5±563	394.7±68.7	465.1 ±100.2
CRP mg/dl	1.61 ± 3.4	0.3 ± 0.4	0.4 ± 0.4	1.3 ± 23	5.3 ± 6.1
SI μg/dl	67.3 ± 40.8	88.6 ± 34.3	82.6 ± 41	62.3 ± 40.5	41.6 ± 28.4
TP g/dl	7.4 ± 0.7	7.68 ± 0.49	7.65 ± 0.58	7.36 ± 0.67	6.89 ± 0.85
AIG %	2.8 ± 1.2	1.9 ± 0.3	2.3 ± 0.5	2.8 ± 0.9	4.2 ± 1.8

The next step was to identify and reduce selected features that are highly intercorrelated. As a result of the feature selection stage, 13 continuous parameters (WBC, PLT, MONO, ESR, fibrinogen, SI, TP, A1G, HGB, RBC, PDW, CRP, number of stools/day) and seven categorical parameters were included in further analysis.

The initial dataset of 356 patient records was randomly divided into a training set (285 records) and test set (71 records) to build the classifiers. Thirty patient records were added independently to constitute the validation set. As a result of the feature selection step, three NN models were trained using the selected parameters as inputs. All three models were evaluated against several activation functions in order to maximize performance.

The first NN model was developed using all 20 variables to predict whether a patient has endoscopic remission (Mayo 0 or 1) or active endoscopic disease (Mayo 2 or 3). On the train set, the model had an ACC of 92.63% (95% CI, 0.89–0.95; $p < .001$) with an SE of 90.22%, and SP of 93.78%, a PPV of 87.37% and NPV of 95.26% and an AUC of 0.92. On the test set, the model achieved a good performance with an ACC of 94.37% (95% CI, 0.862–0.9844; $p<.001$), an SE of 88%, an SP of 97.83%, a PPV of 95.65%, an NPV of 93.75% and an

AUC of 0.9291. On the validation set, model predictions were similar in performance with ACC 93.33% (95% CI, 0.7793–0.9918; p< .001), SE 92.86%, SP 93.75%, PPV 92.86%, NPV 93.75% and AUC 0.933. The performance of the first NN model is shown in Table 1.10.

Table 1.10. First classifier performance metrics.

Actual	Train set		Test set		Validation set	
	Predictions		Predictions		Predictions	
	Remission	Activity	Remission	Activity	Remission	Activity
Remission	83	12	221		13	1
Activity	9	181	345		1	15
ACC	92.63%		94.37%		93.33%	
95% CI	(0.8896, 0.9538)		(0.862, 0.9844)		(0.7793, 0.9918)	
P value	< .001		< .001		< .001	
SE	90.22%		88%		92.86%	
SP	93.78%		97.83%		93.75%	
PPV	87.37%		95.65%		92.86%	
NPV	95.26%		93.75%		93.75%	
AUC	0.92		0.9291		0.933	

The second NN model was built to predict the same binary outcome (endoscopic remission or activity) as the first classifier using only the 12 biological input parameters in order to investigate a model that uses only objective data. On the train and test set, the model achieved a good performance: ACC 87% (95% CI, 0.8255–0.9069; p < .001), SE 89.13%, SP 86.01%, PPV 75.23%, NPV 94.32%, AUC 0.9084 on the train set and ACC 88.73% (95% CI, 0.79–0.9501; p < .001), SE 88%, SP 89.13%, PPV 81.48%, NPV 93.18%, AUC 0.9191 on the test set. Performance on validation set was rather moderate: ACC 83.33% (95% CI, 0.6528–0.9436; p <.001), SE 78.57%, SP 87.5%, PPV 84.62%, NPV 82.35%, AUC 0.8482 (Table 1.11).

The multiclass predictor (third NN model) was developed using all 20 variables to predict the endoscopic Mayo score. The model's output is a categorical variable with four possible values ranging from 0 to 3. The model had a good performance on the train set with an ACC of 89.44% 8304 (95% CI, 0.8526–0.9276; p < .001) and a multivariate predictor AUC of 0.9541. Model performance was moderate on the test set (ACC 76.06%, CI = 0.6446–0.8539; multivariate AUC 0.8726) and on the validation set (ACC 80%, CI = 0.6143–0.9229; multivariate AUC 0.9175) (Table 1.12).

Discussions.This study was the first NN developed for predicting endoscopic disease activity in UC based on routinely available clinical parameters. We demonstrated that using only standard, non-costly, non-invasive clinical and biological data can differentiate active UC from inactive UC. Our selected biological parameters' clinical relevance is confirmed by numerous studies in which standard inflammatory biomarkers such as WBC, CRP, ESR, fibrinogen, PLT and A1G have already been studied as markers for disease activity in UC (Fengming, Jianbing, 2014). HGB - reflecting both gastrointestinal bleeding and inflammation - has also been found to correlate with endoscopic activity (Miranda-García et al, 2016). Neural models have also included clinical parameters since several studies assessing composite

markers consisting of clinical and biological data have shown superiority over biological data alone in discriminating active and inactive IBD (Dragoni et al, 2021).

Table 1.11. Second classifier performance metrics.

Actual	Train set		Test set		Validation set	
	Predictions		Predictions		Predictions	
	Remission	Activity	Remission	Activity	Remission	Activity
Remission	82	27	225		11	2
Activity	10	166	341		3	14
ACC	87%		88,73%		83.33%	
95% CI	(0.8255, 0.9069)		(0.79, 0.9501)		(0.6528. 0.9436)	
P value	< .001		< .001		< .001	
SE	89.13%		88%		78.57%	
SP	86.01%		89.13%		87.5%	
PPV	75.23%		81.48%		84.62%	
NPV	94.32%		93.18%		82.35%	
AUC	0.9084		0.9191		0.8482	

Table 1.12. Multiclass predictor performance (third classifier).

	Train set				Test set				Validation set			
	Mayo predictions				Mayo predictions				Mayo predictions			
Actual Mayo	0	12		3	0	12		3	0	12		3
0	69	2	1	0	12	1	0	0	8	110		
1	2	65	8	0	3	7	2	1	0	5	10	
2	0	3	58	9	0	123		2	0	0	5	1
3	0	1	4	62	0	1	6	12	0	0	2	6
ACC	89.44%				76.06%				80%			
95% CI	(0.8526, 0.9276)				(0.6446, 0.8539)				(0.6143, 0.9229)			
P value	< .001				< .001				< .001			
Multivariale predictor AUC	0.9541				0.8726				0.9175			

The first classifier proposed in our study used eight clinical parameters and 12 routine biological tests to differentiate endoscopic active UC from inactive UC with a high ACC of 94.37%, and SE of 88%, an SP of 97.83% and an AUC of 0.9291 on the test set and ACC 93.33%, SE 92.86%, SP 93.75% and AUC 0.933 on the validation set. The performance of our model in discriminating endoscopically active UC (Mayo 2 or 3) from inactive (Mayo 0 or 1) proved to be superior compared to FC, the most widely used biomarker in clinical practice (Rokkas et al, 2018).

The second classifier presented in our paper used only the 12 routine biological tests to predict the same binary output that differentiates inactive from active disease with an ACC 88.73%, SE 88%, SP 89.13%, AUC 0.8857 on the test set, and ACC 83.33%, SE 78.57%, SP 87.5%, AUC 0.8304 on the validation set. The results obtained on the test set are similar to the results FC, as is clear from Rokkas's meta-analysis (a SE of 88% in our study vs. a pooled SE of 87.3% for FC in the meta-analysis). However, our classifier obtained higher values for SP (89.13% in our study vs. 77.1% for FC in the meta-analysis). Additionally, a high NPV of 93.18% obtained by the second NN model on the test set could reliably rule out active UC. The performance metrics obtained on the validation set were slightly lower, except for SP, which was higher than the pooled results of FC reported in the meta-analysis. Thereby, the second model could successfully be used in clinical practice, considering that the results of the second classifier (ACC 88.73% on the test set) are roughly similar to the FC performance in detecting active disease. Moreover, considering that our model uses cheaper, more stable, immediately available laboratory tests compare to FC, future research on larger datasets remains of interest for further validation of the proposed classifier.

Third classifier used all 20 parameters to predict the endoscopic Mayo score with an ACC of 76.06% (95% CI, 0.6446–0.8539; $p < .001$) and a multivariate AUC of 0.8726 on the test set and an ACC of 80% (95% CI, 0.6143–0.9229; $p<.001$) and multivariate AUC of 0.9175. To date, there are no studies that aimed to predict the endoscopic Mayo score using only non-invasive biomarkers. Although binary classification into active/inactive disease is of compelling importance, there are studies to suggest that differentiating between endoscopic Mayo 0 and 1 may have a better impact on predicting future outcomes (Barreiro-de Acosta et al, 2016). For this reason, results obtained by the multiclass predictor in our study could open the path for further research in estimating complete endoscopic Mayo subscore by non-invasive ML methods.

Our study had some limitations. Firstly, our dataset's small size and the fact that the independent validation set is from a single center entail rigorous external validation with data from other centers. Secondly, the imbalanced distribution of Mayo classes in the initial dataset of 356 records (with Mayo 2 class containing three times more records than each of the other groups) predisposes to calculation biases. However, the SMOTE function in R was used to reduce these biases significantly. Thirdly, FC was not documented for a direct real-time comparison with the proposed NN classifiers' performance. In the future, these drawbacks could be overcome by employing studies on a larger number of patients in a center with greater accessibility that would permit organizing a cohort with a balanced distribution of Mayo classes. Next trials would improve models' performance by incorporating more ML algorithms and real-time comparisons with the documented FC levels. Further improving and validating automatic learning methods in this area may lead to more frequent monitoring of UC patients, significantly fewer invasive procedures, less exposure to inherent risks and more comfort for the patients. Intensified UC monitoring may lead to the early tracing of subclinical inflammation.

Conclusions **.**Our study has proposed a non-invasive AI/ML – based method to predict UC activity accurately. Our neural network model represents a significant advance in the noninvasive assessment of inflammation in UC, leading to further research and possible future use in clinical practice.

1.3.4. Developing a neural network model for a non-invasive prediction of histologic activity in IBD

Background&Aim. If in the previous study we created an AI model capable of estimating endoscopic activity in UC, this time our goal was even more ambitious, wanting to predict histological remission, both in UC and CD. Recent data from the literature show that histological healing predict better relapses, corticosteroid use, hospitalizations, colectomy rates, compared to mucosal healing (Christensen et al, 2020). We aimed to develop a NN model for non-invasive prediction of histologic activity in IBD using inexpensive, routinely available clinical and biological parameters and assess the model's generalizability by testing it on unseen data, anticipating the future utility in clinical setting and decision-making.

Material and methods. An observational retrospective single-center cohort study was conducted on a sample of 486 (371 UC, 115 CD) patient records (Popa IV et al, 2021). Pre-diagnosed and newly diagnosed confirmed UC and CD patients who underwent a colonoscopy with biopsy for disease assessment were included. According to the European consensus guidelines, UC and CD diagnosis is established by clinical, biochemical, stool, endoscopic, cross-sectional imaging, and histological investigations (Maaser et al, 2019). Documented clinical parameters were age, gender, smoking status, number of stools/day and presence of diarrhea, tenesmus, lower gastrointestinal bleeding (LGB), abdominal pain, weight loss, asthenia, and pallor. Smoking status was represented as a categorical variable with 3 possible values: 0: smoker, 1: nonsmoker, and 2: former smoker. The number of stools/day was recorded as a continuous variable. The presence of diarrhea, tenesmus, LGB, abdominal pain, weight loss, asthenia, and pallor was represented as binary categorical variables (1: indicating presence and 0: the absence of referred symptom). Laboratory markers are the same with those used in the previous study: red blood cells, white blood cells (WBC), platelets (PLT), hemoglobin (HGB), hematocrit (HCT), mean corpuscular hemoglobin concentration (MCHC), plateletcrit (PCT), platelet distribution width (PDW), mean platelet volume (MPV), platelet large cell ratio (PLCR), neutrophils (NEUT), lymphocytes, monocytes (MONO), C reactive protein (CRP), erythrocyte sedimentation rate/h (ESR), fibrinogen, serum iron (SI), ferritin, total proteins (TP), albumin, alpha 1 globulins (A1G), alpha 2 globulins (A2G), beta 1 globulins, beta 2 globulins, gamma globulins, glucose. As to date, there is no validation or standardization of the histological definition of remission in IBD, in our paper, histological healing was defined as the absence of erosions/ulcerations and neutrophils both in the crypts and lamina propria, according to the latest proposed definitions (Chateau et al, 2020). A patient was considered to have histologic remission if no neutrophils were detected in the worst affected biopsy specimen. Similarly, the active histologic disease was considered when erosions/ulcerations were present, or neutrophils were detected in the biopsy sample either in the crypts or in lamina propria. Each bioptic sample in our study was analyzed independently by 2 experienced pathologists, with good expertise in IBD diagnosis. The lack of consensus was resolved by a third independent experienced pathologist.

Initial data of 341 UC patient records were randomly divided into a training set of 273 records (80%) and a test set of 68 records (20%) such that variables distributions in each set were similar to those in the original dataset. The other 30 patient records from the same medical center were added independently to be used as a validation set. Similarly, initial data of 103 CD patient records were randomly divided into a training set of 82 records (80%), and a

test set of 21 records (20%) and other 12 independent patient records from the same center constituted the validation set. Four multilayered perceptron classifiers were developed based on the training sets by dr. Iolanda Popa. Two binary classifiers were built for UC predictions. UC classifiers were used to predict whether a UC patient has histologic activity or remission based on all 14 parameters chosen by the feature selection method (first UC classifier) or based only on biological parameters (second classifier). The other 2 binary classifiers were built for CD predictions. CD classifiers were used to predict whether a CD patient has active or inactive histologic disease based on 2 clinical and 4 biological parameters (first CD classifier) or based on 6 biological parameters (second CD classifier). Classifiers trained only on biological data were built as it is of interest to construct and evaluate a model based only on objective data. Developed NNs were evaluated on the test set and validation set on accuracy (ACC) of classification. Where applicable, the area under the receiver operating characteristic curve (AUC), sensitivity (SE), specificity (SP), and positive and negative predictive values (PPV and NPV) were also determined.

Results. Selected clinical characteristics and laboratory findings for all patient records, and each activity class are summarized in Table 1.13 (UC) and Table 1.14 (CD).

Using ANOVA with Holm adjustment, feature selection for UC initially included 17 continuous variables with a significant difference between active histology group and remission group: number of stools/day, WBC, HGB, HCT, MCHC, PLT, MONO, NEUT, MPV, PLCR, PCT, ESR, fibrinogen, SI, CRP, A1G, and A2G. Significance was established at $p < .0001$. Next, highly intercorrelated features were identified and removed. Four strong correlations with a Pearson coefficient ≥ 0.9 were identified between WBC and NEUT, HGB and HCT, PLT and PCT, MPV, and PLCR. Thus, the following 4 parameters were removed from the analysis: NEUT, HCT, PCT, and PLCR. Feature selection in CD was based on domain knowledge and included 7 continuous variables: number of stools/ day, WBC, CRP, fibrinogen, ESR, PLT, and SI. Considering that the number of CD patient records in our database is much smaller than in the UC dataset, features selected for CD also have to be fewer. All categorical parameters were successively fitted with the histology activity variable using the chi-square test, both in UC and CD. The categorical parameter with the best chi-square test statistic was chosen to be included in the analysis for each disease. LGB was the selected categorical feature in UC ($X2$ (1, N = 371) = 184.54, $P < .001$) and diarrhea in CD ($X2$ (1, N = 115) = 30.684, $P < .001$). As a result of the feature selection stage, 14 parameters in UC (number of stools/day, LGB, WBC, HGB, MCHC, PLT, MONO, MPV, ESR, fibrinogen, SI, CRP, A1G, A2G) and 8 parameters in CD (number of stools/day, diarrhea, WBC, CRP, fibrinogen, ESR, PLT, SI) were included in further analysis. Two NN models were trained for UC predictions and 2 for CD predictions using the selected parameters as inputs.

For UC the initial dataset of 341 patient records was randomly divided into a training set (273 records) and a test set (68 records). Thirty patient records were added independently to constitute the validation set. The first NN model was developed using all 14 variables to predict whether a UC patient has histologic activity or remission. The model had 95.24% ACC in the train set, 95.59% in the test set and 96.67% in the validation set, and AUC 0.971, 0.976, 0.991 respectively (Table 1.15). In the second UC NN model we have used only the 12 biological input parameters in order to investigate a model that uses only objective data. The second model performance metrics are presented in Table 1.16.

Table 1.13. Clinical and biological parameters for all UC patient records and each histologic activity class.

	All	Histology	
		Remission	Activity
Number of records	371	76	296
Gender (male:female)	247:124	46:29	201:95
Age (years)	44.8±14	43.9±12.3	45±14.4
Number of stools/day	5.1 1 3.9	1.4±0.8	6±3.8
LGB	250 (67.4%)	1 (1.3%)	249 (84.1%)
WBC. *10³/µL	8.2 + 3.3	6.5±1.5	8.6±3.4
HGB, g/dL	13.612	14 6± 1.2	13.4±2
MCHC. g/dL	3311.5	336±1.3	32.9±1.5
PLT,. *10³/µL	313.6± 111.7	259.5±78.1	327.3±114.9
MONO, *10³/µL	0.68 ± 0.3	0.5±0.2	0.7±0.4
MPV fl	10.3±1	10.8±0.8	10.1±1.1
ESR, mm/h	15.9±19.8	4.6 13.2	18.9±21.2
Fibrinogen, mg/dL	389.4±1 83.5	336±71.7	405.5±80.2
CRP. mg/dL	1.6±3.4	0.2±0 2	2±3.8
SI, µg/dL	72.5±42	97.3±36.6	66±40.9
A1G, %	2 8±1.2	2±0.3	3±1.3
A2G, %	11.3±2.5	10.2±1.6	11.6±2.6

LGB, lower gastrointestinal bleeding, WBC, white blood cells, HGB, hemoglobin, MCHC, mean corpuscular hemoglobin concentration; PLT, platelets; MONO, monocytes; MPV, mean platelet volume; ESR. erythrocyte sedimentation rate/h; CRP, C reactive protein; SI, serum iron; A1G, alpha 1 globulins (A1G); A2G,

Table 1.14. Clinical and biological parameters for all CD patient records and each histologic activity class

	All	Histology	
		Remission	Activity
Number of records	115	33	82
Gender (male:female)	64:51	20:13	44:38
Age (years)	38.4±11.2	41.8±11.2	37±10.9
Number of stools/day	3.7±3	1.8 ± 1.1	4.5±3.1
LGB	59 (51.3%)	3(9.1%)	56 (68.3%)
WBC. *10³/µL	8.9±3.7	7.1±1.8	9.7±4.1
PLT,*10³/µL	353±145.4	268.8±46.2	385.9±157.4
ESR, mm/h	16.4±18.1	5.1±4.4	20.4±19.5
Fibrinogen, mg/dL	408.3±91.7	351±50	432.6 ± 94.8
CRP. mg/dL	3.1±5.4	0.3±0.2	4.3±61
SI, µg/dL	67±45	108.4±42.8	51.6±35.2

WB, white blood cells; PLT, platelets; ESR. erythrocyte sedimentation rate/h; CRP. C reactive protein; SI, serum iron

Table 1.15. The First UC histology classifier performance metrics

	Train Set		Test Set		Validation Set	
	Predictions		Predictions		Predictions	
Actual	Remission	Activity	Remission	Activity	Remission	Activity
Remission	47	13	14	2	12	0
Activity	0	213	1	51	1	17
ACC	95.24%		95.59%		96.67%	
95% CI	(0.9199,0.9744)		(0.8764, 0.9908)		(0.8278, 0.9992)	
P value	<.001		<001		<001	
SE	100%		93.33%		92.31%	
SP	94 25%		96.23%		100%	
PPV	78.33%		87.5%		100%	
NPV	100%		98.08%		94.44%	
AUC	0.9719		0.9786		0.991	

ACC, accuracy; SE, sensitivity: SP. specificity; PPV, positive predictive values: NPV, negative predictive values; AUC, area under the receiver operating characteristic curve

Table 1.16. Second UC histology classifier performance metrics.

	Train Set		Test Set		Validation Set	
	Predictions		Predictions		Predictions	
Actual	Remission	Activity	Remission	Activity	Remission	Activity
Remission	45	20	11	4	11	2
Activity	2	206	4	49	2	15
ACC	91 94%		88 24%		86 67%	
95% CI	(0.8805,0.9488)		(0.7813, 0.9478)		(0.6928, 0.9624)	
P value	<.001		<001		<.001	
SE	95.74%		73.33%		84.62%	
SP	91.15%		92.45%		88.24%	
PPV	69 23%		73.33%		84.62%	
NPV	99.04%		92.45%		88.24%	
AUC	0.9508		08629		0.9231	

ACC, accuracy; SE, sensitivity: SP. specificity; PPV, positive predictive values: NPV, negative predictive values; AUC, area under the receiver operating characteristic curve

The other 2 binary classifiers were built for CD predictions. CD classifiers were used to predict whether a CD patient has active or inactive histologic disease based on 2 clinical and 4 biological parameters - number of stools/day, diarrhea, CRP, fibrinogen, ESR, PLT (first CD

classifier) or based on 6 biological parameters - WBC, CRP, fibrinogen, ESR, PLT, SI (second CD classifier).

The two models performance metrics are presented in Table 1.17 and Table 1.18.

Table 1.17. The first CD histology classifier performance metrics

	Train Set		Test Set		Validation Set	
	Predictions		Predictions		Predictions	
Actual	Remission	Activity	Remission	Activity	Remission	Activity
Remission	20	7	5	1	5	0
Activity	1	54	1	14	1	6
ACC	90.24%		90.48%		91.67%	
95% CI	(0 8168, 0.9569)		(0.6962, 0.9883)		(0 6152,0.9979)	
P value	<001		<001		<001	
SE	95.24%		83.33%		83.33%	
SP	88 52%		93.33%		100%	
PPV	74.07%		83.33%		100%	
NPV	98.18%		93.33%		85.71%	
AUC	0.9305		0.9444		0.8889	

ACC, accuracy; SE, sensitivity: SP. specificity; PPV, positive predictive values: NPV, negative predictive values; AUC, area under the receiver operating characteristic curve

Table 1.18. The second CD histology classifier performance metrics

	Train Set		Test Set		Validation Set	
	Predictions		Predictions		Predictions	
Actual	Remission	Activity	Remission	Activity	Remission	Activity
Remission	20	8	4	1	5	0
Activity	1	53	2	14	1	6
ACC	8902%		85.71%		91.67%	
95% CI	(0.8018, 0.9486)		(0.6366, 0.9695)		(0.6152,0.9979)	
P value	<001		<001		<001	
SE	95.24%		66.67%		83.33%	
SP	86.89%		93.33%		100%	
PPV	71.43%		80%		100%	
NPV	98.15%		87.5%		85.71%	
AUC	09641		0 6889		1	

ACC, accuracy; SE, sensitivity: SP. specificity; PPV, positive predictive values: NPV, negative predictive values; AUC, area under the receiver operating characteristic curve

Discussions. Our paper proposed for the first time the use of AI for estimating histological activity / remission in both UC and CD. We showed that using only standard, non-costly, non-invasive parameters, it is possible to differentiate active histologic disease in IBD from inactive status. The evaluation of histological disease activity is especially critical because persistent microscopical inflammation in quiescent endoscopic disease increases the risk of disease relapse, corticosteroids, hospitalization, colectomy (Bryant et al, 2014). FC is the most

studied and most used non-invasive biomarker in the assessment of endoscopic and histological activity (Zittan et al, 2016; Dulai et al, 2019). However, FC has a number of disadvantages: significant variability across platforms (which makes it difficult to establish a cut-off value), intra-individual day-to-day variation in FC concentrations, decreasing of FC levels at room temperature after stool collection. Additionally, FC is not allowed in Asian and some Western countries and is more expensive than routine laboratory investigations (Dulai et al, 2019).

Compared to other studies that assessed FC performance in histological activity assessing, our models had similar or superior performance. For example, one paper evaluated the performance of FC to predict histologic activity in IBD (both UC and CD) and achieved an AUC of 0.898, an ACC of 85%, SE 77%, SP 100%, PPV 100%, NPV 68% for an FC cut-off level of 200 ng/mL (Vieira et al, 2009). Another study assessed FC in estimating histologic remission in colonic IBD and obtained an AUC of 0.95 (SE: 0.03; 95% CI, 0.88-1.02; P < .001), SE 100%, SP 77%, PPV 81.2%, NPV 100%, for a FC cutoff level of 100 μg/g (Zittan et al, 2016).

Conclusions. In the context of current costly and invasive monitoring methods for IBD, our study proposes a cost-efficient, non-invasive, AI/ML-based tool to predict histologic disease activity in IBD with reasonable accuracy. Our NN model represents a significant upsurge in the noninvasive assessment of IBD microscopical inflammation, leading the way to possible future use in clinical practice. Further validations on external datasets are needed to ensure generalizability and further prospective studies are needed to evaluate whether the proposed classifiers can be used as biomarkers able to replace endoscopies and biopsies.

1.3.5. A Machine Learning Model Accurately Predicts Ulcerative Colitis Activity at One Year in Patients Treated with Anti-Tumor Necrosis Factor α Agents

Background & Aim. A third model of AI was created to estimate endoscopic activity at 1 year in UC patients treated with anti- Tumor Necrosis Factor α (anti-TNF) agents. Anti-TNF and other modern biological regimens have revolutionized IBD treatment by their ability to achieve subclinical (endoscopic and histologic) remission, corticosteroid therapy discontinuation, reduction in hospitalization and surgery rates, long-term remission, and a good quality of life (Park, Jeen, 2015; Holdam et al, 2016). However, the high costs of biological agents and their potential side effects (mostly related to opportunistic infections and malignancies) have led to intense debates over the most opportune timing for starting or discontinuing the therapy, increasing the dose, or switching to another biological regimen and deciding on the most appropriate management of the lack or loss of response (Rubin, 2015; Moss, 2015; Waljee et al, 2019). AI/ML may be a solution for identifying prognostic factors in response to treatment in IBD patients. Transcriptomic analyses on purified CD8 T cells and/or whole blood or other multi-omics ML approaches have been described for predicting IBD treatment outcomes with good performance (Biasci et al, 2019; Zarringhalam et al, 2014). However, multi-omics techniques are based on costly investigations that are not widely available, making them hardly applicable for routine use. Promising ML solutions for predicting disease outcome after treatment with Vedolizumab (Waljee et al, 2018) or Azathioprine (Hardalaç et al, 2015) based on standard clinical parameters have been proposed in IBD. To date, no study based on routinely available clinical data has described ML models for predicting anti-TNF response in UC patients. We aimed to build a machine learning model for predicting disease activity and risk of relapse at one year follow-up in UC patients treated with anti-TNF agents using only standard clinical variables. After the model's validation on independent, external, and sufficiently large cohorts of patients, the proposed ML solution may prove useful in assisting the clinicians' decisions to increase the dose or change the biological agent.

Material and Methods. An observational retrospective single-centre cohort study was conducted on a sample of 55 UC patient records (Popa IV et al, 2020). Confirmed UC patients under maintenance therapy with an anti-TNF agent (Infliximab - 5 mg/kg every eight weeks or Adalimumab - 40 mg every two weeks) who underwent a colonoscopy for disease assessment at the initial evaluation and one year follow-up were considered. Only patients in clinical remission at the initial evaluation were included. Patients were excluded if they were in evidence with concurrent disorders (infections, autoimmune and inflammatory conditions, cirrhosis, neoplasia, and hemodialysis) capable of influencing medical parameters, if they presented clinical relapse at the initial evaluation or if changes were made to the therapeutic regimens between the two visits. No patient received other concomitant immunomodulatory treatment.

Laboratory parameters documented were: red blood cells (RBC), white blood cells (WBC), platelets (PLT), hemoglobin (HGB), hematocrit (HCT), mean corpuscular hemoglobin concentration (MCHC), plateletcrit (PCT), platelet distribution width (PDW), mean platelet volume (MPV), platelet large cell ratio (PLCR), neutrophils (NEUT), lymphocytes, monocytes (MONO), C reactive protein (CRP), erythrocyte sedimentation rate/1 h (ESR), fibrinogen, serum iron (SI), ferritin, total proteins (TP), albumin, alpha one globulins (A1G), alpha two globulins (A2G), beta one globulins, beta two globulins, and gamma globulins. Colonoscopy with biopsy was performed in all patients. According to the colonoscopy findings, a patient was considered to have endoscopic remission if the Mayo score was 0 or 1. Similarly, active disease was considered for Mayo scores 2 or 3.

Initial data of 50 UC patient records were randomly divided into a training set of 40 records (80%) and a test set of 10 records (20%), such that variables distributions in each set were similar to those in the original dataset. The other five patient records from the same medical centre were added independently to be used as a validation set. Endoscopic activity classes (active/inactive) were not equally represented in the train and test set. However, the validation set had a balanced distribution of the disease activity classes. One multilayered perceptron classifier was developed based upon the training set. The classifier was built to predict whether a UC patient will present endoscopic remission or active disease at the one year follow-up if the therapeutic strategy is left unchanged. The developed neural network was evaluated on the test set and validation set according to the ACC. The AUC, SE, SP, PPV, NPV were also determined.

Results. Using ANOVA with Holm adjustment, the feature selection step initially included six continuous baseline variables with a significant difference between the active disease group and the remission group at one year: NEUT, PDW, MPV, PLCR, CRP, and A1G. Significance was established at $p < 0.05$. Next, highly intercorrelated features were identified and removed. Three strong correlations with a Pearson coefficient ≥ 0.9 were identified between PDW and MPV, PDW, and PLCR, MPV, and PLCR. Thus, the following parameters were removed from the analysis: MPV and PLCR. As a result of the feature selection stage, four parameters (NEUT, PDW, CRP, and A1G) were included in further analysis.

Baseline clinical findings, laboratory parameters and endoscopic activity at one year for all patients are presented in Table 1.19.

The neural network model was developed using the baseline endoscopic activity and all four selected variables as inputs to predict whether a UC patient will have an active or inactive endoscopic disease at one year, under the same therapeutic regimen. Model performance metrics are shown in Table 1.20.

Table 1.19. Baseline parameters for all patient records and each endoscopic activity class at one year

Baseline Parameters	AH	Endoscopic Activity at One Year	
		Inactive	Active
N umber of records	55	42	13
Gender (male:female)	40:15	32:10	8:5
Age (years)	44.3 ± 10.5	43.7 ± 11.4	46 ± 6.4
Baseline endoscopic activity	Inactive 39 Active 16	35 7	4 9
NEUT*KP/pL	4.59 ± 2	3.32 ± 1.13	5.7 ± 2.37
PDW fL	12.9 ± 1.9	13.2 ±2.1	11.8 ± 1
CRP mg/dL	0.35 ± 0.4	0.3 ± 0.32	0.55 ± 0.4
A1G %	2.1 ±0.33	2 ± 0.32	2.31 ± 0.27

* signifies "multiplied by".

Table 1.20. The classifier's performance metrics.

	Train Set		Test Set		Validation Set	
Actual	Predictions Remission	Activity	Predictions Remission	Activity	Predictions Remission	Activity
Remission	27	0	6	1	3	0
Activity	6	7	0	3	0	2
ACC	85%		90%		100%	
95% CI	(0.70,0.94)		(0.56, 0.99)		(0.48,1.00)	
p value	<0.001		<0.001		<0.001	
SE	82%		100%		100%	
SP	100%		75%		100%	
PPV	100%		86%		100%	
NPV	54%		100%		100%	
AUC	0.91		0.92		1.00	

ACC (Accuracy); CI (Confidence Intervals); AUC (Area under the receiver operating characteristic curve); SE (Sensitivity); SP (Specificity), PPV (Positive predictive value); NPV (Negative predictive value).

ROC curves proving model performance on the train, test, and validation sets are shown in Fig. 1.6.

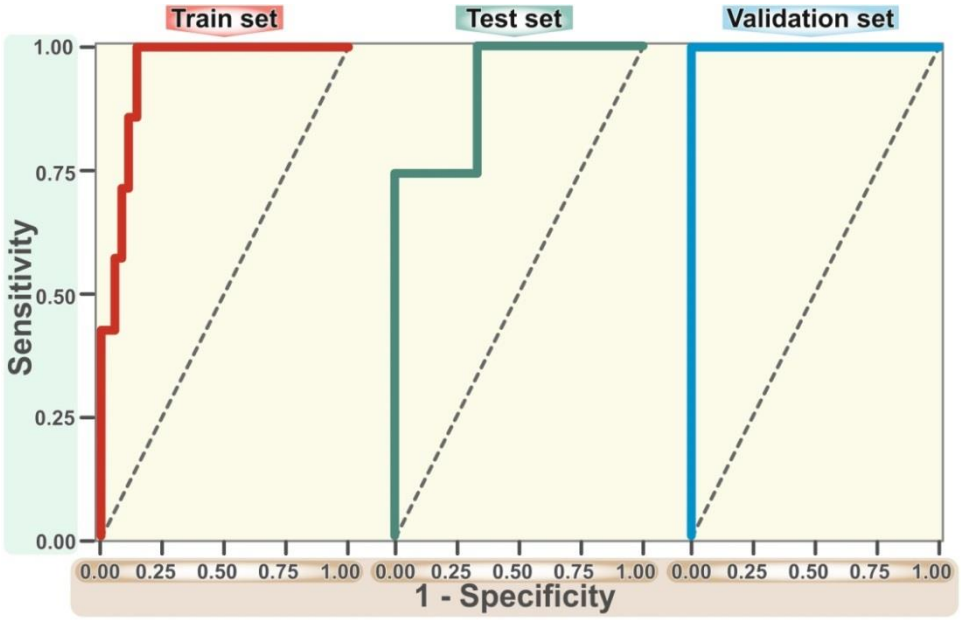


Fig. 1.6. Classifier's performance to predict endoscopic remission vs. relapse at one year

Discussions. Our study is the first neural network developed for UC patients treated with anti-TNF agents for predicting endoscopic disease activity and risk of relapse at one year using standard baseline parameters. We demonstrated that it is possible to accurately predict anti-TNF response at one year in UC patients using machine learning methods. Present-day guidelines indicate that the best future activity prediction is based on current endoscopic activity (Magro et al, 2017; Lamb et al, 2019). Our solution brings a new perspective by incorporating additional parameters besides baseline endoscopy to predict future disease outcomes. Although biological therapy has been instrumental in achieving endoscopic and histological healing, the inadequate response to biologics delays the disease's resolution, exposes patients to unnecessary toxic drug effects, and wastes medical resources. Therefore, the growing interest in monitoring the therapy aims to identify appropriate end-points for successful treatment and timely discontinue or switch the therapy in those likely to relapse or unlikely to respond (Ben-Horin et al, 2015). The proper therapeutic decision is essential, even more so as inopportune discontinuation may trigger the development of anti-drug antibodies that can lead to future response loss (Kothari et al, 2017). Comparing the baseline activity with the predicted one and acknowledging the risk of relapse, the clinician may decide whether increasing the dose, switching to other biologic agents, or discontinuing the therapy is the most appropriate decision. Our model achieved an ACC of 85% with 82% SE, 100% SP and an AUC of 0.91 on the trainset. On the test set, the classifier obtained an excellent performance with an ACC of 90%, SE of 100%, SP of 75%, and AUC of 0.92. On the validation set, the model predictions achieved the maximum performance with a 100% ACC, SE, and SP and an AUC of 1.

A few other studies aimed to predict endoscopic remission at one year. A study that used FC (cut-off of ≤121 μg / g) to predict endoscopic remission one year after initiation of anti-TNF therapy had inferior performances compared with our proposed model (70% SE and 70% SP) (Guidi et al, 2014).

Conclusions. Our proposed ML solution proved to accurately predict disease activity at one year in UC patients treated with anti-TNF agents using routinely available clinical parameters. Acknowledging the risk of relapse could lead to increasing the dose or switching to other biological agents. After rigorous validation on large, external datasets, our ML approach could significantly impact clinical practice by helping the physician decide on the most appropriate therapeutic option concerning the management of anti-TNF biologics.

The main points of the studies regarding the role of AI in IBD patients are resumed in Table 1.21.

Table 1.21. Main points regarding the role of artificial intelligence in IBD patients

SCIENTIFIC/CLINICALRELEVANCE
• AI is a new tool in modern medicine, able to improve diagnostic performance and estimate prognostic factors in various diseases
• In IBD management there is an urgent need for non-invasive markers
• With the help of AI / ML we created 3 predictive models: ✓ estimation of endoscopic activity in patients with UC ✓ estimation of histological remission in patients with both UC and CD ✓ estimation of endoscopic remission in patients with UC at 1 year of anti-TNF therapy
• All three models had outstanding performance in terms of accuracy, sensitivity, specificity, positive and negative predictive value, the area under the curve
• The performance of AI models was superior to the biomarkers currently used (eg fecal calprotectin)
• After rigorous validation on large, external datasets, our ML approachcould significantly improve IBD management
• Our research paves the way for new AI models used in gastroenterology

Chapter 2.
INTERDISCIPLINARY APPROACH IN INFLAMMATORY BOWEL DISEASES

2.1. INTRODUCTION

IBD is one of the relevant examples of the need for interdisciplinary approach of modern medicine. IBD diagnosis implys the involvement of the gastroenterologist but also of the radiologist and the pathologist. Collaboration with the surgeon is essential in case of intestinal complications of the disease. The extraintestinal manifestations (EIM) are present in a percentage of 6-47% and affect multiple organs: joints, skin, or eyes, but also other organs, such as the liver, lung, and pancreas (Singeap et al, 2021). Anemia and osteoporosis are common complications with multifactorial etiopathogenesis. Thromboembolic events can complicate the course of hospitalized patients with flares. Infections and malignancies are important issues when we consider biological therapy. Last but not least, patients with IBD have a special psychological profile, a low QoL, with an increased frequency of anxiety and depression (Guillo et al, 2020). All this makes the multidisciplinary approach a sine qua non condition of the management of IBD patients (Rogler et al, 2021).

I will further present the main contributions to this research direction.

2.2. EXTRAINTESTINAL MANIFESTATIONS IN IBD

2.2.1. State of the art

EIMs are complications of IBD that occur outside of inflammation of the digestive tract. Different studies, on different patient populations, with different definitions and follow-up periods, indicate an extremely variable prevalence of EIM from 6% to 47% (Ott, Schölmerich, 2013). Most authors estimate that 1/3 of IBD patients have at least one EIM (Singeap et al, 2021). The most common are the joints manifestations, followed by the hepato-biliary, skin and eyes. Some of them evolve in parallel with intestinal flare (peripheral arthritis, erythema nodosum, episcleritis), while others are independent: ankylosing spondylitis (AS), uveitis. They can occur both before and after the IBD diagnosis. A quarter of patients associate multiple EIM (Vavricka et al, 2015). Their presence influences the therapeutic decision and considerably affects the patients QoL (Rogler et al, 2021). The pathogenic mechanisms are similar to IBD, through the interaction of genetic factors with those of the environment and the intestinal microbiota, with the activation of the abnormal immune response (Hedin et al, 2019).

Musculoskeletal manifestations are the most common EIM of IBD, part of a wider group named spondylarthropathies.

Peripheral arthropathies can forego the onset of the gut clinical signs.Their prevalence in UC is smaller (5-10%) than in CD (10-20%) (Orchard et al, 1998). The main characteristic is their non-erosive, non-deforming pattern.Type1**:** pauci-articular (less than five joints) is defined by migratory non-deforming and non-erosive large-joint arthropathy predominantly of the lower limbs. It is associated with HLA B27,-B35, HLA-DRB1-0103 in up to 65% of the patients.The acute episode is self limiting (mean duration is 5 weeks) and runs in parallel with the IBD. Type 2**:** polyarticular (more than five joints affected) is characterized by symmetrical persistent small joint polyarthropathy.The course is independent of the underlying IBD and can last for several months (Orchard et al, 1998).The genetic

background is strongly correlated with HLA B44, MHC class I chain-like gene A (on chromosome 6) (Orchard et al, 2000).

Axial arthropaties are not correlated with disease activity of IBD. Sacroiliitis is non-progresive and is not associated with HLA B27.This form is associated with enthesitis, tenosynovitis, dactylitis even in the absence of arthritis (Salvarani et al, 2000).

Osteoporosis is another extraintestinal complication of IBD. The main role in the ethiopathogenesis is assumed to the connection between osteoprotegerin – RANKL - RANK (Vidal et al, 1998). Osteoclastogenesis is induced by a surface receptor (RANK) located on osteoclasts. Its ligand (RANKL) is induced by proinflammatory cytokines. On the other hand, osteoblasts produce osteoprotegerin which prevents ligation of RANKL to RANK, so the bone loss is stoped.

Osteomalacia is determined by a prolonged and severe vitamin D deficiency. Often appear after multiple intestinal resections in severe cases of CD (Compston et al, 1978).

Mucocutaneous manifestations can be divided in three classes of injuries relaying on the ethiopathogenic mechanism (Marineata et al, 2014)**.**

Granulomatous lesions and IBD have the same histological pattern. Perianal/peristomal ulcers and fistulas frequently appear in patients with CD**.**

Reactive skin manifestations of IBD usually are diagnosed in parallel with intestinal disease activity. Aphthous stomatitis follows the colonic localization of IBD. Pyoderma gangrenosum may run an independent course of the underlying IBD activity. Commonly involves the skin with a predilection for the lower limbs, but also may occur around surgical stoma or in any area of the skin. Erythema nodosum appears asraised, warm, tenderery nodules tipically in the pretibial areas. Histologicaly it is a septal panniculitis due to immune complex deposition. It was proved a genetic association between erythema nodosum and a certain HLA region of chromosome 6 (Antonelli et al, 2021). Sweet's syndrome is a neutrophilic dermatosis which appears as painful erythematous nodules or plaques. It is often associated with fever and leukocytosis (Marzano et al, 2013).

Nutritional-deficient cutaneous manifestations due to nutritional malabsorption include acrodermatitis enteropathica determined by zinc deficiency, stomatitis, glossitis, angular cheilitis, pellagra, scurvy, purpura.There are also a lot of skin manifestations secondary to IBD treatment: infusion reactions, paradoxical reactions, infections, malignancies (Nigam et al, 2020).

Ocular manifestations. There are two kinds of manifestations depending on their immunopathogeny. Immune-related conditions such as: episcleritis, scleritis, uveitis, corneal disease or related to drug exposure: cataract, glaucoma. A high risk for developing these conditions is the presence of genotypes HLA B27, B58, DRB 10103. Usually are flaring and subsiding at the same time as the bowel inflammation (Mintz et al, 2004).

Hepatobiliary and pancreatic manifestations. *Primary sclerosing cholangitis* is a chronic, slowly progressive cholestatic disorder characterized by inflammation and fibrosis of intrahepatic / extrahepatic bile ducts. It is a premalignant condition for cholangiocarcinoma and determine a higher risk of colonic dysplasia/carcinoma (Yarur et al, 2014). *Hepatobiliary complications* include: cholelithiasis (more frequent in CD and caused by the malabsorption of bile salts from the inflamed terminal ileum), fatty liver due to steroid therapy or poor nutrition, liver abcesses, portal vein thrombosis, pylephlebitis. *Pancreatic manifestations* can take two forms: acute or chronic pancreatitis. Acute pancreatitis is induced by drugs such as salycilates, azathioprine, 6-mercaptopurine. Usually it has a mild course with a remission of the inflammatory processes after discontinuing the therapy. Chronic pancreatitis is characterized by a decreased exocrine function due to circulating inflammatory mediators or to autoantibodies against pancreatic tissue (Suk Lee et al, 2018).

Urinary system manifestations. IBD patients present quite frequent minimal, subclinical, glomerular inflammatory changes. Interstitial nephritis and tubular proteinuria is often attributed to CD (Gasche, 2000). Glomerulonephritis can lead to nephrotic syndrome and renal failure and is directly related to the activity of the bowel disease. Urinary complications include nephrolithiasis and urinary tract fistulas. Nephrolithiasis appear more frequent in CD than in UC. Renal amyloidosis appers more often in ileal CD.

Pulmonary manifestations. The most common manifestation is bronchial inflammation and suppuration with or without bronchiectasis. Lung parenchymal diseases include bronchiolitis obliterans with organizing pneumonia, unspecified interstitial lung disease, noncaseating granulomatous inflammation and fibrosis, parenchymal nodules and granulomata, alveolitis and alveolar consolidation. Pleural diseases include pneumothorax, pleural thickening, pleuritis and pleural effusion (Faller et al, 2000). Drug-related lung disease can be interpreted as complications.

Cardiovascular manifestations. Heart involvement is rare. It is seen more frequently in men and in those with UC. These manifestations are not related to the activity of the bowel disease. Cardiac involvement may present as ischemic heart disease, pericardial effusion, myopericarditis (Kristensen et al, 2013). It is estimated a prevalence of 1-7% of thromboembolism in IBD, 2-3 times higher compared to the general population, similar in UC and CD (Alkim et al, 2017). The most common are deep vein thrombosis in the lower limbs and pulmonary embolism, but other locations are possible: cerebral, portal vein, mesenteric vein, retinal veins. Cases of arterial thrombosis (cardiac, cerebral, mesenteric) have also been reported, especially in young patients in the postoperative period, as well as cases of migratory thrombophlebitis (Irving et al, 2005).

Neurological manifestations include central and peripheral nervous system involvement. Peripheral neuropathy is one of the most common complications (Nemni et al, 1987). It has also been found an inflammatory myopathy.

EIM may produce greater morbidity than the underlying intestinal disease. The distinction between disease and treatment side effects can be sometimes extremely difficult. Early recognition of these EIM should help guide therapy that will reduce overall morbidity in affected patients.

In this research direction I have published the following papers:

1. Cardoneanu A, **Mihai C***, Rezus E, Burlui A, Popa I, Prelipcean CC. Gut Microbiota Changes in Inflammatory Bowel Diseases and Ankylosing Spondylitis. *J Gastrointest Liver Dis*2021; 30 (1): 46-54. IF 2,008
2. Cardoneanu A, Cijevschi Prelipcean C, Danciu M, **Mihai C**, Dranga M, Gavrilescu O, Rezus E. Looking beyond gut inflammation in inflammatory bowel disease. *Romanian Journal of Morphology & Embriology* 2018; 59(4): 1097-1105. IF=0.912
3. Dumitrescu G, **Mihai C**, Dranga M, Prelipcean CC. Serum 25-hydroxyvitamin D concentration and inflammatory bowel disease characteristics in Romania.*World J Gastroenterol* 2014;20(9):2392-6. FI=2.369
4. Cardoneanu A, Cijevschi Prelipcean C, **Mihai C**, Gavrilescu O, Popa I, Ungureanu I, Popa R, Rezus E. Two different diseases, one treatment. Proceedings XXXVIth National Congress of Gastroenterology, Hepatology and Digestive Endoscopy. Cluj Napoca 8-11 iunie 2016. Editor: Dan Dumitrascu. FILO diritto editure. www.gastro2016.medical-congresses.ro.: 92-95.
5. Cardoneanu A, Duceac LD, Rezus E, **Mihai C**, Dranga M, Gavrilescu O, Cijevschi Prelipcean C. A new reality: gut microbiota and inflammatory systemic diseases. *Rev Med Chir Soc Med NatIasi*2017; 121 (2): 291-295.
6. Cardoneanu A, Rezuș E, Gavrilescu O, Dranga M, Cijevschi Prelipcean C, **MihaiC**. Extra

intestinal manifestations in inflammatory bowel disease - results from Northeastern Romania**.** *Rev Med Chir Soc Med Nat Iasi*2017; 121 (3):479-485.
7. Marineaţă A, Rezuş E, **Mihai C**, Cijevschi Prelipcean C. Extra-intestinal manifestations and complications in inflammatory bowel disease. *The Medical-Surgical Journal* 2014;118(2): 279-288.
8. Dumitrescu G, **Mihai C**, Dranga M, Cijevschi Prelipcean C. Bone mineral density in patients with inflammatory bowel disease from north-eastern Romania. *The Medical-Surgical Journal* 2013; 117 (1): 23-28.

2.2.2. Epidemiology of extraintestinal manifestations in IBD in the Northeastern region of Romania

Background & Aim.The aims of this study were to:

- develop specific clinical and epidemiological data on patients diagnosed with IBD who associate EIM and intestinal complications in the Northeastern (NE) region of Romania;
- establish the risk factors associated with the occurrence of EIM and intestinal complications;
- establish correlations between EIM, intestinal complications and IBD characteristics (localization of intestinal inflammation, disease phenotype);
- assess the link between intestinal complications and EIM.

Material and methods

We performed a retrospective case-control study, which included 517 patients with IBD (CD, UC or undifferentiated colitis) diagnosed between 1975 and 2016 in the Northeastern (NE) region of Romania (Cardoneanu et al, 2018). The patients were extracted from the national database (IBD Prospect). The inclusion criteria were: age over 18; patient consent and signing the informed consent; certain diagnosis of CD, UC or undifferentiated colitis. Exclusion criteria were: uncertain diagnosis of CD, UC or undifferentiated colitis; the patient's refusal to be included in the national database. The Montreal classification was used to classify IBD by phenotype and to localize intestinal inflammation. The demographic characteristics of patients (age, gender, ethnicity, environment, occupation, smoker status) and of IBD (year of diagnosis, phenotype and location of the disease, presence and number of EIM as well as the presence of intestinal complications, the following treatment), were extracted from the national database.

Among EIM, it has been taken into consideration articular manifestations (arthritis or sacroiliitis/ankylosing spondylitis AS), dermatological manifestations (erythema nodosum, pyoderma gangrenosum), ophthalmologic signs (uveitis/episcleritis), hepatobiliary manifestations (primary sclerosing cholangitis – PSC) and urinary involvement (oxalic nephrolithiasis, kidney amyloidosis, urinary tract infections). The diagnosis of arthritis was based on clinical symptoms (pain, joint swelling) and on rheumatological examination made by a specialist doctor who excluded the presence of other associated autoimmune pathologies. Sacroiliitis was highlighted by pelvis radiography or magnetic resonance imaging (MRI). Patients diagnosed with AS have met the 1984 modified New York Diagnostic Criteria (van der Linden et al, 1984). The skin manifestations have been diagnosed by a dermatologist (clinically or skin biopsy). Eye manifestations were evaluated by an ophthalmologist. The PSC diagnosis included abnormal liver tests and cholangio MRI ± liver biopsy. Reno-urinary manifestations were diagnosed by serum and urine tests (urine culture, urine analysis), ultrasonography or renal biopsy (in the case of suspicion of renal amyloidosis). Among intestinal complications, there were considered: abscesses, intestinal or perianal fistula, intestinal stenosis, toxic

megacolon, intestinal perforation, lower digestive bleeding or the presence of malignancies. All patients included were periodically monitored clinically and paraclinically (blood tests, colonoscopy with biopsy and pathological examination). The obtained data were centralized in the Statistical Package for the Social Sciences (SPSS) 18.0 database.

Results

The study included 517 patients with IBD of which only 513 had all data required for the statistical analysis (Table 2.1).

Table 2.1. Characteristics of the study group

	IBD, *n(%)*	CD, *n*(%)	UC, *n (%)*	*P*
No. of patients	517(100)	135 (26.1)	368 (71.2)	0.001
Males/females	294 (56.9)/223 (43.1)	66(48.9)/69 (51.1)	221 (60.3)/147 (39.7)	0.016
Average age [years]	48.24	44.52	49.65	0.003
Area of origin: urban/rural	341 (66.7)/172 (34.9)	95 (70.4)/40 (29.6)	240 (65.2)/128(34.8)	0.536
Smokers/ex-smokers/ non-smokers	77 (15)/164 (31.8)/276 (53.2)	34 (25.2)/31 (23/70 (51.9)	37 (10.1)/131 (35.6)/200 (54.3)	0.001
Disease activity: mild/moderate/severe	226 (44 2)/242 (47.4)/43 (8.4)	56 (41.5)/73 (54.1)/6 (4.4)	165 (44 8)/166 (45.1)/37 (10.1)	0.062
Form of CD: L1/L2/L3/L4	NA	34 (25.2)/52 (38.5)/45 (33.3)/4 (3)	NA	0.818
Phenotype of CD: B1/B2/B3	NA	84 (62 2)/40 (29 6)/11 (8.1)	NA	0 026
Form of UC: E1/E2/E3	NA	NA	71 (19.3)7203 (55.2)794 (25.5)	0.012

IBD: Inflammatory bowel disease; CD: Crohn's disease; UC: Ulcerative colitis; *n:* No. of cases; L1: Ileitis; L2: Colitis; L3: Ileocolitis, L4: Upper gastrointestinal tract; B1: Inflammatory; B2: Stricturing; B3: Penetrating. E1: Proctitis; E2: Left-sided colitis; E3: Pancolitis; NA: Not applicable

UC predominated against CD cases (n=368 vs. n=135). Only 10 patients were diagnosed with undifferentiated colitis. Female gender (51.1% vs. 48.9%) predominated in the group of CD patients, while, in the UC group, male gender prevailed (60.3% vs. 39.7%) (p=0.016). UC patients had an older age than the rest of the cases (p=0.003). CD summarized 135 cases. Colonic involvement (L2) (n=52, 38.8%) predominated, followed by ileocolitis (L3) (n=45, 33.6%). Eighty-four (63.4%) of these patients had an inflammatory phenotype (B1) and 40 (28.4%) a stricturing form. The ileal inflammation was identified in 27.4% of patients with a non-stricturing form of CD and in 27.3% of those with a penetrating form of disease. Most commonly, the colonic location of intestinal inflammation was identified in patients with a stricturing disease (42.1%), and the rarest in those with a penetrating form of CD (27.3%). Ileocolitis was most commonly associated with the penetrating phenotype (45.5%). Involvement on the upper gastrointestinal tract was present in 3.6% of patients with non-stricturing disease and in 2.6% of those having a stricturing phenotype. Patients diagnosed with UC have totalized 368 cases. Among these cases, left-sided disease (n=203, 55.16%), then pancolitis (n=94, 25.5%) predominated. Most patients with UC and those diagnosed with CD had a moderate form of intestinal inflammation (n=242, 47.4%).

Over 90% of IBD cases (n=484, 93.6%) were on medication at the time of enrollment [Mesalazine – 5- Aminosalicylic Acid (5-ASA), Azathioprine (AZA), Methotrexate, tumor necrosis factor-alpha (TNF-α) blockers – Infliximab (IFX), corticosteroids (CS) and Budesonide, antibiotics – commonly Rifaximine, probiotics]. Of these, 223 (46.07%) patients were treated with 5-ASA and 216 (44.62%) had combined therapy, most cases (n=136, 62.96%) being treated with 5-ASA and CS.

In the study group, 51 cases with IBD and EIM were identified, having a prevalence of 9.9% (Table 2.2).

Table 2.2. EIM classification in patients with IBD depending on disease phenotype

EIM	CD (n=27)		UC (n=24)		*P*	OR	RR	95% CI
	n	%	*n*	%				
Articular manifestations								
Arthritis	16	59.3	10	41.7	0.209	2.04	1.4_{CD}	0.82-2.39
SI/AS	4	14.8	8	33.3	0.118	0.35	$1\ 63_{UC}$	0.94-2.81
Cutaneous manifestations								
Erythema nodosum	1	3.7	1	4.2	0.932	0.89	1.07_{UC}	0.26-4.4
Pyoderma gangrenosum	3	11.1	1	4.2	0.345	2.88	1.47_{CD}	0.78-2 76
Hepatobiliary manifestations								
PSC	0	0	1	4.2	0.216	-	2.17_{UC}	1.61-2.94
Ocular manifestations								
Uveitis	2	7.4	1	4 2	0.619	1.84	$1\ .28_{CD}$	0.55-2.98
Renal manifestations								
Oxalate nephrolithiasis	2	7.4	1	4.2	0.619	1.84	1.28_{CD}	0.55-2.98
Multiple urinary infections	5	18.5	3	12.5	0.553	1.59	1.22_{CD}	0.66-2.25

EIM: Extraintestinal manifestations; IBD: Inflammatory bowel disease, CD: Crohn's disease, UC: Ulcerative colitis; OR: Odds ratio. RR Relative risk, CI: Confidence interval; *n:* No. of cases; SI/AS: Sacroiliitis/ankylosing spondylitis; PSC: Primary sclerosing cholangitis.

The most common EIMs were musculoskeletal manifestations (7.4%), followed by renal manifestations (2.2%), cutaneous manifestations (1.2%), ocular (0.6%) and hepatobiliary manifestations (0.2%). EIMs occurred with a higher frequency in patients diagnosed with CD than UC (52.9% vs. 47.1%) ($p<0.001$). No patients with undifferentiated colitis presented EIM ($p=0.289$). Over 50% of cases of IBD and EIM belonged to female gender (52.9%, $p=0.142$), higher in the CD group (55.6% vs. 50%, $p=0.692$). Mean age was slightly higher in patients who had EIM (49.31 vs. 48.13 years, $p=0.595$). Ten (19.6%) patients were active smokers, over half – 28 (54.9%) non-smokers and 13 (25.5%) former smokers ($p=0.465$). By logistic regression, it was confirmed that active smokers had a 1.3 times higher risk to develop EIM than non-smokers (OR=1.306, $p=0.497$). Former smokers presented a risk of 0.758 (OR=0.758, $p=0.431$), so smoking status may be a protective factor for the occurrence of EIM. Patients with CD and EIM (n=27) exhibited a risk of 3.687 times higher than the rest of the cases for developing EIM ($p < 0.001$, OR=3.687, 95% CI 2.04–6.65). In these patients, ileocolitis predominated (n=11, 40.7%, $p=0.361$). Based on the statistical analysis, UC may be considered a protective factor for the occurrence of EIM ($p < 0.001$, OR=0.305, 95% CI 0.169–0.549).

Most patients with IBD and EIM (n=49, 96.1%) were on medical treatment. By comparison with the group without EIM, patients with EIM had frequently CS (7.8% vs. 1.1%, $p=0.017$) and infliximab therapy (5% vs. 11.8%, $p=0.145$). Patients without EIM received more frequently 5-ASA and antibiotics (46.1% vs. 19.6%, $p=0.569$). CS therapy exhibited a 10.8-fold increased risk for EIM compared to the treatment-free group ($p=0.017$, OR=10.8, 95% CI 1.541–75.699).

Musculoskeletal manifestations were the most common EIM (n=38, 74.5%, p=0.001). Peripheral involvement – arthritis (n=26, 68.42%) predominated, followed by axial damage – sacroiliitis/AS (n=12, 31.58%) (p=0.001). Patients with CD had a 3.48-fold greater risk of developing joint manifestations than the rest of the patients (p<0.001, OR=3.478, 95% CI 1.779–6.801). In both CD and UC patients, arthritis cases prevailed over sacroiliitis/AS (68.42% *vs.* 31.58%). Patients with CD had a 5-fold higher risk of developing arthritis (*p*<0.001, OR=5.009, 95% CI 2.21–11.34). Neither CD, nor UC patients, had a confirmed risk of developing sacroiliitis/AS (*p*=0.468, OR=1.565, 95% CI 0.463–5.293 for CD) (*p*=0.586, OR=0.714, 95% CI 0.211–2.413 for UC). The cases of arthritis and CD (*n*=16) mainly correlated with colitis (*n*=7, *p*=0.723) and ileocolitis (*n*=7, *p*=0.321). UC patients with arthritis (*n*=10) were linked to pancolitis (*n*=5, *p*=0.072, OR=3.023; 95% CI 0.855–10.69), proctitis (*n*=3, *p*=0.392) and left-sidedcolitis (*n*=2, *p*=0.024, OR=0.196, 95% CI 0.041–0.938).Uveitis was highlighted in two patients with CD and in an UC case. All cases with ocular signs also showed peripheral articular manifestations – arthritis. Pyoderma gangrenosum was more common in CD patients than in UC cases (n=3 vs. n=1) and was associated with articular manifestations. PSC was highlighted in one patient with UC and did not associate other EIM. Renal manifestations occurred with a higher frequency in CD and were associated with the presence of other EIM.

A strong point of the study was the correlation of the presence of EIM with intestinal complications, a fact little studied in the literature.The association between intestinal complications and EIM was highlighted in 15 (22.7%) patients (p=0.007), being more common in CD cases than in UC (n=12 vs. n=3) (Table 2.3).

Table 2.3. The association between intestinal complications and EIM

IBD				Intestinal complications	EIM
Disease phenotype	*Location of inflammation*	*Form of IBD*	*Severity of disease*		
CD	Lt	B2	moderate	intestinal stenosis	arthritis
CD	L3	B2	moderate	intestinal stenosis	multiple urinary tract infections
UC	E2	-	mild	Hdi	multiple urinary tract infections
CD	L3	B2	moderate	intestinal signal	arthritis + uveitis
UC	E1	-	mild	Hdi	arthritis
CD	L2	B1	mild	abscesses + fistula	arthritis + uveitis + oxalic nephrolitiasis+ multiple urinary tract infections
CD	L3	B1	moderate	Hdi	arthritis
CD	L3	B2	moderate	intestinal stenosis	arthritis
CD	L2	B2	moderate	intestinal stenosis	arthritis + *pyoderma gangrenosum*
CD	L2	B2	severe	intestinal stenosis	arthritis
CD	L3	B1	severe	fistula	multiple urinary tract infections
CD	Lt	B2	moderate	abscesses	multiple urinary tract infections
CD	L2	B3	mild	fistula	oxalic nephrolitiasis
UC	E3	-	mild	malignancy	oxalic nephrolitiasis
CD	L3	B1	mild	fistula +Hdi	SI/AS

EIM: Extraintestinal manifestations; IBD: Inflammatory bowel disease; CD: Crohn's disease; UC: Ulcerative colitis; L1: Ileitis; L2: Colitis; L3: Ileocolitis; E1: Proctitis; E2: Left-sided colitis; E3:Pancolitis; B1: Inflammatory; B2: Stricturing; B3: Penetrating; Hdi Inferior digestive hemorrhage;SI/AS: Sacroilitis/ankylosing spondylitis

The risk of developing intestinal complications was found to be 3.35 times higher in patients with EIM as compared to the rest of the cases (p<0.001, OR=3.358, 95% CI 1.72–

6.55). Of the intestinal complications, intestinal stenosis and lower bleeding predominated, while the most common EIM were articular manifestations (with a predominance of peripheral manifestations – arthritis). The stricturing phenotype and ileocolitis in CD apparently favored the association between intestinal complications and EIM (without statistical significance – very few cases).

Discussions

This study brings important data on the epidemiological and clinical characteristics of patients with IBD in the NE region of Romania. It also highlights the association and correlations between IBD and EIM, as well as those regarding intestinal complications. Most of the obtained results are consistent with the data published in the literature.

In the NE region of Romania, the EIM prevalence in patients with IBD was 9.9%, being relatively low compared to other geographical areas. Numerous clinical studies have analyzed the incidence of EIM in patients with IBD. The prevalence of EIM varies greatly, ranging from 6% to 47% (Bernstein et al, 2001; Ott et al, 2013). The most common EIMs were musculoskeletal manifestations, followed by renal, cutaneous, ocular and hepatobiliary manifestations. Our results coincide with data from other published studies that support the fact that, in patients with IBD and EIM, the highest incidence is assigned to articular manifestations (Isaacs, 2008). On the second place are mucocutaneous manifestations, followed by ophthalmological symptoms (Zippi et al, 2014).

In the study group, CD patients showed more frequently articular manifestations, pyoderma gangrenosum, uveitis and oxalic nephrolithiasis, while in patients with UC there was a higher ratio of PSC, in accordance with other studies in the literature (Yarur et al, 2014; Levine, Burakoff, 2011).

Both EIM and intestinal complications showed a higher incidence among CD patients, in accordance with other studies in the literature (Vavricka et al, 2011). Interestingly, compared to data published nationwide (Singeap et al, 2021) in our study we identified a lower prevalence of EIM. This can be explained by the predominance of UC in our geographical area, EIM being more frequently associated with CD. We cannot sustain that a particular phenotype of CD favors the appearance of EIM. However, those having EIM had a more frequent ileo-colonic disease.

Following the smoking status of patients with IBD and EIM, it was argued that active smoking is considered to be a risk factor for the development of EIM, while former smokers developed some protection. Lakatos et al. (Lakatos et al, 2005) reported the association between smoking and the occurrence of EIM. The study published by Ott et al. supports the increased risk of smokers diagnosed with CD to develop EIM (Ott et al, 2014). It has been shown that CS therapy has been a risk factor for the onset of EIM, while patients without EIM were given more frequent 5-ASA.

This study also focused on highlighting the correlations between EIM and intestinal complications. The obtained results after statistical analysis are promising, even if the number of the analyzed subjects was small. The risk of developing intestinal complications was found to be3.35 times higher in patients who also had EIM compared to the rest of the patients. The association between EIM– complications was much more common in CD cases.

Conclusions.

The prevalence of EIM in our study was lower in our study compared to data from the literature. The most common EIM were joint (peripheral arthritis), followed by renal, cutaneous, ocular, hepatobiliary manifestations. CD represents the phenotype of IBD, which has a higher incidence both for EIM and intestinal complications.Increased risk of intestinal complications in patients with EIM highlights the role of intestinal inflammation as a pathogenic substrate of EIM.

2.2.3. Vitamin D and osteoporosis in IBD

Background & Aim.

Low bone density is a condition frequently associated with IBD. The pathogenesis of bone demineralization is multifactorial. Even though the induction mechanisms have not been completely elucidated, the main risk factors are the duration of inflammatory bowel disease, protein-caloric malnutrition, prolonged corticotherapy, calcium and vitamin D deficiency, sex hormones deficit and tobacco consumption (Siffledeen et al, 2004). The aim of our study was to determine the degree of bone demineralization in patients with IBD and to identify the main causes leading to this condition (Dumitrescu et al, 2013).

Material and methods.

A prospective descriptive study was carried out on a series of 143 patients with IBD, hospitalized at the Institute of Gastroenterology and Hepatology in Iaşi. The demographic parameters (age, gender, place of residence, type of disease, duration, extension of lesions and degree of activity) as well as the treatment (corticotherapy – months of treatment and cumulative prednisoneequivalent doses) were analyzed. Additionally, the body mass index (BMI) (kg/m²), smoking status and intensity (estimated in packs per year), history of fractures, and, in women, obstetrical antecedents were assessed. Patients presenting other possible causes of bone demineralization and vitamin D deficiency (bowel resections, thyroid or parathyroid pathology, hypogonadism, diabetes mellitus, neoplasia, renal or hepatic failure, patients with menopause for more than 5 years) were excluded.

Osteoporosis was assessed by osteodensitometry (Dual Energy X-rays Absorptiometry = DEXA) at the lumbar spine (L1-L4) and the upper end of the femur. The results were expressed as a T-score: standard deviations (SD) below or above the mean value for a younger subject in the general population). World Health Organization classification of bone mineral density was used: normal (T-score > 1SD), osteopenia (T-score between -1 SD and -2.5 SD) and osteoporosis (T-score < -2.5).

The following biochemical values were determined: serum calcium (mg/dl), ionic calcium(mmol/l), alkaline phosphatase (UI/l), 25 hydroxy-vitamin D (ng/ml).

Results

In UC patients osteodensity at the lumbar spine and proximal end of the femur revealed: 34 patients (32.69%) had normal bone mineral density, 50 patients (48.07%) presented osteopenia, and 20 patients (19.23%) osteoporosis. In CD patients there were 11 patients with normal mineral density, 22 patients (56.41%) with osteopenia, and 6 patients(15.38%) with osteoporosis (Fig. 2.1.)

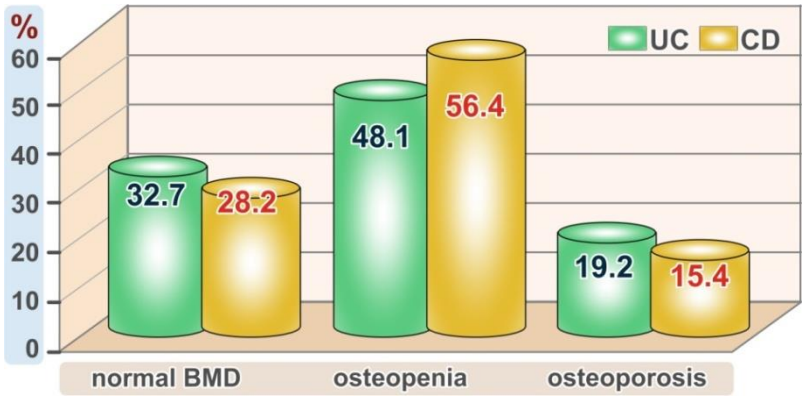


Fig. 2.1. Bone mineral density in patients with IBD

Bonemineraldensitywaslowerin the patients with CD compared to thosewithUC.T-scoreforbothlumbarspineand hipwaslowerinCD compare with UCpatients(lumbarspine:CD=-1.60±1.00;UC=-1.00±1.13,proximalendofthefemur:CD=-0.93±0.84;UC=-0.31±0.90).

Smoking in UC and CS in CD were the main factors associated with low bone mineral density (Table 2.4).

Table 2.4. Risk factors for low bone mineral density in IBD patients

Parameters	UC	Crohn'sdisease	p
BMI<18.5kg/m^2	12/104	4/39	>0.05
Smoker	62/104	22/39	<0.05
Averagetobaccoconsumption(PY)	15.93	11.12	<0.05
Averagediseaseduration(months)	49.58	37,64	>0.05
CSTreatment	44/104	24/39	<0.05
AverageCSdose (prednisoneequivalentdose-g/patient)	2.82±2.4	8,37±21,65	<0.05

Although we found lower levels of 25-OH Vitamin D in CD compared to UC patients, the levels did not corelated with low bone mineral density. In another study about 25-OH vitamin D in IBD patients we have demonstrated that the vitamin D levels were significantly lower in the CD patients with moderate to severe disease activity compared to the CD patients in remission or with mild disease activity (16 ± 6 ng/mL vs 26 ± 7 ng/mL; 16 ± 6 ng/mL vs 31 ± 9 ng/mL, respectively, $P < 0.05$). Vitamin D levels in the UC patients were not influenced by disease activity and no correlation was observed with the inflammation markers tested (C-reactive protein, fibrinogen, and erythrocyte sedimentation rate). No association was observed between vitamin D levels and smoking status or ongoing medication (5ASA, steroids, and anti-TNFα). Newly diagnosed IBD patients had lower vitamin D levels than patients with established cases, though these differences were not significant (UC: 22 ± 9 ng/mL vs 26 ± 12 ng/ mL; CD: 18 ± 6 ng/mL vs 27 ± 11 ng/mL, respectively). Although no association was found between the season during which the visit was scheduled and vitamin D levels, the UC patients assessed during the winter tended to have lower levels than those assessed during the summer (22 ± 9 ng/mL vs 28 ± 13 ng/mL, respectively) (Dumitrescu et al, 2014).

Discussions

Our study revealed low bone density in 67.3% of the patients with UC and in 71.8% of the patients with CD. 19% of the UC patients and 15.4% of the CD patients had osteoporosis.Vitamin D deficiency was significantly higher in the CD patients (35.7%) compared to UC patients (25.6%). Although low vitamin D concentrations have been reported in IBD there are contradictory data regarding the correlation between 25-hydroxyvitamin D levels and IBD activity (Leslie et al, 2008). Despite the high prevalence of vitamin D deficiency in IBD patients, serum vitamin D levels were only associated with IBD activity in CD patients. Newly diagnosed IBD patients tended to have lower vitamin D levels, though the small number of patients prohibited this difference from reaching statistical significance. It has been suggested that vitamin D deficiency in IBD patients is related to inadequate absorption[24]. Although we demonstrated an increased incidence of vitamin Ddeficiency in IBD patients, it is unclear whether the low vitamin D level is due to the IBD and associated inflammation of the gut, or if the IBD is a consequence of the immune disorders induced by the vitamin D deficiency. Recent studies have demonstrated that vitamin D deficiency in IBD

can increase disease recurrence, IBD-related hospitalization or surgery, and deterioration of QoL (Kim et al, 2020).

Conclusions

Our research has shown an increased incidence of osteoporosis and osteopenia in patients with IBD, more commonly in those with CD compared to those with UC. The main risk factors associated with low bone mineral density were smoking and corticosteroid treatment. Low levels of vitamin D have been linked to active CD, but not to a decrease in bone mineral density.

2.2.4. Connections between IBD and ankylosing spondylitis: gut microbiota changes

Background & Aim.

IBD and ankylosing spondylitis (AS) are chronic disorders sharing common etiopathogenetic mechanisms. Microscopic intestinal inflammation was found in 40-60% of AS patients (Van Praet et al, 2013). IBD can be considered the result of the interaction between genetic factors that determine susceptibility and environmental factors that influence the composition of the intestinal microbial flora, thus leading to an abnormal mucosal response.The results of the published studies have demonstrated that IBD dysbiosis is characterized by a reduction in the diversity of bacterial species (especially anaerobic bacteria) followed by an increase in the concentration of entero-adherent bacteria (Nishida et al, 2018; Khan et al, 2019). On the other hand, in AS gut dysbiosis is also represented by a decreased total number ofbacterial species,dominated by pro-inflammatory pathogens(Breban et al, 2019; Costello et al, 2015). If gut dysbiosis is a cause or an effect of systemic inflammation or the missing link between these two diseases remains to be determined. The aim of the study was to evaluate the composition of intestinal microbiota and to characterize gut dysbiosis in patients having IBD (CD, UC) or AS. Special attention was given to the intestinal microbiota analysis in patients who presented the association between IBD and AS.

Material and methods

We conducted a prospective, case-control study in two academic centers in Northeastern Romania, one of gastroenterology and the other of rheumatology (Cardoneanu et al, 2021). The study enrolled in 124 cases between April 2016 and March 2017. The control group belonged to Rheumatology Clinic, Rehabilitation Clinical Hospital from Iasi, and included healthy people.

The inclusion criteria were: age over 18; the patient's signed consent to be included in the study; certain diagnoses of CD, UC, AS or association between IBD and AS. Diagnosis of IBD (CD or UC) was based on clinical symptoms, colonoscopy, and histopathology examination. The Montreal classification was used to classify IBD by phenotype and to localize intestinal inflammation. Patients diagnosed with AS met the 1984 modified New York diagnostic criteria. The subjects were asked to complete a questionnaire regarding dietary habits and antibiotic or probiotic use in the last three months. Exclusion criteria consisted of patient refusal to participate in this study, uncertain diagnosis of CD, UC or AS, colorectal cancer, serious infections (tuberculosis, *Clostridioides difficile*), other comorbidities, use of antibiotics, probiotics, or restrictive diets in the last 3 months.

The enrolled cases were distributed as follows: (1) the group with CD - 20 cases, (2) the group with UC - 27 cases, (3) the group with AS - 28 cases, (4) the group with IBD and AS - 17 cases and (5), the control group - 32 cases.

CD patients were divided into L1-ileitis, L2-colitis, and L3-ileocolitis. UC patients were divided into E1-proctitis, E2-left colitis, and E3-pancolitis and AS patients were grouped in axial disease and peripheral disease. For evaluating disease activity, CDAI (Crohn Disease Activity Index) scores were calculated for patients with CD, the Mayo score was used for UC

cases, and the BASDAI (Bath Ankylosing Spondylitis Disease Activity Index) and BASFI (Bath Ankylosing Spondylitis Functional Index) scores were used for AS cases.

Intestinal microbiota analysis was performed using real-time polymerase chain reaction (real-time PCR) in the stool samples. From each patient included in the study, 20 g of faeces were obtained. The stool samples were transported as quickly as possible (within the same day) to the microbiology laboratory and were frozen at a temperature of -80 ° Celsius for a maximum of one week until DNA extraction. DNA extraction of the stools and real-time PCR were done in the Microbiology Laboratory of University of Medicine and Pharmacy "Grigore T. Popa" Iasi. The PCR reaction targeted the following bacteria: total bacteria, *Bacteroides, Bifidobacterium, Clostridium coccoides (XIVa) (C. coccoides), Clostridium leptum (IV) (C. leptum), Faecalibacterium prausnitzii (F. prausnitzii), Lactobacillus, Escherichia coli* (*E. coli*). The β-globin gene was the internal control. The primer's structures were taken from the article published by Wang et al. (Wang et al, 2014). In order to reduce the quantitative error of detected bacteria and to characterize changes in bacterial copies, the abundance of the 16S rRNA gene was calculated from standard curves. Specific bacterial groups were expressed as a percentage of total bacteria identified using universal primers.The obtained data were centralized in the SPSS 22.0 database. Statistical analysis used both descriptive and analytical methods at 95% significance level (CI 95%). Some of the statistical methods used were: ANOVA and chi-square tests, linear regression, Odds Ratio. For comparisons between groups having a non-linear distribution, the Mann-Whitney U test and Kruskal-Wallis method were used. A p-value of less than 0.05 ($p<0.05$) was considered statistically significant.

Results

The characteristics of the patients included in our study are presented in Table 2.5.

The total bacteria was lower in all studied groups (CD, UC, AS, IBD+AS) compared to the control group. The lowest intestinal microbiota was highlighted in patients with CD, followed by IBD + AS cases (Fig. 2.2.). Patients with UC showed a higher total bacteria compared to CD and AS patients.

Specific bacterial counts were calculated as a percentage of the total bacterial counts of each study group. The diversity of intestinal microbiota for each study group is presented in Fig. 2.3. UC and CD had a similar profile, with an increased percentage of *Bacteroides* and *E. coli* and a decreased percentage of *C. coccoides*, *C. leptum* and *F. prausnitzii* compared to the control group. Patients with AS had similarities with the control group, while patients with associated IBD and AS seemed to have a similar bacterial distribution with IBD patients, with the highest percentage of *Bacteroides* from all studied groups.

In patients with CD, statistically significant data regarding the location of the disease was observed only for total bacteria, *Bifidobacterium*, and *Lactobacillus.* The number of total bacteria was significantly increased in the L2 form of CD ($p=0.034$), while the percentages of *Bifidobacterium* ($p=0.010$) as well as *Lactobacillus* group ($p=0.023$) were higher in the L3 form of CD. In UC cases, significant correlations were highlighted only for *Bacteroides*. In the E2 form of UC, the quantity of *Bacteroides* was much higher compared to the E3 form ($p=0.004$). Other bacterial groups also presented a numerical growth in the E2 form of UC.

Concerning AS patients, significant correlations were observed only for the *Bifidobacterium* species, significantly increased in the axial form compared to peripheral disease ($p=0.035$). Many other bacterial groups were numerically increased in the axial form of AS (except *Bacteroides*), but without significant differences.

Table 2.5. Characteristics of the study group

	CD (n=20)	UC (n=27)	AS (n=28)	IBD+AS (n=17)	Control (n=32)
Gender (n,%)					
Female	11 (55%)	12 (44.4%)	11 (39.3%)	5 (29.4%)	20 (62.5%)
Male	9 (45%)	15 (55.6%)	17 (60.7%)	12 (70.6%)	12 (37.5%)
Age, years					
Median (SD)	51.2	48.93 (9,7)	52.1 (13,6)	52.4 (8.9)	61.5(10)
Range	46-55	45-52	46-57	47-57	57-65
IBD extension (n)					
El	NA	5	NA	NA	NA
E2	NA	12	NA	8	NA
E3	NA	10	NA	NA	NA
LI	3	NA	NA	NA	NA
L2	6	NA	NA	5	NA
13	11	NA	NA	4	NA
Disease activity					
CDAI (Median)	251.47	NA	NA	159.11	NA
Mayo (Median)	NA	5.88	NA	4.37	NA
BASDAI (Median)	NA	NA	4.83	4.01	NA
BASFI (Median)	NA	NA	9.11	4.34	NA

CD: Crohn's disease; UC: ulcerative colitis; AS: ankylosing spondylitis; IBD: inflammatory bowel disease; SD: standard deviation; E1I: proctitis; E2: left colitis; E3: pancolitis; LI: ileitis, L2: colitis; L3: ileocolitis; CDAI; Crohn's Disease Activity Index; BASDAI: Bath Ankylosing Spondylitis Disease Activity Index; BASFI: Bath Ankylosing Spondylitis Functional Index; NA: not assessed.

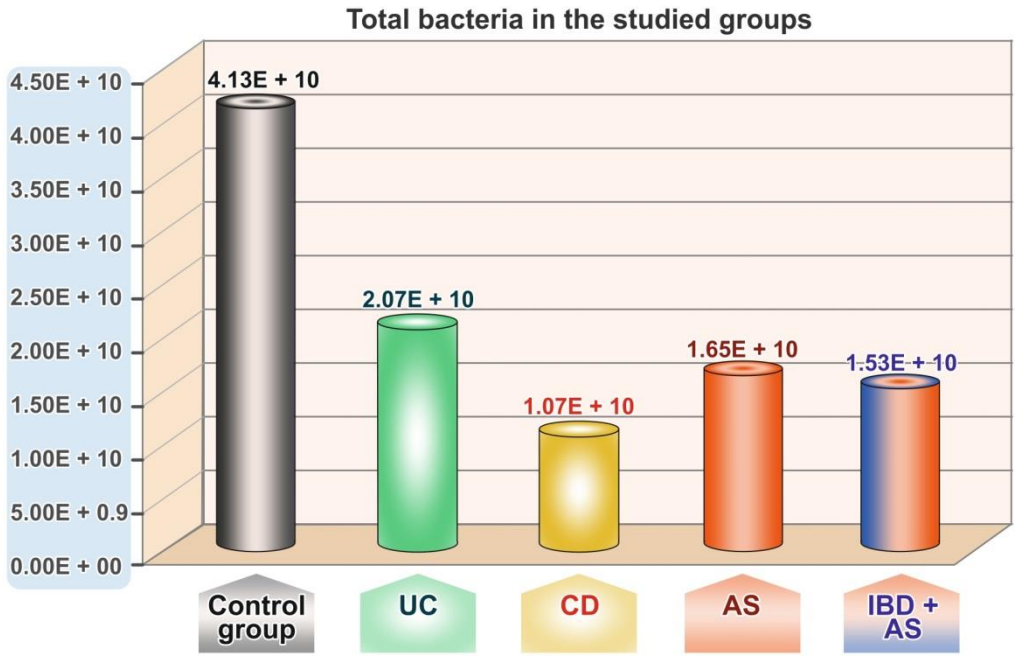


Fig. 2.2. Quantification of total bacteria in studied groups - log10CFU (faeces/g); CD: Crohn's disease; UC: ulcerative colitis; AS:ankylosing spondylitis; IBD: inflammatory bowel disease.

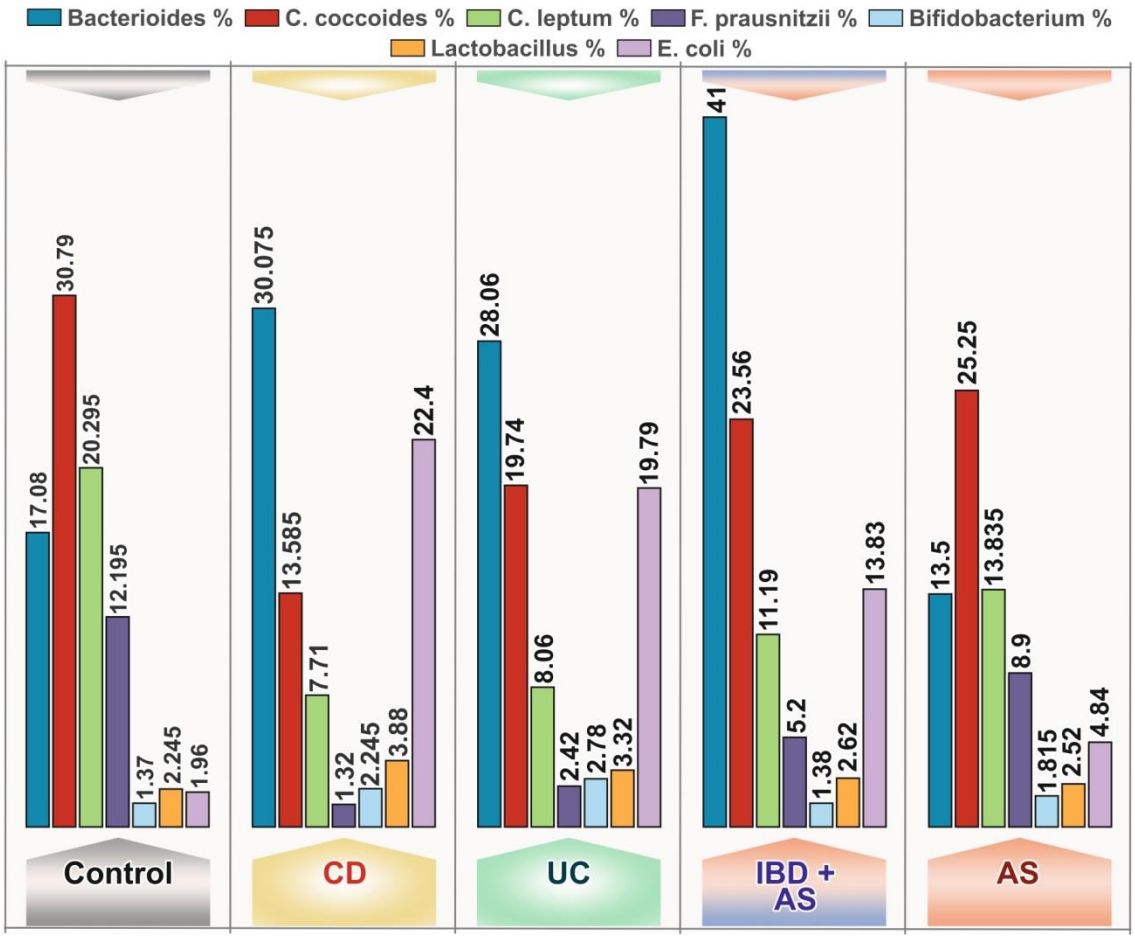


Fig. 2.3. Specific bacterial counts calculated as a percentage of the total bacterial counts of each study group. Crohn's disease, A: ulcerative colitis, B: ankylosing spondylitis, C: inflammatory bowel disease (CD or UC) + ankylosing spondylitis, D: control group.

Statistically significant inverse correlations were demonstrated between the CDAI score and the total bacterial group (p=0.023, r=-0.507), respectively *Bacteroides* (p=0.021, r=-0.511). Positive Spearman correlations (but without statistical significance) were found between CDAI score and *C. coccoides*, *Bifidobacterium* and *Lactobacillus*. Significant associations were observed between the Mayo score and *Lactobacillus* (p=0.001), respectively *E. coli* (p=0.001). In the group of AS cases, no significant correlations were observed between BASDAI and BASFI and the bacterial groups. However, significant associations have been demonstrated in IBD+AS patients. Thus, the BASDAI score was inversely correlated with the total bacterial group (p=0.010, r = -0.606). In addition, the BASFI score correlated with all bacteria (p=0.001, r = -0.764), *F. prausnitzii* (p=0.010, r = 0.606), *Bifidobacterium* (p=0.016, r = 0.575), *Lactobacillus* (p=0,001, r=0,843) and *E. coli* (p=0.016, r = 0.575). Table 2.5 shows the correlations between intestinal microbiota composition and disease activity scores.

Discussions

This was the first Romanian study that analyzes the composition of intestinal microbiota, using faeces real-time PCR, in patients with IBD, AS, and IBD associated with AS. The PCR reaction targeted the following bacteria: total bacteria, *Bacteroides, Bifidobacterium, Clostridium coccoides (XIVa) (C. coccoides), Clostridium leptum (IV) (C. leptum), Faecalibacterium prausnitzii (F. prausnitzii), Lactobacillus, Escherichia coli* (*E. coli*). Our analysis has been focused on the main bacteria found in the microbiome from all the main phyla: *Firmicutes* (*Clostridiales, F. prausnitzii, Lactobacillus*), *Bacteroidetes* (*Bacteroides*), *Actinobacteria* (*Bifidobacterium*), and *Proteobacteria* (*E. coli*). The main disadvantage is that

many other species involved in IBD and/or AS gut dysbiosis (e.g. *Ruminococcus*, *Prevotella*, *Faecalibacterium*, etc) were not analyzed.

Table 2.5. Correlations between intestinal microbiota composition and disease activity scores

	CDAI (CD group)		MAYO (UC group		BASDAI (AS group)		BASDAI (IBD+AS group)		BASE (AS group)		BASH (IBD+AS group)	
	r	p	r	p	r	p	r	p	r	p	r	p
All bacteria	-0.507	**0.023**	-0.061	0.451	-0.336	0.080	-0.606	**0.010**	-0.018	0.927	-0.764	**0.001**
Bacteroides %	-0.511	**0.021**	0.2	0.375	-0.371	0.052	-0.318	0.148	-0.061	0.758	-0.160	0.539
C. Coccoides %	0.198	0.402	-0.239	0.90	0.111	0.573	-0.265	0.304	0.053	0.788	0.050	0.849
C. leptum %	-0.409	0.079	-0.225	0.463	0.430	0.077	-0.265	0.304	0.010	0.960	0.050	0.849
F. prausnitzii %	-0.056	0.814	-0.152	0.90	-0.371	0.052	0.764	**0.001**	0.104	0.597	0.606	**0.010**
Bifidobacterium %	0.171	0.471	0.189	0.318	0.183	0.351	0.063	0.810	0.048	0.809	0.577	**0.016**
Lactobacillus %	0.053	0.823	0.139	**0.001**	0.331	0.085	0.488	**0.047**	0.019	0.924	0.843	**0.001**
E. coli %	-0.016	0.956	0.156	**0.001**	0.309	0.110	0.063	0.810	-0.010	0.960	0.577	**0.016**

CDAI= Crohn Disease Activity Index, BASDAI=Bath Ankylosing Spondylitis Disease Activity Index. BASFI= Bath Ankylosing Spondylitis Functional Index. CD= Crohn's disease, UC=ulcerative colitis, AS=ankylosing spondylitis, IBD=inflammatory bowel disease, r=Spearman correlation

The total bacteria was lower in all investigated cases compared to the control group. Corroborating the presented results, we can divide the studied groups into 3 categories according to the importance of intestinal dysbiosis: groups with a significant dysbiosis: CD and UC, the group with an intermediate dysbiosis: IBD associated with AS, and the low dysbiosis group: patients with AS. The link between the pathological gut and joint inflammation is not fully understood. Recent studies identified genes that encode proteins involved in the IL-23/Th 17 T-cell differentiation in both IBD and AS patients (Fragoulis et al, 2016). Gut – T cells activated by antigens can migrate to the joints and induce inflammation. It has been shown that leukocytes populations from the inflamed gut can bind to synovial vessels and settle into the joint, using multiple adhesion molecules (αEβ7 integrins, vascular adhesion protein-1, intracellular adhesion molecule-1 (ICAM-1/CD54) (Fragoulis et al, 2019).Our study suggests that other mechanisms, more than gut dysbiosis, could be involved in the pathogenesis of AS compared to IBD patients.

A quantitative and qualitative (biodiversity) reduction in gut microbiota in IBD patients is supported by numerous published studies (Willing et al, 2010; Wills ES et al, 2014). The study published by Frank et al. (Frank et al, 2007) pointed out important changes in intestinal microbiota in patients with IBD. The authors noticed significant bacterial decreases especially in the phyla *Firmicutes*and *Bacteroidetes*, both in CD and UC cases.

In IBD patients, both CD and UC, we noticed a decreased percentage of *C. coccoides*, *C. leptum*, and *F. prausnitzii*, followed by an increased percentage of *Bacteroides* and *E. coli*. *F. prausnitzii* produces butyrate and plays an important role in epithelial barrier integrity and immune modulation. A decrease in the *C. leptum* groups, especially *F. prausnitzii*, has been reported in many studies (Machiels et al, 2014; Fujimoto et al, 2013; Varela et al, 2013). Regarding *Bacteroides*, the results of the studies are contradictory. Some of them reported a reduction of the *Bacteroides* group in IBD patients, especially in CD patients (Gevers et al, 2014; Aomatsu et al, 2012) but others, similar to our results, demonstrated an increased amount of this phylum in IBD (Andoh et al, 2011; Rehman et al, 2016). An increased percentage of *E. coli*, again similar to our results, has been demonstrated in many other studies (Duboc et al, 2017; Knoll et al, 2017).

In patients with CD, statistically significant data regarding the location of the disease were observed only for total bacteria, *Bifidobacterium*, and *Lactobacillus.* In UC cases, significant correlations were highlighted only for *Bacteroides*. It could be inferred that, in CD patients, the extension of the intestinal inflammation is a significant factor for intestinal dysbiosis. Our results are consistent with the data published in the literature. The study of Vrakas (Vrakas et al, 2017) confirms the worsening of intestinal dysbiosis in an extended and active form of CD. On the other hand, in UC cases, most bacterial groups showed a numerical growth in E2 form. The results of different studies are contradictory. Some support the worsening of intestinal dysbiosis in patients with an extensive and active UC (Vrakas et al, 2017). However, Pascal's study (Pascal et al, 2017) presents the augmentation of dysbiosis in proctitis. Many factors may explain these discrepancies: sample source (biopsy or stool), disease activity (flare or remission), medication, comorbidities, diet, smoking status, body mass index, methods used to analyze the microbiota, etc (Matsuoka, Kanai, 2015).

The number of studies regarding intestinal microbiota analysis in AS patients is lower, but they also support the presence of intestinal dysbiosis characterized by a decrease in total bacterial diversity (Stebbings et al, 2002; Matzkies et al, 2012). In 2017 Wen published an article that highlights the particular microbial profile in AS patients (Wen et al, 2017). In this study, the AS patients showed increases in the amount of *Prevotella melaninogenica*, *Prevotella copri*, and *Prevotella sp. C561* and decreases in *Bacteroides spp*. Similar to our results, *Bifidobacterium* was increased in AS patients (Wen et al, 2017). Many other studies noticed a higher amount of the *Bifidobacterium* genus, including *B. bifidum* species in AS patients (Breban et al, 2017; Stoll et al, 2018). Again similar to our results, *Bacteroides* genera were found to be higher in AS patients by several other studies (Stoll et al, 2018; Zhang et al, 2015).

Our study also analyzed the composition of intestinal microbiota in patients having the association of IBD and AS. We found that the level of dysbiosis in IBD associated with AS was intermediate, less than in IBD and more than in AS. Compared with AS, we found an increase of *Bacteroides* and a decrease of *F. prausnitzii, C. coccoides, C. leptum*. Compared with IBD, we found an increase of *Bacteroides* and *E.coli* and a decrease of *F. prausnitzii*. Many other studies have demonstrated a decrease of *Fecalibacterium* in patients with both diseases (IBD and AS) (Breban et al, 2017). Salem meta-analysis (Salem et al, 2019) focused on similarities and differences in gut microbiome in patients with chronic rheumatic inflammatory diseases and IBD. The authors noticed an increase in *Firmicutes* genera *Lactobacillus* and *Staphylococcus*, *Actinobacteria,Bifidobacterium*, and *Proteobacteria* genera such as

Pseudomonas, *Klebsiella*, and *Proteus*, whereas *Firmicutes* phyla, *Faecalibacterium*, *Roseburia* genera, and *Verrucomicrobia* phylum were decreased in both chronic rheumatic diseases and IBD.

Last but not least, our study investigated the link between intestinal microbiota and the activity of the diseases considered. Our results support the close relationship between disease activity and the degree of intestinal dysbiosis. The higher CDAI and Mayo scores are, the more important intestinal dysbiosis becomes. We observed significant associations between the Mayo score and *Lactobacillus.* Our results are similarto those of Wang [19] who found that *Bifidobacterium* and the *Lactobacillus* group were increased in active IBD patients. In patients with AS, no significant correlations were observed between activity scores (BASDAI, BASFI) and the bacterial groups. Only for the group with AS and IBD association, there were significant correlations between the composition of gut microbiota and BASDAI, respectively BASFI scores. An increase in AS activity corroborated with a decrease in functionality can be associated with an increase in pro-inflammatory bacteria.

An interesting finding of our study is the unexpected significant correlation between the *Lactobacillus* and *Bifidobacterium* with UC activity, BASFI, and BASDAI scores in the IBD associated with AS group, and, respectively AS and BASFI scores in the combination group. Salem noticed an increase in *Lactobacillus* and *Bifidobacterium* in both IBD and chronic rheumatic diseases (Salem et al, 2019). More than that, *Lactobacillus R. Gnavus* was more present in mice with arthritis and colitis compared with an arthritis - only group (Liu et al, 2016). Further studies are necessary to determine if it is an epiphenomenon due to ecological niche competition or if there are some specific species involved or it is a microbial signature in IBD associated with AS. Until then we have to be more cautious when prescribing probiotics containing *Lactobacilli* and *Bifidobacterium* to patients with active IBD associated with active AS.

Our study has several limitations: it analyzed a small number of bacterial populations (3 species, 2 genera, 1 phylum) and therefore cannot sufficiently characterize the entire microbiota (all bacteria, but also virus or fungi); for microbiome analysis, we used RT-PCR, which is less specific than 16S rRNA gene sequencing or shotgun metagenomics sequencing. Next generation sequencing or shotgun metagenomics are considered superior when compared to RT-PCR, with a higher power to detect novel genes and a higher sample throughput. However, RT-PCR remains a viable method, with a familiar workflow, and it has been successfully applied for quantification of bacterial DNA in many previous studies regarding microbiota changes. We analyzed only faeces samples, not colonic biopsies; we didn't compare various treatments (immunomodulators, biologics) that can influence the composition of the microbiota. With all these limitations, as far as we know, it is the first study which directly compares the composition of gut microbiota in IBD, AS, and association IBD + AS patients.

Conclusions

Our results support the presence of a link between intestinal bacterial composition, IBD, and AS. Intestinal dysbiosis in patients with CD and UC is quite similar, being more pronounced compared to the control group or AS patients and it is correlated with disease activity. In the association of AS with IBD dysbiosis is less significant compared to IBD, but it is associated with higher rheumatic disease activity scores. *Bifidobacterium and Lactobacillus* were found to be increased in the association between active IBD and active AS. Further studies are needed for a better understanding of the interactions between the microbiome and immune system within the microbiota-joint–gut axis.

Main points of this area of research (EIM manifestations in IBD patients) are presented in Table 2.6.

Table 2.6. Main points of studies of EIM in IBD patients

SCIENTIFIC/CLINICAL RELEVANCE
• EIM have a low prevalence in our geographical region, the most common being the articular ones.There are more common in CD compared to UC. The correlation with intestinal complications is an argument for intestinal inflammation as a common etiopathogenic link
• Osteopenia/osteoporosis are common complications in IBD, related especially with smoking and corticosteroids.
• Low levels of vitamin D have been identified especially in active CD, but did not correlate with bone mineral density
• Intestinal dysbiosis is associated with both IBD and AS. In the association of IBD with SA, dysbiosis is intermediate, but it is associated with the more severe articular disease. *Bifidobacterium and Lactobacillus* (commonly used as probiotics!) were found to be increased in the association between active IBD and active AS.

2.3. IMPAIRMENT OF QUALITY OF LIFE IN IBD PATIENTS

2.3.1. State of the art

QoL is perhaps the most important outcome in IBD management. More than 20 years ago, it was proven that IBD was likely to impact the QoL (Casellas, 2001). Since then, various instruments approved for QoL assessment have been used. Health-related QoL was defined as the functional effect of a disease and its treatment on a patient, as perceived by the patient. The patient's perception on his/her own health is vital for the understanding of the way in which (s)he carries out activities at home, at school, or at work IBD symptoms (abdominal pain, diarrheal stools or constipation, nausea, weight loss, and marked asthenia) are manifested especially in disease activity, with the activity and severity of the disease being the main determinants of QoL (Knowles et al, 2018; Knowles et al, 2018).QoL is especially affected in patients with active disease but, even in remission, is lower than the general population (Mikocka-Walus, 2008).

The need for a QoL assessment emerged in order to reach the medical goal of improving the general well-being of patients. This is useful in quantifying the effects of the treatment, describing the nature and severity of a condition and assessing prognosis, but also in identifying the most appropriate type of therapy, comparing the benefits and side effects of different types of treatment (Sajid et al, 2008). International Organization for the Study of IBD has been trying since 2013 to set therapeutic targets in IBD (STRIDE - The Selecting Therapeutic Targets in Inflammatory Bowel Disease). The latest recommendations, developed by expert consensus based on accumulated evidence, include the evaluation of QoL as an important therapeutic target, both in CD and in UC (Turner et al, 2021).

The results of my research have been materialized in the following papers:

1. Dranga M, Boiculese LV, Popa IV, Floria M, Gavril IO, Bărboi OB, Trifan A, Prelipcean Cijevschi C, **Mihai C**, Gavrilescu O. Anemia in Crohn's Disease—The Unseen Face of Inflammatory Bowel Disease. *Medicina* 2021; 57: 1046. IF = 2.43
2. Gavrilescu O, Cijevschi Prelipcean C, Dranga M, Soponaru C, **Mihai C**. The specialized educational and psychological counseling in inflammatory bowel disease patients – a target or a challenge? *Turk J Gastroenterol* 2020; 31(11): 760-6. IF=1.852
3. Gavrilescu O, **Mihai C**, Anton Paduraru DT, Moisa S, Ciubara A, Cijevschi Prelipcean C. Impact of Inflammatory Bowel Disease on Quality of Life. *Revista de cercetare si*

interventie sociala 2015; 50: 80-95. IF=0.424
4. Gavrilescu O, Dranga M, Cardoneanu A, **Mihai C**, Prelipcean Cijevschi C. Irritable bowel disease versus Crohn's disease – impact on quality of life. ISI Proceedings. Meeting of the Romanian Society of Neurogastroenterology with Rome IV Regional Central – East European Meeting, Neurogastro 2017: 87-91.
5. Gavrilescu O, Cijevschi Prelipcean C, Drug V, Toader E, Mihai B, Dranga M, Badea M, **Mihai C**. Assessment of quality of life in patients with irritable bowel syndrome versus ulcerative colitis.Proceeding of 49th Annual Scientific Meeting of the European Society of Clinical Investigation, 27-30 May 2015, Cluj Napoca, Romania: 191-196.
6. Cardoneanu A, Rezus E, **Mihai C**, Drug V, Cijevschi Prelipcean C. The impact of inflammatory bowel disease and ankylosing sponyilitis on patients functionality and quality of life – a point of view. Proceeding of 49th Annual Scientific Meeting of the European Society of Clinical Investigation, 27-30 May 2015, Cluj Napoca, Romania: 89-92.
7. Gavrilescu O, **Mihai C**, Dranga M, Jigaranu O, Cijevschi Prelipcean C. Weight status and quality of life in inflammatory bowel diseases. *Rev Med Chir Soc Med Nat Iasi* 2017; 121 (1): 46-56.
8. Gavrilescu O, Dranga M, Mihai C, Cijevschi Prelipcean C. Quality of life in Crohn's disease patients.*The Medical-Surgical Journal* 2015;119(2): 340-345.

2.3.2. QoL in IBD patients

Background & Aim.

Over the last period, there has been growing awareness on the chronic nature of IBD and its underlying psycho-social implications. The psycho-social impact of chronic diseases is always difficult to assess, especially due to the multifactorial nature of the psychological and social interactions, where a patient's disease is just one of the important variables (Pizzi et al., 2006). Various instruments approved for QoL assessment have been used, including questionnaires specific to certain diseases. Studies have pointed to the fact that an improvement in the patients QoL is inversely proportional to the activity of the disease (Casellas et al, 2000; Lixet al, 2008). Consequently, irrespective of the type of treatment – medical or surgical – remission contributes to improving QoL. Disease remission seems to increase the QoL score in all fields, not just in the field of abdominal symptoms, but also in systemic, social symptoms, or the physical and emotional function. However, the QoL score is still more reduced in the patients with IBD in remission, compared to the general population (MikockaWalus et al, 2008). Our study aimed to assess the impact of IBD (UC, CD) on the patients' QoL and to identify the significant changes in QoL depending on the developmental particularities of IBD and the epidemiological parameters.

Material and methods

A prospective study was conducted over a period of 36 months (October 2011 – October 2014) at the Institute of Gastroenterology and Hepatology, Iasi (Gavrilescu et al, 2015). The study was conducted on 254 patients diagnosed with IBD, 187 patients with UC and 67 patients with CD. The UC or CD diagnosis was supported by complete hematological, biochemical tests, endoscopic and imagistic examinations, as well as histopathology confirmation. The activity of the disease was quantified by means of the UCDAI/ CDAI score. The quality of life was assessed using the questionnaire IBDQ-32 (Inflammatory Bowel Disease Questionnaire), which is one of the most widely used questionnaires in QoL assessment. The questionnaire contains 32 questions grouped into four fields: bowel symptoms (diarrhea, abdominal pain, rectal bleeding, and urgency), systemic symptoms (fatigue, sleep disorders), emotional functions (depression, irritability, anger) and social functions

(absenteeism, affected social status, sexual activity). The answers were marked on a scale from 1 (the worst) to 7 (the best). The total score ranged between 32 and 224. The lowest the score, the most affected the QoL.

Results

The total IBDQ score was slightly more reduced in the patients with UC compared to the patients with CD. Of the 4 subscales of the IBDQ score, the scores highlighting the emotional and social component were significantly more reduced in the patients with UC, while the scores for the bowel symptoms were significantly more reduced in the patients with CD (Table 2.7.).

Table 2.7. IBDQ score in UC vs. CD patients

IBDQ Score	UC Group(n=187) Average score± SD(extreme)	CD Group (n=67) Average score ± SD(extreme)	P values for FANOVA test
Total score	134.74±32.52 (66-200)	138.82±31.84(70-200)	0.376
Bowel symptoms	33 68±8 15(15-51)	29.78±7 81 (14-46)	**0.001**
Systemic symptoms	33.77±8.26(17-52)	32.39±8 01 (16-50)	0.239
Emotional functions	33.02±9.36(11-54)	37.85±9 07(16-58)	**0.001**
Social functions	34.45±8.77(17-55)	38 42±7 99 (22-53)	**0.001**

In both the patients with UC and with CD, the correlations between the UCDAI, respectively CDAI score and the IBDQ score were indirect, highlighting reduced values of IBDQ in patients with a severe activity of the disease (Fig. 2.4.). The severe flare of the disease had a negative impact on the patients' QoL.

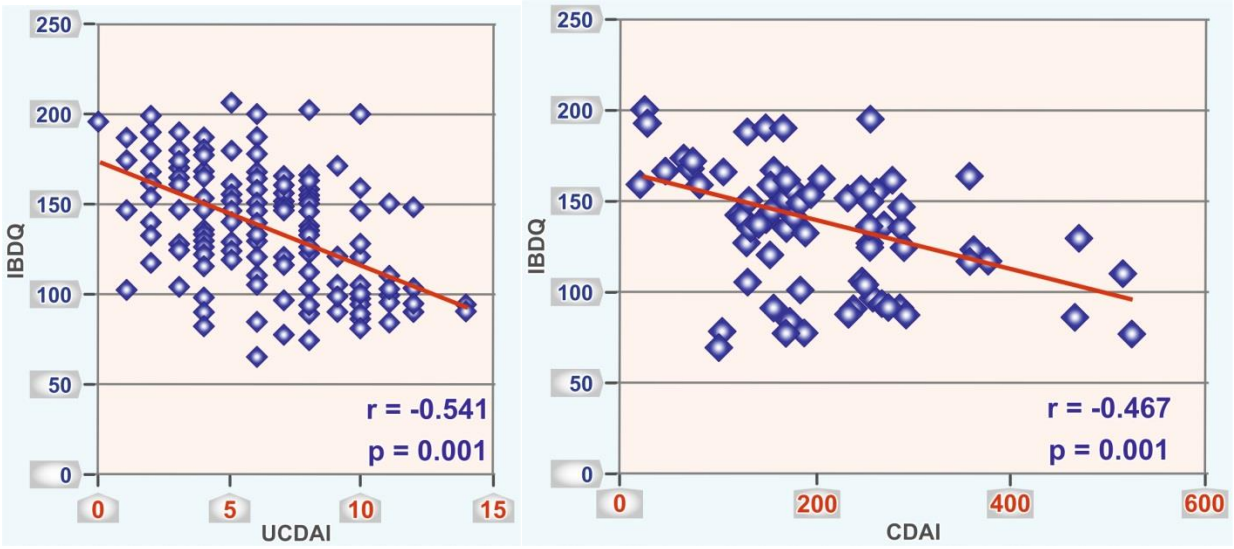


Fig. 2.4. Correlation of the IBDQ scores with the UC/ CD activity

Different parameters were analyzed (age, sex, smoker status, background environment, marital status, treatment followed), following their influence on QoL.

The age of the patients with UC was in low direct correlation with the IBDQ score; slightly higher IBDQ values were noticed in older patients, but no statistically significant differences were highlighted (r= +0.109; R=0.0118; p=0.138). Similarly, the age of the patients with CD did not correlate with the IBDQ score (r = + 0.002; R = 0.000006; p = 0.987). The lowest IBDQ score was recorded in a single patient aged under 20 years old (87), and the highest score was recorded in a single patient aged over 80 years old (158).

In the patients with UC, the IBDQ score presented values ranging from 66 to 207; the extreme values were recorded in the male patients where the group average was 136.97±34.68, slightly higher compared to the one recorded in the female group, with an average of 131.56±29.08 (p = 0.264). In the patients with CD, both the average IBDQ score and its subgroups were slightly higher in the female patients with CD (p>0.05).

In both groups, the average IBDQ score did not present significant differences between the average values recorded in smokers (UC: 133.85±32.89, CD: 133.62 ±36.63), compared to non-smokers (UC:135.32±32.41, CD: 143.47±27.44) (UC: p=0.764, CD: p=0.228).

Most patients came from the urban environment (UC = 66.8%, CD = 76%). The average IBDQ score was slightly higher in the patients with UC from the urban environment (137.50±31.71) compared to the number registered for the patients from the rural environment (129.19±33.67) (p = 0.100). In the sample of patients with CD, the overall IBDQ score registered a slightly increased average value in the patients from the rural environment, without registering significant differences between the background environments (p = 0.363).

Marital status did not influence QoL significantly in any of the two groups, although the IBDQ score was slightly higher in married patients.

As far as QoL depending on the type of treatment underwent is concerned, both in the patients with UC and in those with CD, corticotherapy administered during the disease activity periods was associated with lower IBDQ scores. Considering the fact that corticotherapy is administered solely during the disease activity periods, the low values of the IBDQ score can be interpreted also within the severe activity periods of the disease. Moreover, the patients undergoing biological therapy had a higher IBDQ score (Tables 2.8. and 2.9.). In this sample of subjects, biological therapy was used for both inducing and maintaining remission, but the number of patients who achieved remission when assessing QoL was higher. Therefore the high QoL scores in this group of patients can be due to the weaker, or even absent symptoms.

Table 2.8. IBDQ score in patients with UC based on type of treatment

IBDQ score	Corticotherapy			Biological therapy		
	Yes	No	P	Yes	No	P
Total score	128.37±33.39	137.33±31.92	0088	142.67±40.78	134.34±32.14	0455
Bowel symptoms	31.76±8.54	34 41±7 90	0044	35 67±10 26	33.54±8.06	0447
Systemic symptoms	31.94±8.86	34 51±7.92	0050	36.67±10.64	33 62±8.13	0 282
Emotional functions	31.67±9.26	33 57±9.37	0.208	35.33±11.95	32.90±9.23	0.449
Social functions	33.02±9.48	35.04±8.34	0.154	35.44±9.61	34.40±8.75	0.730

Table 2.9. IBDQ score in patients with CD based on type of treatment

IBDQ score	Corticotherapy			Biological Therapy		
	Yes	No	P	Yes	No	
Total score	131.29±32.87	144 23±29 99	0.099	158 00±2414	135.05±31 74	0.027
Bowel symptoms	28 14±7 89	30.95±7.68	0.150	35.64±6 39	28 63±7 62	0.006
Systemic symptoms	30.86±8.11	33.85±7.88	0.082	38.64±6.92	31.16±7.80	0.004
Emotional functions	35.89±9.74	39.26±8.36	0.134	40.64±7.20	37.30±*9.31	0266
Social functions	36.54±8.16	39.77±7.58	0.100	42.82±5 90	37 55±803	0.043

Discussions

Similarly to the data obtained from the literature, the present study confirms the fact that the disease activity is the factor likely to have the highest impact on QoL in IBD patients.In the present study, both the UC and CD had a negative impact on all the aspects related to the QoL, which is likely to favour the development of depressive states and anxiety during and after periods of disease activity. The total IBDQ score in patients with UC was relatively low, compared to the sample of patients with CD. Equally, the subscores for the emotional and social components of QoL were significantly lower ($p<0.05$).

Nevertheless, there are studies which demonstrated that patients with CD present more severe psycho-social dysfunctions, a lower level of wellness, anxiety and depressive states, as well as deeper effects on QoL compared to UC (RombergCamps et al, 2010; Simren et al, 2002). Since the disease activity is a factor likely to have a stronger impact on QoL in patients with IBD, in our study, lower QoL scores in UC patients can be attributed to the higher number of patients with severe forms of UC compared to the patients with severe forms of CD.

In the present study, the variables age, gender, smoking and marital status did not influence the total IBDQ score and subscores although some studies have shown that QoL is more affected in female compared to male patients with IBD (Irvine, 1995). In our study, corticotherapy administered during the disease activity periods was associated with lower QoL scores, while the patients under biological therapy with Ac anti-TNF- α had a higher QoL score.Nevertheless, considering the fact that corticotherapy is administered only in the disease activity periods, used for inducing remission, the low QoL scores in this sample of patients can be considered secondary to their symptomatology, and not necessarily to corticotherapy. On the other hand, biological therapy in this sample of patients was used for both inducing and maintaining remission, but the number of patients who achieved remission with biological therapy when assessing QoL was higher. Therefore the high QoL scores in this group of patients can be due to the weaker, or even absent symptoms.Under these circumstances, QoL damage could be considered secondary to disease severity and not to therapy (Kalafateli et al, 2013).

Conclusions

To conclude, the disease activity represents the factor likely to have the stronger impact on QoL in patients with IBD. The general perception on the health state in these patients is more pessimistic during the disease activity periods. The patients with UC presented relatively low IBDQ scores compared to the sample of patients with CD. Additionally, in the patients with CD, the bowel symptoms had a higher negative impact on the patients' QoL compared to UC. Age, sex, background environment, smoking status and marital status did not influence

significantly the QoL status. QoL assessment is helpful in identifying the patients requiring specialised support. Additionally, it has an important role in understanding the real impact of the disease on the patients, as well as in redefining the strategies for improving QoL in patients with IBD.

2.3.3. QoL in CD vs diarrhea – IBS patients

Backround & Aim.Both the organic and the functional disorders can harm patients, having a negative impact on their QoL. The present study aimed to assess and compare the impact of CD – an organic disorder, and that of IBS-d (diarrhea-predominant IBS) – a functional disorder, on the patients' QoL.

Material and methods. An analytical prospective case-control study was conducted at the Institute of Gastroenterology and Hepatology Iași (Gavrilescu et al, 2017). The study included 135 patients, distributed into 2 groups: group A formed of 72 patients with CD and group B – 63 patients with IBS-d. The CD diagnosis was sustained by complete hematologic-biochemical tests, colonoscopy, computed tomography and histopathologically confirmed. The disease activity was quantified through the CDAI score. The IBS-d diagnosis was sustained by the ROME III criteria, excluding organic disorders (hematologic-biochemical explorations, copro-parasitological examination, stool culture and colonoscopy – normal). Quality of life was assessed by means of the SF-32 questionnaire (Short Form-32), one of the most frequently used generic instruments used for measuring the physical and mental state. SF-32 uses eight scales, grouped into two generic concepts: physical and mental health. Physical health was assessed by the first 4 scales (physical function, physical activity reduction, somatic pain, general health state, vitality), while mental health was assessed by the last 4 scales (general health state, vitality, social function, emotional function and mental health state). The scores vary from 0 to 100, in such a way that the higher the score, the better the patient's functioning in the respective field. The interpretation was carried out both on each dimension individually, and based on the two general scores: the physical and the general component.

Results

The two groups had a similar component in terms of age, sex, smoking status, background. The only statistically significant difference was related to marital status (the married status prevailed among the patients with IBS-d). According to the results of the SF-36 questionnaire, the patients with CD presented a significantly lower score of the QoL physical component ($p< 0.001$), compared to the patients in the IBS-d group (Table 2.10.).

Table 2.10. Quality of life in patients with CD compared with diarrhea-IBS patients: physical and mental components

SF-32Functions	Group A(n=72) Average score±SD(extreme)	Group B (n=63) Average score±SD(extreme)	P values for F_{ANOVA} test
SF-physical component	61.25±5.00 (54-66)	68.95±5.27 (63-75)	**0.001**
SF-mentalcomponent	60.03±3.56 (57-65)	60.81±1.85 (58-63)	0.120

Three of the subscale scores reflecting the physical state of the QoL (physical state, general health, vitality) were significantly lower in the patients with CD, compared with those

with IBS-d (p< 0.001). The disorder these patients suffer from seems to limit their physical activities that suppose moderate or high effort. The values of the score that reflect the social activity were also lower compared with the IBS-d group (p<0.001). For the patients with IBS-d, the mental component of the quality of life was more deeply influenced. The scores of the subscales assessing the mental component of quality of life (vitality, social function, emotional role and mental health) were significantly lower compared with the patients in the CD group (p< 0.001) (Table 2.11).

Table 2.11. Quality of life in patients with CD compared with diarrhea- IBS patients: the subscale scores

SF-32 Function	Group A (n=72) Averagescore±SD(extreme)	Group B (n=63) Average score±SD(extreme)	P values for F_{ANOVA} test
Physical functions	19.64±4.53 (14-25)	23,46±2.99 (20-27)	**0.001**
Physical role	5.78±1.73 (4-8)	5.94±0.76 (5-7)	0.502
Somatic pain	7.17±3.18 (3-10)	7.71±0.85 (7-9)	0.187
General health	14.67±2.37 (13-18)	16.62±1.22 (16-19)	**0.001**
Vitality	14.0±0 (14-14)	15.22±1.88 (12-17)	**0.001**
Social functions	5.31±0.46 (5-6)	6.48±0.50 (6-7)	**0.001**
Emotional role	5.39±0.93 (4-6)	3.54±0.50 (3-4)	**0.001**
Mental health	20.67±0.95 (20-22)	18,95±0.68 (18-20)	**0.001**

Discussions.The results of the study prove the importance of estimating QoL both in the patients with CD – organic disorder, and in those with IBS-d – functional disorder. Both diseases had a negative impact on the patients' QoL.The patients with IBS-d seem to be more receptive to the impact of gastrointestinal symptoms on the psychosocial variables, compared with the patients with CD, whose QoL is affected more by the physical component of the disease.

The data obtained in the present study corresponds to the data from the studies applying the same test, or various other tests designed to assess QoL. A study conducted in Croatia by Tkalcić Met al demonstrated the fact that patients with IBS have a more increased level of anxiety and irritability compared to the patients IBD (Tkalcić et al, 2010). There are studies on IBD which emphasised the negative effect on all aspects related to QoL, affecting especially the emotional behaviour and favouring the development of depressive states and anxiety during and after the disease activity periods (Drossman et al, 1989). Although psychic stress and disease severity constitute the most important parameters likely to affect QoL, there are additional psychological variables, such as somatisation – which has an important role in the score (Vidal et al, 2008). Both the general and the specific IBD tests have shown that the symptoms frequency is related to the decrease in QoL (Fuller-Thomson et al, 2006).

Conclusions. The present study showed that QoL is affected in both the patients with IBS-d and those with CD. Compared to IBS-d, which influences more the mental QoL component, in the studied groups, CD had higher impact on QoL through the reduction of physical activities.

2.3.4. Anemia and QoL in CD's patients

Background & Aim. Anemia is the most frequent complication of IBD, with a prevalence that varies between 6% and 74% (Wilson et al, 2004).Clinically, anemia can affect important QoL components, such as the exercise capacity, the cognitive function, and the ability to carry out social activities (Guagnozzi, Lucendo, 2014). In addition, anemia increases the number of hospitalizations and even mortality rates (Gisbert et al, 2009). Starting from these prerequisites, we conducted a study to estimate the impact of anemia on QoL in patients with CD.

Material and methods.

A prospective study was conducted over a period of 3 years (1 January 2016–31 December 2019) (Dranga et al, 2021). The study included 134 patients with CD—both patients with a history of CD and patients who were at their first diagnosis—who were evaluated during this period in a Romanian tertiary center. At the time of the assessment, the following parameters were recorded: age, marital status, occupational status, and smoking obtained from anamnesis. A complete clinical examination was performed for each patient, investigating, in particular, abdominal sensitivity, the possible presence of the palpable masses, and articular and mucocutaneous modifications, which could point to an extra-intestinal manifestation. Upon hospitalization, hematological and biochemical tests were performed, investigating specifically hemoglobin and inflammatory markers. The positive diagnosis and the extent of the disease were established by colonoscopic examination and confirmed by the histopathological examination. All patients underwent total colonoscopy, and in the cases where the small intestine involvement was suspected, additional examinations were required (small bowel capsule endoscopy, computed tomography, and magnetic resonance imaging). The extension and behavior of the disease were determined according to the Montreal classification. The clinical activity was evaluated according to the Crohn's disease activity index (CDAI). Clinical remission was defined for a CDAI score of ≤150. Patients associated with hematological disorders (anemia), kidney and liver disorders, and neoplasm, which might have influenced the studied parameters, were excluded from the study. Anemia was defined according to the World Health Organization at a concentration of Hb < 12 g/dL in non-pregnant women and< 13 g/dl in men (Liu, Kaffes, 2012). QoL was assessed by means of the IBDQ-32 questionnaire (Inflammatory Bowel Disease Questionnaire). As we described above the questionnaire has 32 questions structured into four fields: IBDQ1—gastrointestinal symptoms (diarrheal stools, abdominal pain, rectorrhagia, and rectal tenesmus), IBDQ2—systemic symptoms (fatigue and sleep disorders), IBDQ3 - emotional functions (depression, irritability, anger, and sexual activity) and IBDQ4—social functions (absenteeism and affected social status). The responses in the questionnaire were graded from 1 (the worst) to 7 (the best). The total score is included in the range 32–224. Moreover, we computed the sub-scores for each distinct field (emotional functions, 12–84; gastrointestinal symptoms, 10–70; systemic symptoms, 5–35; and social functions, 5–35). The lower this score is, the more QoL is affected.Statistical application SPSS 18.0 for Windows was used in order to process the data.

Results.

The study included 134 patients with CD. The average age of the patients was 43.57 ± 15.57, and the patients were equally male and female. Most patients came from the urban environment. A quarter of the patients were in remission. The most frequent disease form was colonic and ileo-colonic CD and inflammatory phenotype. Anemia was more frequent in

women and in the patients who underwent corticosteroid therapy. There were statistically significant correlations between the presence of anemia and the CDAI value. Moreover, anemia was significantly more present in patients with active disease. QoL was significantly influenced in the patients presenting anemia and those with active disease (Table 2.12.).

Table 2.12. Relationship between clinical and demographic characteristics of CD patients and the total score obtained in the IBDQ-32 questionnaire

Characteristic	No. of Participants	IBDQ-Total Mean (95% Confidence Interval)	Significance
Gender			
Female	68	142.41 (131.75-153.07)	0.33
Male	66	135.12 (123.50-146.75)	
Marital status			
Married	98	139.20(130.28-148.13)	0.90
Single	36	137.78 (120.84—154.71)	
Area of residence			
Urban	104	137.71 (128.58-146.85)	0.40
Rural	30	142.67(127.23-158.11)	
Location			
LI	16	147.38(128.06-166.69)	
L2	62	137.90(126.09-149.72)	0.33
L3	46	135.78(121.22-150.35)	
L4	10	144.80 (99.82-189.78)	
Behavior			
BI	70	134.57(124.28-144.86)	
B2	48	145.50(131.13-159.87)	0.09
B2	16	137.38(110.47-164.28)	
CDAI			
Remission	34	152.06(133.12-170.99)	<0.001
Activity	100	134.32(126.16-142.48)	
Anemia			
Present	60	127.03 (115.70-138.37)	<0.001
Absent	74	148.38 (138.47-158.29)	

Note: *p* significances were computed by meansof nonparametric Mann-Whitney U test for two sets, respectively,and the Kruskal-Wallis testwas used for more than two sets. The p-values below 0.05 are marked in bold.

When analyzing the univariate influence that the two factors (disease activity and anemia) had on the QoL, we noticed that patients with anemia who were in remission had IBDQ3 and IBDQ4 significantly affected compared to patients without anemia.

In order to check the codependence of the variables activity and anemia in terms of QoL impact, we used multiple regression. The effect of the disease activity (CDAI) on QoL is important in multiple regression. The reduction of the coefficient for the adjusted form is not significant—for example, for total IBDQ from 0.132 to −0.112, thus, with (0.132–0.112)/0.132 ×100% = 15.1%. Anemia had a lower quantifiable effect on QoL, quantified by total IBDQ (Table 2.13).

Discussions

The data regarding the prevalence of anemia in CD in the world are heterogeneous, and they differ depending on the studied region and the studied population (hospitalized or ambulatory patients). A recent meta-analysis estimates the prevalence of anemia to a 27% ratio in patients with CD, higher in hospitalized patients (70%) compared to outpatients (15%) (Filmann et al, 2014). According to recent data published for the western and central regions in Romania, anemia prevalence in CD patients is as high as 35.65% (Lupu et al, 2015). In our

study, anemia prevalence was 44.8% higher than in other regions of the country. This could be explained by several factors: this is a poor region; the study was conducted in a tertiary establishment in which severe forms of the disease are treated; and the study was conducted on hospitalized patients, and three-quarters of them had flare-ups. Nevertheless, we can notice a decrease in prevalence over the last years, due to a more efficient management of this manifestation. Similar to other studies in the literature, our study also demonstrates a higher rate of anemia in patients with active disease. Decreased iron level, which, in time, may lead to iron deficiency due to ineffective erythropoiesis, is caused by decreased intake (inappetence and digestive intolerance), chronic intestinal loss, and, rarely, by decreasing iron uptake (lesions at duodenal or jejunal level) (Bohm, 2021). Moreover, during activity flares, inflammatory anemia can occur, due to the modifications of cytokines and acute phase proteins. Besides the modulation of the iron homeostasis, the cytokines directly influence hematopoiesis by inhibiting the erythroid cells. Moreover, TNF α decreases iron incorporation into erythrocytes, thus leading to anemia (Aberra et al, 2003). It is noticed that patients with anemia had more severe forms of the disease (CDAI), with all of these mechanisms being more pronounced in these forms.

Table 2.13. Effect on quality of life scores by univariate and multiple regressions

Dependent Variable (Y)	Coefficient of UCDAI Covariate (CI 95%)	Coefficient of Anemia Covariate (CI 95%)	Level of Significance–*p* *t*-Test of Covariates	
			UCDAI	Hb
IBDQ total^{UV}	−0.132 (−0.175 to −0.089)	-	<0.001 *	-
IBDQ total^{UV}	-	−21.345 (−31.579 to −11.111)	-	<0.001 *
IBDQ total^{MR}	−0.112 (−0.159 to −0.064)	−10.561 (−21.110 to −0.011)	<0.001 *	0.05 *
IBDQ1 ^{UV}	−0.030 (−0.041 to −0.020)	-	<0.001 *	-
IBDQ1 ^{UV}	-	−5.208 (−7.746 to −2.670)	-	<0.001 *
IBDQ1 ^{MR}	−0.025 (−0.037 to −0.013)	−2.794 (−5.450 to −0.137)	<0.001 *	0.039 *
IBDQ2 ^{UV}	−0.031 (−0.042 to −0.019)	-	<0.001 *	-
IBDQ2 ^{UV}	-	−5.109 (−7.751 to −2.467)	-	<0.001 *
IBDQ2 ^{MR}	−0.026 (−0.038 to −0.013)	−2.640 (−5.412 to 0.131)	<0.001 *	0.062
IBDQ3 ^{UV}	−0.037 (−0.050 to −0.025)	-	<0.001 *	-
IBDQ3 ^{UV}	-	−5.705 (−8.649 to −2.76)	-	<0.001 *
IBDQ3 ^{MR}	−0.033 (−0.046 to −0.019)	−2.566 (−5.595 to 0.462)	<0.001 *	0.096
IBDQ4 ^{UV}	−0.034 (−0.044 to −0.023)	-	<0.001 *	-
IBDQ4 ^{UV}	-	−5.284 (−7.853 to −2.715)	-	<0.001 *
IBDQ4 ^{MR}	−0.029 (−0.041 to −0.017)	−2.497 (−5.131 to 0.138)	<0.001 *	0.063

Note: *—statistical significance, UV—univariate analysis, and MR—multiple regression.

Similar to the literature data the disease activity had a significant QoL impact estimated by IBDQ in the patients included in the sample (Zhou et al, 2010; Kalafateli et al, 2013). This is mainly due to clinical manifestations, which are more severe during the periods of disease activity. Additionally, disease activity disruptions may influence the onset or worsening of psychiatric disorders such as anxiety or depression, which is an additional factor impairing the QoL in these patients (Von Wietersheim, Kessler, 2006).

In our study, QoL was affected both globally (IBDQ) and in each of the sub-scores. The patients in activity flare presented more frequently gastrointestinal symptoms (diarrheal stools, abdominal pain, rectorrhagia, and rectal tenesmus) (IBDQ1), and had significantly increased fatigue and sleep disorders (IBDQ2). In addition, the emotional function was more frequently and more intensely affected (depression, irritability, anger, sexual activity) (IBDQ3), all of which lead to increased periods of absenteeism and, implicitly, social impairment (IBDQ4). Another factor that significantly affected QoL in these patients was the presence of anemia. There are several studies in the literature that have shown that anemia has a negative impact on the QoL in patients with IBD (Wells et al, 2006; Pizzi et al, 2006). When analyzing patients by categories of activity, we noticed that patients in remission with anemia presented significantly more frequent and more important emotional symptoms ($p = 0.008$) compared to patients without anemia. Similarly, social life was more impaired in these patients (more frequent periods of absenteeism and social activities impairment) ($p = 0.029$) compared to those without anemia. This shows that anemia does not depend on the activity of the disease and its impact on QoL.

Later on, we performed a multiple regression data adjustment to reveal the effect that each of the two variables (disease activity and anemia) had on QoL. The effect of CDAI is also significant in univariate analysis and in the adjusted form (multiple regression). We notice that the effect of anemia is not always significant, but it has a measurable impact on total IBDQ. This is lower than the effect of disease activity quantified by the CDAI score, with the effects being independent from one another. From a practical point of view, our study has demonstrated that both the treatment of flare and the treatment of anemia are needed to increase the QoL of CD patients (Fig. 2.5.)

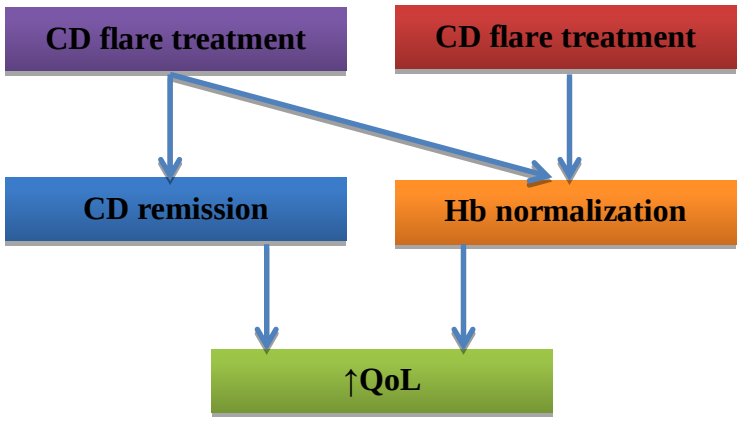


Fig. 2.5. Practical management for improving quality of life in CD patients with anemia. CD: Crohn's disease; Hb: hemoglobin; Qol: quality of life

Conclusions

Anemia has high prevalence in the northeastern region of Romania. Anemia was more common in patients undergoing corticosteroid treatment and in those with active disease. Both anemia and disease activity had a strong negative and independent impact on QoL. In the case of patients in remission, emotional symptoms were more frequent and more prevalent in patients with anemia compared to those without anemia. Social activities were significantly modified in these patients; similarly, absenteeism periods were more frequent compared to the patients without anemia. Based on these data, in order to improve quality of life, we consider that anemia should be corrected according to the existing guidelines (ECCO) in all patients, not only in those with disease activity.

2.3.5. The specialized educational and psychological counseling in IBD patients

Background & Aim.

This paper (Gavrilescu et al, 2020) was the result of a research grant obtained through competition at "Grigore T. Popa" University of Medicine and Pharmacy Iasi.

Despite having a low mortality rate, IBD has significant effects on QoL. New and emerging therapies have improved the overall outcomes, symptoms, and mucosal healing, altering the natural history of the disease (Lonnfors et al, 2014). Psychological interventions have been found to benefit patients suffering from chronic diseases. Recent studies have concluded that psychological therapies, especially cognitive behavioral therapy (CBT) might have small short-term beneficial effects on depression scores and QoL among patients with IBD but the results are still contradictory (Stapersma et al, 2020). Our study aimed to evaluate whether specialized educational and psychological counseling (SEPC) influences disease activity and QoL among patients with IBD.

Material and method. We conducted a randomized controlled trial with 60 patients diagnosed with IBD. We included adult patients with previously diagnosed IBD (clinical, biological, endoscopic, imagistic, or histological diagnosis). Patients with undetermined colitis, colorectal cancer, other neoplasia, or psychiatric disorders were not included in the study. After enrollment, the patients' medical records, including FC levels and demographic data, were collected and compiled into a database. All patients also completed 2 questionnaires: the IBD questionnaire-32 (IBDQ-32) and the Big Five Inventory (BFI). Patients were randomized (1:1) into 2 groups. Group A (experimental group) included 30 patients who received SEPC and group B (control group) included 30 patients treated according to the current medical practices. All patients were reassessed after 12 months (using medical records, FC levels, and the IBDQ-32). FC values >50 μg/g were considered to indicate intestinal inflammation. The educational counseling consisted of 1 session per month for 6 months for each group of 7–8 patients. In these sessions, issues related to etiopathogenesis, symptomatology, diagnosis, paraclinical diagnosis complications, nutrition, and treatment of IBD were presented to patients by gastroenterologists in an accessible manner. Psychological counseling was based on cognitive behavioral techniques. The SEPC group met weekly at a tertiary hospital for 6 months for 2-hour sessions carried out by a qualified clinical psychologist. The psychologist's training and supervision were provided by „Alexandru I Cuza" University.

Results. After SEPC, there was no improvement in disease activity as estimated by FC levels (Table 2.14.) but the QoL of patients in group A was significantly improved (Table 2.15.). The highest mean difference between the initial and final IBDQ scores was found among patients whose main personality trait was openness to experience (48.58±28.80), and the lowest mean difference between these 2 scores was found among patients whose main personality trait was closedness to experience (3.33±2.97); this difference was statistically significant (p=0.009) (Fig. 2.6.).

Discussions. Most of the previous studies has used activity scores, which may include subjective items, to evaluate disease activity. In our research, disease activity was estimated using the level of FC, which is a non-invasive biomarker known for its ability to assess disease activity among patients with IBD. Similar to other studies in the literature, our study did not show any improvement in disease activity following SEPC (Knowles et al, 2013; Mikocka-Walus, 2017).QoL was significantly improved by the SEPC. No statistically significant differences were observed in the IBDQ1 (gastrointestinal symptoms) or IBDQ2 (systemic symptoms) sub-scores. This can be explained by the fact that the SEPC had a low impact on

disease activity in general. In contrast, statistically significant differences were found in the IBDQ3 (emotional functions) and IBDQ4 (social functions) sub-scores. We noticed that at the end of the therapy, patients were much more aware of what it meant to live with IBD, more easily accepted the permanence of this disease in their lives, and were better prepared to coexist with the disease. In addition, social activities improved with patients becoming more active in their social lives.

Table 2.14. Fecal calprotectin evaluation in the study groups

	Group A			Group B		
	First evaluation (n=30)	Re-evaluation (n=30)	p[a]	First evaluation (n=30)	Re-evaluation (n=30)	P
Fecal calprotectin(µg/g)	149.10±120.79	95. ± 69.41	0.002	174.40±149.98	140.70±130.26	0.096

Group A (experimental group): group B (control group): p=0.002a statisticallysignificant.

Table 2.15. Evaluation of the IBDQ score

Characteristic	Group A			Group B		
	First evaluation (n=30)	Re-evaluation (n=30)	P	First evaluation (n=30)	Re-evaluation (n=30)	P
IBDQ total means=standard deviations	144.23±31.14	168.90±24.47	0.001a	141.57±34.54	138.43±34.04	0.852
IBDQ1 means=standard deviations	49.93±13.18	53.67±12.33	0.061	46 .67±13.76	46.87±13.74	0.909
IBDQ2 means=standard deviations	23.93±7.16	27.17±6.46	0.101	22.07±6.«7	22 83±6 31	0.923
IBDQ3 means=standard deviations	50.77±16.10	62.27±10.24	0.001a	54.60±16.04	51.17±15.87	0.426
IBDQ4 means=standard deviations	21.63±5.71	26.83±5.13	0.001a	22.70±6.10	21.00±5.82	0.372

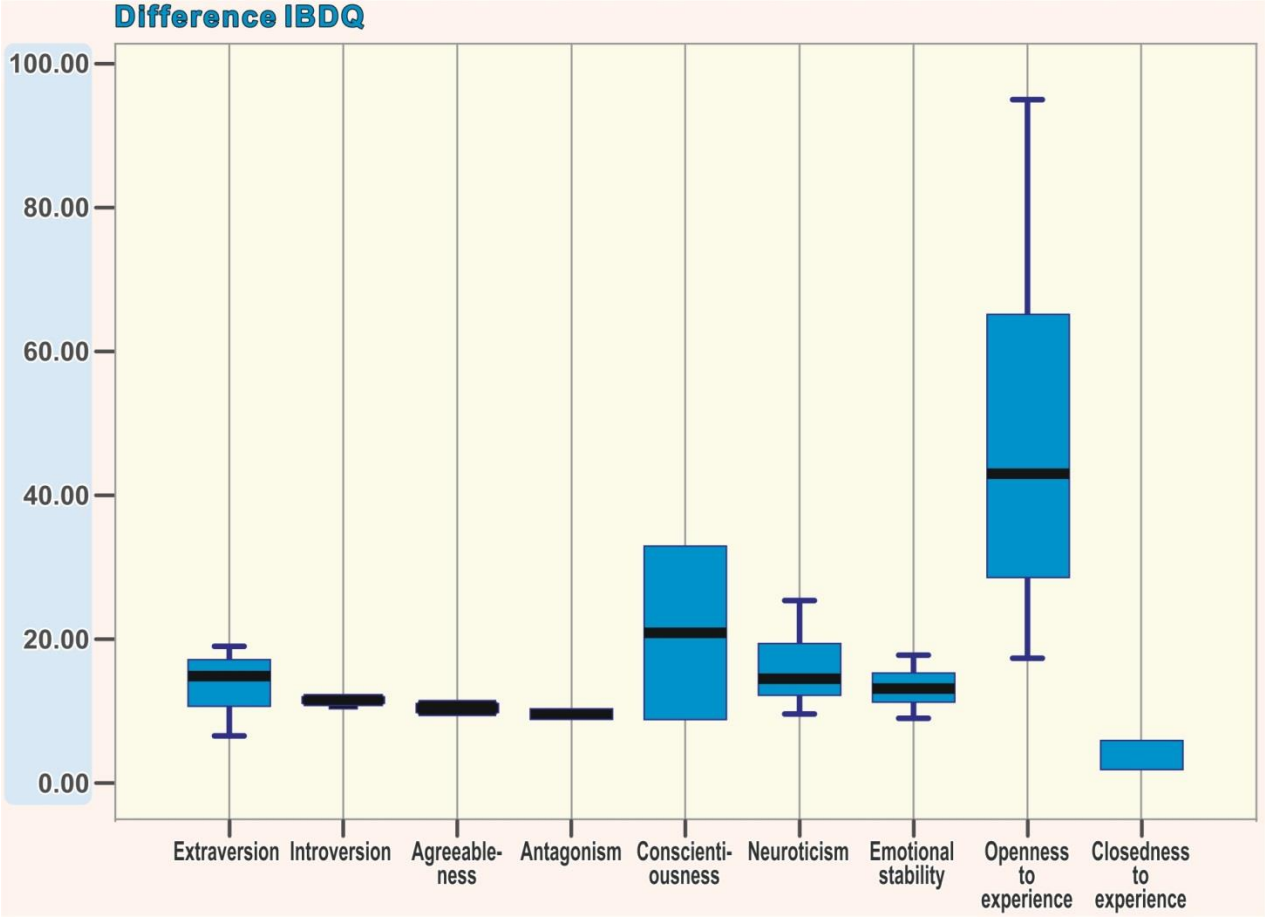


Fig. 2.6. Average difference between the initial and final inflammatory bowel disease questionnaire scores

Existing findings regarding the role of psychological stress management methods for the evolution of IBD are controversial (Timmer et al, 2011). Eccleston et al. (Eccleston et al, 2012) evaluated the effectiveness of psychological interventions among adolescents with IBD and reported promising results. Additional findings by Schoultz et al. (Schoultz et al, 2015) showed the effectiveness of this therapy for patients with IBD. Although the psychological component of the etiology of IBD is currently uncertain, the disease is associated with significant psychological disorders. However, despite the association between IBD and psychological disorders, which has been demonstrated by many studies, very few patients with IBD who also have mental disorders or psychiatric symptoms undergo psychotherapy. A Dutch study showed that < 40% of patients with IBD received psychotherapy because of low addressability and limited access to psychotherapists (Bennebroek Evertsz' et al, 2012). After reviewing 17 studies, Goodhand et al. (Goodhand et al, 2009) concluded that CBT was effective for mood disorders and for improving patients' QoL. McCombie et al. (McCombie et al, 2013) analyzed all relevant Cochrane reviews and selected 18 final studies to identify different types of therapy treatments. CBT was found to be the most efficient, by far, with more positive outcomes than those of counseling and psychotherapy.

Our study had 2 major limitations, which were the small group of patients involved and the short follow-up period. The main strength and novelty of this study is the stratification of patients by personality traits, allowing for identification of the subgroup for whom CBT and educational counseling were the most effective. The results obtained show that this type of intervention is especially effective for patients with a dominant personality trait of openness to experience or neuroticism. To the best of our knowledge, this is the first prospective study to

stratify patients using the BFI. Some existing studies have used QoL assessment questionnaires to identify patients who may respond to psychological therapies (McCombie et al, 2013). Apart from these aspects, our study showed that CBT did not influence the activity of IBD as evaluated objectively using FC levels.

Conclusions. Patients with IBD have an impaired QoL. Although there was no improvement in disease activity after the SEPC, this therapy improved patients' QoL in terms of both emotional and social functions, especially among patients who have the dominant personality trait of openness to experience or neuroticism. The effect of these therapies on disease activity may have been low because of the short period of application. Future studies are needed to assess the long-term effects and identify patients who may benefit from CBT and educational counseling as an integral part of their therapeutic management.

The main points in studies regarding the QoL in IBD patients are presented in Table 2.16.

Table 2.16. Main points regarding QoL in IBD Patients

SCIENTIFIC/CLINICAL RELEVANCE
• Patients with IBD have a reduced QoL. Improving it is one of the main therapeutic goals
• The main factor influencing QoL in both UC and CD is disease activity
• QoL is reduced in both IBD (CD) and IBS-d. Physical activity is more affected (statistically significant) in CD while mental component is more affected in IBS.
• The presence of anemia further affects QoL in patients with CD, even during the remission period of the disease
• The specialized educational and psychological counseling improves QoL in IBD patients without influencing the activity of the disease

2.4. OTHER ASPECTS OF INTERDISCIPLINARITY IN IBD

2.4.1. State of the art

In the following I will briefly present some of the published reviews that analyzed other aspects of interdisciplinarity in IBD. These can be considered as supporting elements in conducting future research in the field of IBD.

The main publications in this research field are the followings:

1. Popa IV, Diculescu M, **Mihai C**, Cijevschi-Prelipcean C, Burlacu A. COVID-19 and Inflammatory Bowel Diseases: Risk Assessment, Shared Molecular Pathways, and Therapeutic Challenges. *Gastroenterology Research and Practice* 2020, 1–7. doi:10.1155/2020/1918035 IF= 1,806
2. **Mihai C**, Stefanescu G, Gogalniceanu P, Anton C, Dranga M, Jigareanu O, Balmus OM, Timofte D, Ciobica A,Cijevschi Prelipcean C. Special features of *Clostridium difficile* colitis in patients with inflammatory bowel disease. *Archives of Biological Sciences* 2015; 67(1): 147-153. IF=0.367
3. Cijevschi Prelipcean C, **Mihai C**, Gogalniceanu P, Anton C, Anton E. Current aspect regarding the connection between pregnancy and inflammatory bowel disease. *Archives of Biological Sciences* 2014; 66(3) 1047-1054. IF=0.648
4. Cijevschi Prelipcean C, **Mihai C**, Iacob R, Preda C, Dranga M, Diculescu M. A new problem in an East European Country- Inflammatory bowel disease in elderly. Proceedings

of the Central European Gastroenterology Meeting (CEURGEM). Editors: Dumitrascu DL, Krejs GJ, Sporea I. 13-15 Feb 2019, Timisoara: 28-33.
5. Mitu O, Alexandrescu DM, **Mihai C**, Cijevschi-Prelipcean C.Is there a cardiovascular risk in inflammatory bowel diseases?*The Medical-Surgical Journal* 2014;118(4): 918-923.
6. Cijevschi Prelipcean C, **Mihai C**, Gogalniceanu P, Mihai B. What is the impact of age on adult patients with inflammatory bowel disease? Clujul Medical 2013; 1(86): 3-9.
7. **Mihai C**, Cijevschi C, Dranga M, Pintilie I, Georgescu S, Mihai B. Boala Crohn – afecţiune medico-chirurgicală. *Jurnalul de chirurgie* 2010; 6 (2): 181-188.

2.4.2. Pregnancy and IBD

IBD has a high incidence predominantly in young individuals, so it also affects family planning and pregnancy. In this review we summarized a number of issues and challenges that arise from this, such as the chances of having a successful pregnancy, how IBD affects pregnancy, what investigations are needed during pregnancy, as well as what is the correct management of IBD (dietary, medical or surgical) in pregnant women with this disorder. IBD in pregnancy requires a multidisciplinary approach involving close collaboration between patient, gynecologist and gastroenterologist in order to increase treatment compliance and facilitate a successful pregnancy.

2.4.3. *Clostridioides difficile* infection and IBD

Through its specific biological, epidemiological, diagnostic and infection management features, *Clostridioides difficile* infection (CDI) can be considered a major health concern, especially in IBD patients. In this particular infection, many IBD risk factors are triggered due to bowel inflammation, antibiotics use, microbiota changes, immunosuppressive therapy use and surgical intervention. Thus, each flare of IBD must be tested for CDI. Clinical features show different initial infectious stages such as mild, fulminate and refractory. It has been shown that CDI presents recurrent episodes. CDI treatment consists of metronidazole, vancomycin or fidaxomicin, as well as prophylactic measures. It was recently shown that antibiotic doses must be gradually reduced in order to avoid CDI relapses. Fecal transplantation, effective in CDI management, remains a challange in CDI patients with concurrent IBD.

2.4.4. Aging and IBD

The incidence and prevalence of IBD in the elderly are increased as a result of the ageing population. Elderly – onset IBD is defined as disease onset after the age of 60-65 years. An evidence-based approach in this population is limited due to the exclusion of elderly patients from clinical trials. The diagnosis in elderly patients can be challenging due to the large number of conditions that mimic IBD. The treatment approaches are those used in younger patients, but a number of additional factors must be considered, including comorbidities, potential pharmacological interactions and drug side-effects. The risks associated with the use of some IBD medications may be increased in older patients, but so is the risk of undertreated IBD and surgery.

2.4.5. Cardiovascular involvement in IBD

IBD are characterized by an increased thrombembolic risk, given the powerful relation between inflammation and thrombosis. Multiple studies showed that patients with IBD have an up to 3-fold higher risk for developing venous thrombembolic complications compared to general population, this risk being more increased in the hospitalized IBD flares. Thus, latest consensus recommendations indicate prophylaxis for thrombembolism in hospitalized patients with active

IBD but with no clear indications for the management of IBD outpatients. Regarding atherothrombotic risk (myocardial infarction or stroke), up-to-date data are inconclusive. IBD is associated with subclinical atherosclerosis in patients without clinical manifestations of cardiovascular diseases. However, the results of major studies assessing the hypothesis that IBD is strongly associated with atherosclerotic macrovascular events prove to be divergent even if they show positive correlations with cardiovascular diseases especially on different subgroup analysis. These facts should lead in the future to more prospective studies with control groups that have the same cardiovascular risk profile as in IBD populations in order to admit definitively that patients with IBD are exposed to an increased cardiovascular risk.

2.4.6. COVID – 19 and IBD

The novel coronavirus SARS-CoV-2 causing COVID-19 disease is yielding a global outbreak with severe threats to public health. In this paper, we aimed at reviewing the current knowledge about COVID-19 infectious risk status in IBD patients requiring immunosuppressive medication (Popa et al, 2020). We also focused on several molecular insights that could explain why IBD patients appear not to have higher risks of infection and worse outcomes in COVID-19 than the general population in an attempt to provide scientific support for safer decisions in IBD patient care. PubMed electronic database was interrogated for relevant articles involving data about common molecular pathways and shared treatment strategies between SARS-CoV-2, SARS-CoV-1, MERS-CoV, and IBD. Besides, Neural Covidex, an artificial intelligence tool, was used to answer queries about pathogenic coronaviruses and possible IBD interactions using the COVID-19 Open Research Dataset (CORD-19). Few molecular and therapeutic interactions between IBD and pathogenic coronaviruses were explored. First, we showed how the activity of soluble angiotensin-converting enzyme 2, CD209L other receptors, and phosphorylated α subunit of eukaryotic translation initiation factor 2 might exert protective impact in IBD in case of coronavirus infection. Second, IBD medication was discussed in the context of possible beneficial effects on COVID-19 pathogeny, including “cytokine storm” prevention and treatment, immunomodulation, interferon signaling blocking, and viral endocytosis inhibition. Using the current understanding of SARS-CoV-2 as well as other pathogenic coronaviruses immunopathology, we showed why IBD patients should not be considered at an increased risk of infection or more severe outcomes. Whether our findings are entirely applicable to the pathogenesis, disease susceptibility, and treatment management of SARS-CoV-2 infection in IBD must be further explored.

Chapter 3
ULTRASONOGRAPHY - A NON-INVASIVE METHOD OF EXPLORING LIVER DISEASES

3. 1. INTRODUCTION

Another area of research is abdominal ultrasonography. As a gastroenterologist, abdominal ultrasound is widely used in current clinical practice, being practically an extension of the objective examination.After obtaining the competence in ultrasonography, I aimed to continuously improve my examination technique and to acquire new techniques derived from ultrasonography (especially elastometry and contrast-enhanced ultrasound – CEUS). In this sense, I participated in numerous courses and workshops organized both in the country and abroad. I am a member of the Romanian Society of Ultrasound in Medicine and Biology and, since 2021, I am a guest member of the steering committee. I have presented numerous papers at national ultrasonography congresses. For over 10 years I have been a lecturer in the postgraduate course for obtaining competence in ultrasonography and I am part of the examination commission. Practicing for the first time CEUS technique in the university center of Iasi, I was co-opted in several multicenter studies that were published in journals with visibility and with a large number of citations.The paper "Contrast-enhanced ultrasound (CEUS) for the evaluation of focal liver lesions - a prospective multicenter study of its usefulness in clinical practice"published in 2014 in Ultraschall Medicine (impact factor 4.924, 60 ISI citations) received the national research award. The main publications in the field are: 3 ISI articles, 1 ISI proceedings article, 2 articles in other international database.

1. Şirli R, Sporea I, Popescu A, Dănilă M, Săndulescu DL, Săftoiu A, Moga T, Spârchez Z, Cijevschi C, **Mihai C**, Ioanițescu S, Nedelcu D, Iacob N, Miclăuș G, Brisc C, Badea R. Contrast-enhanced ultrasound for the assessment of focal nodular hyperplasia - results of a multicentre study. *Med Ultrason* 2021;23(2):140-146. IF = 1,611
2. Sporea I, Săndulescu DL, Şirli R, Popescu A, Danilă M Spârchez Z, **Mihai C**, Ioanițescu S, Moga T, Timar B, Brisc C, Nedelcu D, Săftoiu A, Enăchescu V, Badea R. Contrast-Enhanced Ultrasound for the Characterization of Malignant versus Benign Focal Liver Lesions in a Prospective Multicenter Experience - The SRUMB Study. *J GastrointestinLiver Dis*. 2019; 28:191-196. IF = 2,351
3. Sporea I, Badea R, Popescu A, Spârchez Z, Sirli RL, Dănilă M, Săndulescu L, Bota S, Calescu DP, Nedelcu D, Brisc C, Ciobâca L, Gheorghe L, Socaciu M, Martie A, Ioaniţescu S, Tamas A, Streba CT, Iordache M, Simionov I, Jinga M, Anghel A, Cijevschi Prelipcean C, **Mihai C**, Stanciu SM, Stoicescu D, Dumitru E, Pietrareanu C, Bartos D, Manzat Saplacan R, Pârvulescu I, Vădan R, Smira G, Tuţă L, Săftoiu A. Contrast-enhanced ultrasound (CEUS) for the evaluation of focal liver lesions - a prospective multicenter study of its usefulness in clinical practice.*Ultraschall Med* 2014; 35(3):259-66. IF= 4.924
4. Stefănescu G, Gîlcă GE, Bălan G, **Mihai C**, Brăilescu R, Drug V, Cijevschi Prelipcean C. Non-invasive Assessment of Liver Fibrosis in Patients with Chronic HCV Infection: Prediction Factors for Discordance between Fibromax and Transient Elastography. Proceedings XXXVIth National Congress of Gastroenterology, Hepatology and Digestive Endoscopy. Cluj Napoca 8-11 iunie 2016. Editor: Dan Dumitrascu. FILO diritto editure. www.gastro2016.medical-congresses.ro: 397-404.

5. **Mihai C**, Cijevschi Prelipcean C. Ultrasonografia – o tehnică a clinicianului?*Jurnalul dechirurgie*2012; 8(2): 115-117.
6. **Mihai C**, Mihai B, Crumpei F, Barr C, Ferariu D, Georgescu S, Cijevschi Prelipcean C. Multiple focal liver lesions – diagnosis challenges. Case report. *Medical ultrasonography* 2011; 13 (1): 72-75.

3.2. CONTRAST-ENHANCED ULTRASOUND – NON-INVASIVE ULTRASOUND METHOD

Next I will refer to the main scientific achievements in the field, namely those related to CEUS.

3.2.1. State of the art

The scientific advances of the latter decades have profoundly transformed medicine, moving it away from a clinical and human side towards a technological one. Cheap, affordable, non-invasive, repeatable, providing multiple information on internal organs, ultrasound has become today almost an integral part of any clinical examination. CEUS is an ultrasonographic technique that uses contrast agents allowing real-time examination of the vascular pattern of the lesions.It has no renal or hepatic toxicity, does not use ionizing radiation and has diagnostic performance similar to computed tomography or magnetic resonance imaging (Sporea et al, 2017). Immediately after a focal liver lesion is found by conventional ultrasound, a CEUS can be recommended, frequently performed in the same room, using the same machine. Last but not least, the cost of the investigation is lower compared to CT or MRI (Tranquart et al, 2009). Unlike contrast agents used in computed tomography or magnetic resonance, agents used in CEUS have a low rate of anaphylactic reactions (1 / 10,000); does not require biological analysis before administration and no fasting status (Tang et al, 2017). The ultrasound contrast agent used in Europe is Sonovue - that contains sulphur hexafluoride with a phospholipid shell. The adverse events following the administration of perfluoro-containing agents are rare and mild as compared with other contrast imaging tech- niques, but it should be specified that severe adverse events after CEUS injections can occur in patients with severe cardiac conditions, such as acute myocardial infarction, class III/IV cardiac insufficiency, or with significant rhythm disorders (Piscaglia, Bolondi, 2006). The device allows a low mechanical index, which produces a non-linear oscillation of the microbubbles.

The first guidelines regarding the use of CEUS were issued in 2004 by the European Federation of Societies for Ultrasound in Medicine and Biology (EFSUMB) (Albrecht et al, 2004), which were revised in 2008 (Claudon et al, 2008), in 2012 (Claudon et al, 2013) and in 2020 (Dietrich et al, 2020), the last two developed in cooperation with the World Federation for Ultrasound in Medicine and Biology (WFUMB), thus with universal validity.

CEUS is recommended as the first contrast-enhanced imaging in patients with renal impairment and in all cases of focal lesions with uncertain etiology on computed tomography or magnetic resonance imaging (Jo PC et al, 2017).

The technique has multiple applications in the liver, spleen, pancreas, kidney, testis, bowel pathologies. For the liver there are 3 phases of the examination: arterial (0-30 seconds after contrast injection) - provides information on the degree and pattern of the arterial vascular supply of a focal liver lesion, portal (30-120 seconds) - represents the arrival of contrast agent through the portal system, resulting in diffuse and maximal enhancement of normal liver parenchyma and venous (from 120 seconds until the disappearance of microbubbles) – represents the clearance of the contrast agent (Dietrich et al, 2020). For the rest of the organs there is only the arterial phase and the venous phase.

The most used application of CEUS is represented by focal liver lesions. These may be accidentally discovered on an ultrasound examination or may be detected during monitoring of patients with chronic liver disease and / or pre-existing focal lesions.

CEUS has notable performances in differentiating benign and malignant liver formations, with a sensitivity of 93% and a specificity of 90% (Friedrich-Rust et al, 2013). Among liver lesions, the diagnostic accuracy is very good for hemangioma, focal nodular hyperplasia and liver metastases. Adenoma, hepatocellular carcinoma, cholangiocarcinoma can create problems of differential diagnosis (Sporea et al, 2017). Other applications of CEUS in hepatology are: characterization of portal vein thrombosis (benign vs. malignant), intraoperatively in case of surgical resection of liver formations, biopsy guidance, intracavitary use to guide interventional techniques, tumor ablation, monitoring of therapeutic response in hepatic malignant tumors (Dietrich et al, 2020).

3.2.2. The role of Contrast-Enhanced Ultrasound for the evaluation of focal liver lesions

Background & Aim

This was the first of the prospective multicenter studies in Romania that evaluated the role of CEUS in the characterization of focal liver lesions.

Abdominal ultrasound is the main investigation that diagnoses focal liver lesions. Accessible and non-invasive, most often ultrasonography can not etiologically frame the identified liver lesions. Computet tomography (CT) and magnetic resonance imaging (MRI) are viable alternatives but the cost price is much higher and side effects are present (renal damage of the contrast substance, ionizing radiation). In some cases, only the histopathological examination obtained by percutaneous puncture or surgical resection can resolve the diagnosis. In this landscape of investigations, CEUS is gaining more and more ground as an essential exploration in the diagnosis of focal liver lesions. Both international multicenter studies (Strobel et al 2008, Tranquart et al, 2008) and Romanian experience (Sporea et al, 2012) have demonstrated the usefulness of CEUS in current clinical practice. Consequently the Romanian Society of Ultrasound in Medicine and Biology initiated this multicenter trial with CEUS, aiming to prospectively establish its value in the assessment of de novo focal liver lesions in clinical practice and to perform a cost-effective analysis.

Material and methods

A multicenter prospective study was conducted over 16 months (February 1, 2011 – June 1, 2012) in 8 university centers (14 individual departments), and the trial was registered at clinicaltrials. gov (Identifier NCT01 329 458). In this multicenter study we managed the cases from the Iasi university center. The study included consecutive patients (older than 18 years) with one to three newly discovered focal liver lesions during B-mode ultrasound, regardless of the lesion size. The following characteristics were documented for each patient: the indication for CEUS study and a short history including the presence of chronic liver diseases or various malignancies. In each included patient, the B- mode examination was followed by the contrast study (CEUS). Contrast-enhanced CT or MRI or histopathologic exam was available for each patient and considered as a "gold standard" for establishing the final diagnosis. The exclusion criteria were the following: patients with contraindication for a contrast-enhanced study (subjects with acute myocardial infarction, with class III/IV cardiac insufficiency, or with significant rhythm disorders, as well as pregnant women); patients diagnosed with simple cysts on B-mode ultrasound (biliary) or those diagnosed with hydatid cysts; patients with known hepatic lesions. A dedicated website was developed (http://study.umfcv.ro) for this study.

The B-mode and the CEUS studies were performed in each patient with the same ultrasound machine. Different machines were used in different centers, but all had capabilities for low- mechanical index examinations (in our center we have used Hitachi machine). CEUS was interpreted by experts from each center, who were blinded to the CT/MRI or histology results.

The number, size, ultrasound pattern and location of the focal liver lesions were documented after B-mode ultrasound. CEUS was performed with convex probes using a low mechanical index (0.09 – 0.11) in order to minimize microbubble disruption. The contrast agent was SonoVue® (Bracco SpA, Milan, Italy) (2.4 ml), which was injected through a peripheral intravenous cannula of sufficientsize, followed by a 10-mL saline flush, as per standard protocol. Lesion enhancement patterns were studied in 3 phases: arterial (10 – 30 seconds after injection), portal (30 – 120 seconds) and late phase (> 120 seconds) according to EFSUMB recommendations (Claudon et al, 2013; Dietrich et al, 2020). The contrast study for each patient lasted 5 minutes after bolus injection and was documented by at least 4 video filesno longer than 30 seconds each, containing: B-mode examination, the arterial phase, the portal phase and the late phase.

The contrast vascular patterns were defined by comparing the enhancement behavior of the tumor as compared with the surrounding liver parenchyma and were classified as (Sporea et al, 2014):

- homogeneous hyperenhancement, meaning that the whole hepatic lesion showed global homogeneous contrast enhancement;
- heterogeneous hyperenhancement, meaning that the lesion presented mixed irregular areas of contrast enhancement;
- rim-like hyperenhancement, meaning a peripheral hyperenhancement of the lesion which was limited to less than 25 % of the tumor's diameter;
- isoenhancement, meaning that the lesion enhanced similarly tothe adjacent parenchyma at the same depth;
- hypoenhancement, meaning that the lesion's enhancement intensity was less than that of the adjacent parenchyma at the same depth;
- wash-out, meaning hypoenhancement in the portal or late phases preceded by hyper- or isoenhancement in the arterial phase.

A CEUS diagnosis was established after the contrast study based on the patterns described in the EFSUMB and SRUMB guidelines regarding the use of CEUS for liver applications (Claudon et al, 2013; Sporea et al, 2017; Dietrich et al, 2020), as follows:

1. Hemangioma: centripetal fill-in enhancement in the arterial phase, partial/complete centripetal filling in portal phase and complete enhancement in the late phase;
2. Focal nodular hyperplasia (FNH): rapid arterial hyperenhancement with typical centrifugal radiating or "spoke-wheel" pattern, followed by homogeneous hyperenhancement in the late arterial phase with the persistence of hyperenhancement in the portal phase and iso/hyperenhancement in the late phase;
3. Adenoma: early and homogeneous hyperenhancement in the arterial phase, isoenhancement in portal phase and iso/hypoenhancement in the late phase;
4. Focal fatty alterations: the same enhancement pattern with respect to the surrounding liver in all vascular phases;
5. Liver cysts: no contrast enhancement in any of the vascular phases;
6. Regenerative nodule: the same vascular pattern as the surrounding liver parenchyma in all three vascular phases;
7 Abscess: rim-like enhancement in the arterial phase, hypo/ isoenhancing rim in portal phase and hypo-enhancing rim in the late phase;
8. Hepatocellular carcinoma (HCC): complete hyperenhancement in the arterial phase, isoenhancement in the portal phase and iso/hypoenhancement in the late phase;
9. Hypervascular metastasis: fast complete hyperenhancement in the arterial phase, hypoenhancement in the portal phase and hypo/non-enhancement in the late phase (Fig. 3.1);
10. Hypovascular metastasis: rim-like hyperenhancement in the arterial phase, hypoenhancement in the portal phase and hypo/non enhancement in the late phase;

11. Cholangiocarcinoma: rim-like hyperenhacement in the arterial phase, hypo/non-enhancement in the portal and late phase.

A CEUS examination was considered conclusive if, following contrast, the focal liver lesion had a typical enhancement pattern according to the guidelines, and inconclusive if the enhancement pattern was not in concordance with these guidelines. The CEUS diagnosis was compared with the final diagnosis which was established based on all available imaging and clinical data: contrast-enhancement CT and/or MRI and/or histology.

Statistical analysis was performed using the MedCalc program (MedCalc Software, version 12.3.0, Belgium). The sensitivity (Se) was calculated as true positive cases divided by the total number of cases in which the disease was present; the specificity (Sp) was calculated as true negative cases divided by the total number of cases in which the disease was absent; the positive predictive value (PPV) was calculated as true positive cases divided by all CEUS positive cases; the negative predictive value (NPV) was calculated as true negative cases divided by all CEUS negative cases and accuracy was calculated as the sum of true positive and true negative cases divided by the total number of cases. For the cost-effectiveness analysis, we compared the costs of three strategies: 1) CEUS as the first imaging method followed by CT or MRI for inconclusive cases; 2) contrast-enhanced CT as the first imaging method in all cases; 3) contrast-enhanced MRI as the first imaging method in all cases. For this analysis we used the mean costs of imaging techniques practiced in Romania: CEUS – 42 Euros (32 Euros – the cost of ½ vial of Sonovue+ 10 Euros – staffing costs); CT – 65 Euros and MRI – 150 Euros.

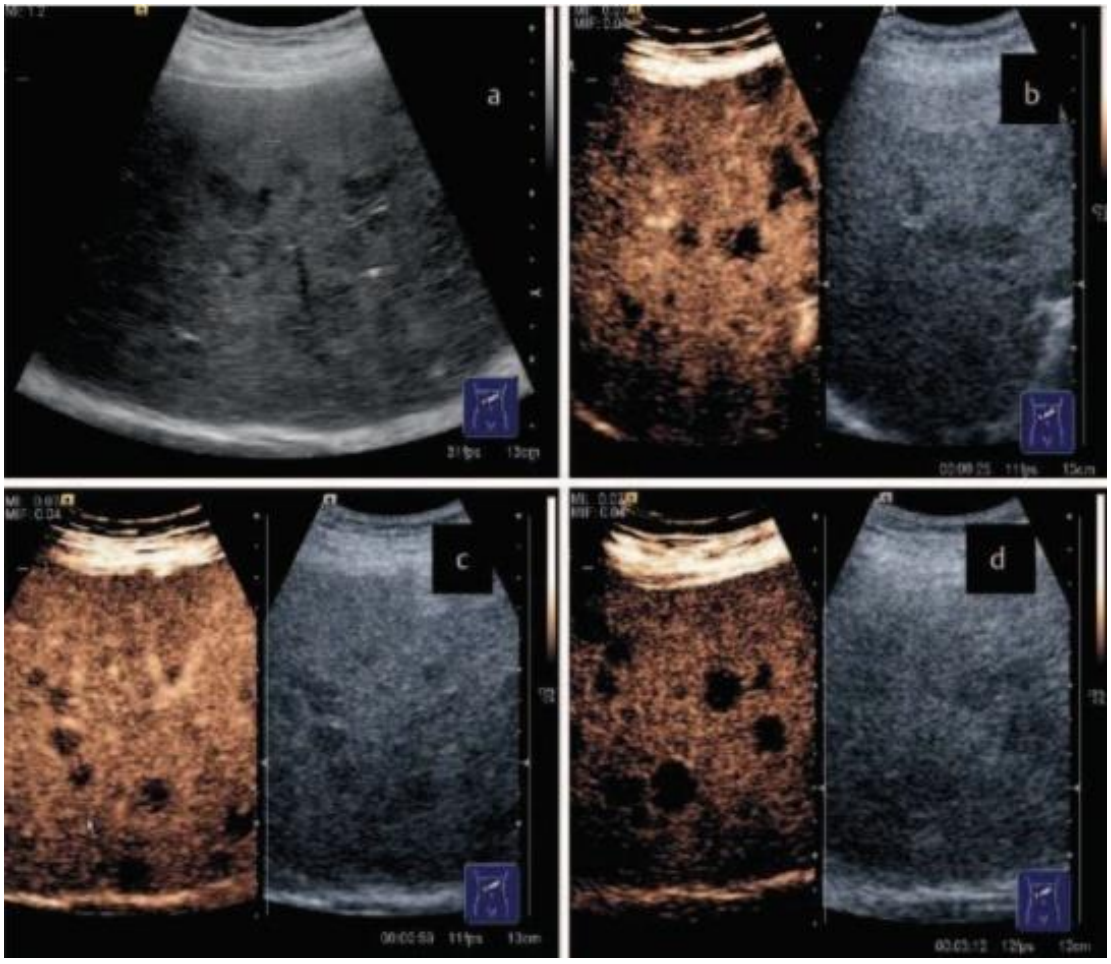


Fig. 3.1.CEUS in malignant lesions (hypovascular metastases). a Conventional ultrasound: multiple hypoechoic liver masses; b Arterial phase: rim enhancement; c Portal phase: wash-out of the rim area; d Late phase: continued wash-out.

Results

Initially, 866 focal liver lesions were included in the online database. From these, the following cases were excluded for not meeting the inclusion criteria: non-hepatic lesions – 4 cases (0.4 %), simple cysts evident on B-mode ultrasound (biliary or hydatid) – 8 cases (0.9 %), patients with more than 3 lesions – 21 cases (2.4 %), no reference method available – 297 cases (34.3 %). Thus, 536 lesions (61.8 %) in 525 patients were included in the final analysis. The final diagnosis was established in most cases by contrast-en- hanced CT/MRI – 379 cases (70.7 %), followed by the histology in 150 cases (27.9 %), and puncture with aspiration from liver ab- scesses in 7 cases (1.4 %).Half of the lesions (53.4 %) were hypoechoic on B-mode ultra- sound, followed by hyperechoic (35.8 %) and isoechoic (10.8 %) lesions, while 59.9 % were inhomogeneous and 40.1 % homogenous. From all 536 lesions analyzed, the final diagnosis established the malignant etiology in 344 cases (64.2 %) and the benign nature in 192 lesions (35.8 %).Patients characteristics are presented in Table 3.1.

Table 3.1. Patients characteristics

Parameter	Data
median age (years)	59(19-89)
gender: - women - men	n = 208(39.6%) n=317(60.4%)
the median size of the examined FLL (cm)	3.5(0.5-18)
number of FLLs examined/patient: - one - two	n = 525(97.9%) n=11(2.1 %)
CEUSindicatiom - incidental finding in subjects without liver pathology - FLL in patients with chronic hepatopathies (including livercirrhosis) - FLL in patients with oncologic history - patients with inconclusive CT/MRI	 n = 237(44.2%) n = 207(38.6%) n= 86(16.1%) n = 6 (1.1 %)
Final diagnosis: - hepatocellular carcinoma (HCC) - metastasis - hemangioma - focal fatty alteration - cholangiocarcinoma - focal nodular hyperplasia (FNH) - regenerating nodules - liver abscess - adenoma - complex cysts - lymphoma - scararea - vascularization disorder	 n = 209(38.9%) n= 109(20.4%) n=102(19.1%) n = 30(5.5%) n = 25 (4.7%) n= 19(3.5%) n= 17(3.1 %) n= 13 (2.5%) n=7(1.3 %) n = 2(0.3%) n= 1(0.2%) n= 1 (0.2%) n=1(0.2%)

The CEUS study was conclusive for benign vs. malignant differentiation in 89.3 % of cases, and inconclusive in 10.7 %. Of the focal liver lesions in which CEUS was conclusive, 56.1 % were malignant and 33.2 % benign.When all included cases were analyzed, considering those that were inconclusive on CEUS as cases wrongly diagnosed, the method had 85.7 % Se, 85.9 % Sp, 91.6 % PPV, 77.1 % NPV and85.8 % accuracy. When we analyzed only the cases categorized as conclusive for benign/malignant differentiation, CEUS had95.7 % Se, 96.4 % Sp, 98 % PPV, 92.6 % NPV and 96 % accuracy.

The size of the FLL was specified in 519 FLLs. This group was divided into lesions ≤ 2 cm: 127 cases (24.4 %), and lesions > 2 cm: 392 cases (75.6 %).The CEUS accuracy for the differentiation between malignant andbenign liver lesions was similar in tumors with a diameter ≤ 2 cm and those with a diameter > 2 cm, in all cases and also in only conclusive CEUS cases: 86.6 % vs. 85.4 %, p = 0.84 and respectively95.5 % vs. 95.9 %, p = 0.93. In our study, only 24 focal liver lesions (4.6 %) had the size smaller or equal with 1 cm. For these lesions, CEUS accuracy for differentiation between malignant and benign was 75 % in all cases and 94.7 % in conclusive CEUS cases. The median size of focal liver lesions in conclusive vs. inconclusive benign/malignant lesions was similar: 3.5 cm (0.5 – 18 cm) vs. 3.5 cm (0.6 –

11 cm), p = 0.50. Also, the proportion of malignant lesions wassimilar in conclusive vs. inconclusive benign/malignant CEUS cases: 64.3 % vs. 68.4 %, p = 0.64.CEUS accuracy for diagnosing different types of benign and malignant FLLs ranged between 79.8 % and 98.3 %, the highest accuracy being obtained for FNH and the lowest for HCC (Table 3.2).

Table 3.2. CEUS performance for different types of focal liver lesions

all cases included, considering those inconclusive on CEUS as wrongly diagnosed						
FLL	**no. of lesions**	**Se(%)**	**Sp(%)**	**PPV (%)**	**NPV (%)**	**Accuracy (%)**
hemangioma	102	8L3	67.1	59.7	95.2	86
FNH	19	94.7	87.6	21.9	99.7	87.8
regenerating nodules	17	76.4	87.2	16.4	99.1	86.9
focal fatty alteration	30	96.6	86.7	30.2	99.7	87.3
liver abscess	13	76.9	88.9	14.7	99.3	86.9
HCC	209	70.3	85.9	76.1	81.9	79.8
metastasis	109	87,1	82.9	56.5	96.1	83.7
cholangiocarcinoma	25	60	85.1	16.4	97.7	83.9
cases categorized on CEUS as conclusive for benign/malignant differentiation						
FLL	**no. of lesions**	**Se(%)**	**Sp(%)**	**PPV (%)**	**NPV (%)**	**Accuracy (%)**
hemangioma	92	90.2	97.6	90.2	97.6	96.2
FNH	19	94,7	98.4	72	99.7	98.3
regenerating nodules	14	92.8	97.4	52	99.7	97.2
focal fatty alteration	28	100	97.7	74.3	100	97.9
Liver abscess	10	100	98.4	62.6	100	98.5
HCC	181	81.2	94.2	89.6	89.2	89.3
metastasis	102	93.1	94,1	81.1	98.1	93.9
cholangiocarcinoma	24	62,5	95.6	42.8	97.9	93.9

The cost-effectiveness analysis showed the following:

a. CEUS in all cases as the first investigations (536 cases x 42 Euro = 22 512 Euro)
+ CT in inconclusive cases (57 cases x 65 Euro = 3705 Euro) = 26127 Euro
+ IRM in inconclusive cases (57 cases x 150 Euro = 8550 Euro) = 31062 Euro
b. CT as first –line investigation in all cases: 536 cases x 65 Euro = 34840 Euro
c. IRM as first – line investigation in all cases: 536 cases x 150 Euro = 80400 Euro

Discussion

Our aim was approximately the same as in the DEGUM (German) and the multicenter French study – STIC (Strobel et al 2008, Tranquart et al, 2008). The reference method for the final diagnosis was dynamic contrast-enhanced CT or MRI in most cases (70.7 %), similarly to

the French multicenter study, but different from the German multicenter study, in which histology was the “gold standard” in most cases.Regarding the "intent to diagnose" and considering all solid focal liver lesions with wash-out in the portal and/or late phase as malignant, and all lesions without wash-out in any vascular phase as benign, CEUS was inconclusive in 10.7 % of cases in our cohort, a percentage higher than that observed in the German multicenter study (6.8 %) (Strobel et al, 2008). The mean lesion size and the proportion of malignant lesions were similar in conclusive and inconclusive CEUS cases.In the present multicenter Romanian study, CEUS has approximately 85 % Se, Sp and accuracy to differentiate between malignant and benign focal liver lesions when all cases were considered, percentages similar to those observed in the French multicenter study (Tranquart et al, 2008) and slightly lower than those presented in the German study (Strobel et al, 2008). Our results showed that CEUS is conclusive in about 90 % of cases in Romanian clinical practice. We observed that Se, Sp, PPVand accuracy for differentiating benign vs. malignant liver tumors were higher than 95 %. Thus, if CEUS examination is conclu- sive (with a typical enhancement pattern according to the EFSUMB guidelines), we can be very confident in our results and another imaging technique is not required for focal liver lesioncharacterization.

The good performance of CEUS for focal liver lesions characterization was also demonstrated in a meta-analysis published in 2011 (Xie et al, 2011), which included 25 studies. The reference method for the final diagnosis was histology in all cases, while CT or MRI was also available in a subgroup of patients. The Se and Sp were similar for CEUS, contrast-enhanced CT and MRI for focal liver lesionscharacterization: 0.87, 0.86, 0.85 and 0.89, 0.82, 0.87, respectively. In another meta-analysis (Friedrich-Rust et al, 2013), which included 45 studies with 8147 focal lesions, CEUS had a summary Se of 0.93 and a summary Sp of 0.90 for differentiating between malignant and benign lesions. In this meta-analysis also, CEUS performed as well as CT/MRI.

Regarding the value of CEUS for the diagnosis of different types of malignant and benign focal liver lesions our results for diagnosing FNH and liver metastasis were similar to those observed in the French multicenter study (Tranquart et al, 2008), in which slightly better results were ob- tained for diagnosing hemangioma and HCC.

When we considered only conclusive CEUS cases, we observed an excellent performance for diagnosing different benign focal liver lesion: focal fatty alteration (100 % Se, 97.7 % Sp, 100 % NPV and 97.9 % accuracy), FNH (94.7 % Se, 98.4 % Sp, 99.7 % NPV and 98.3 % accuracy), hemangioma (90.2 % Se, 97.6 % Sp, 97.6 %. NPV and 96.2 % accuracy) or regenerating nodules in cirrhotic patients (92.8 % Se, 97.4 % Sp, 99.7 % NPV, and 97.9 % accuracy). Our results are in line with other published data (Dai et al, 2008; Liu et al, 2008).

Regarding the characterization of liver masses in oncologic patients, CEUS had an excellent performance with Se, Sp, and accuracy higher than 93 % and NPV > 98 % for diagnosing liver metastases, similar to other published data (Cabassa et al, 2010). The value of CEUS for the characterization of HCC in the CEUSconclusive cases was very good, with Se> 80 %, Sp > 95 % and with PPV, NPV and accuracy > 89 %. Other published studies showed good performance of CEUS for diagnosing HCC in cirrhotic patients (Forner et al, 2008). Our study showed that CEUS is a cost-effective method with very good performance in diagnosis of hemangioma, FNH, focal fatty alteration, liver metastasis, HCC in cirrhotic patients.

Conclusions

In conclusion, CEUS was conclusive for differentiation between malignant and benign focal liver lesions in approximately 90 % of all cases. If only conclusive cases were considered, CEUS proved to be a very accurate method to differentiate malignant vs. benign lesions and also to diagnose different types of focal liver lesions. Therefore, there can be confidence in our results in CEUS-conclusive cases in which other contrast imaging techniques are not required, thus reducing the costs and the time for obtaining the final diagnosis.

Next I will present 2 other SRUMB studies, which used the same methodology and were carried out over a period of 6 years. In the first of these was analyzed the role of CEUS in differentiating benign vs malignant liver lesions and in the second the role of CEUS in the diagnosis of FNH.

3.2.3. The role of Contrast-Enhanced Ultrasound for the characterization of malignant versus benign focal liver lesions

Background & Aim

Starting from the encouraging results of the first Romanian multicenter study on the role of CEUS in the characterization of focal liver lesions, this new study aimed to assess the role of CEUS in differentiating benign from malignant lesions.

Material and methods

This prospective study was conducted by the Romanian Society for Ultrasound and Medicine and Biology (SRUMB) and included 14 Romanian centers. It was performed over a period of 6 years (February 2011- April 2017) and included 2062 focal liver lesions assessed by CEUS.The same methodology was used as in the previous study, being considered as the reference method CT and / or MRI and / or histological examination.

Results

The mean age of studied patients was 52.4 ± 7.5 years. We detected more focal liver lesions in men (1148, 55.7%) with a mean age of 54.7 ± 7.5. The mean size of focal liver lesions was 4.5 ± 3.3 cm. No contrast agent allergic reactions and no other complications were recorded during this study. Out of 2062 FLLs examined by CEUS, 1901/2062 (92.2%) had a CE-CT and/or CE-MRI; 470/2062 (22.7%) of them had histology for the final diagnosis, 293/2062 (14.2%) by biopsy and 177/2062 (8.6%) after surgery.

The lesions analyzed are presented in Table 3.3.

Table 3.3. Types of ocal liver lesions

Hepatocellular carcinomas	685(33.2%)
Hemangiomas	452(21.9%}
Metastases	418(20.8%}
Focal nodular hyperplasias	94(4.5%)
Regenerative nodules	84(4.1%)
Focal fatly infiltrations	70(3.4%)
Cholan giocarcj nomas	57 (2.7%)
Abscesses	45 (2.2%)
Complex cysts (inhomogennus cystic lesions)	43 (2.1%)
Other benign lesions[1]	37(1.8%)
Adenomas	32(1.6%)
Fatty free areas	26(1.3%)
Other malignant lesions**	19(0.9%)

* Other benign lesions: scar area, angiomyolipoma, hamartoma·

** Other malignant lesion: lymphoma, hemangiosarcoma, hepatic epithelioid, hemangioendothelioma.

The most frequent lesion was HCC (Fig. 3.2) 33.2% (685 cases), followed by hemangioma - 21.9% (452 cases) and liver metastases (20.3%) (418 cases).

From the 2062 focal liver lesions included in the study, 57.2% (1179) were malignant and 42.8% (883) were benign. Considering only the diagnosis of malignancy, CEUS managed a correct differentiation of malignant vs. benign lesions in 88.3% (1820/2062) of the cases. For

the lesion-specific diagnosis, CEUS managed a correct diagnosis in 81.4% (1678/2062) of the lesions. CEUS had similar accuracy for diagnosing benign and malignant lesions (both Ac 89.9%) (Table 3.4).

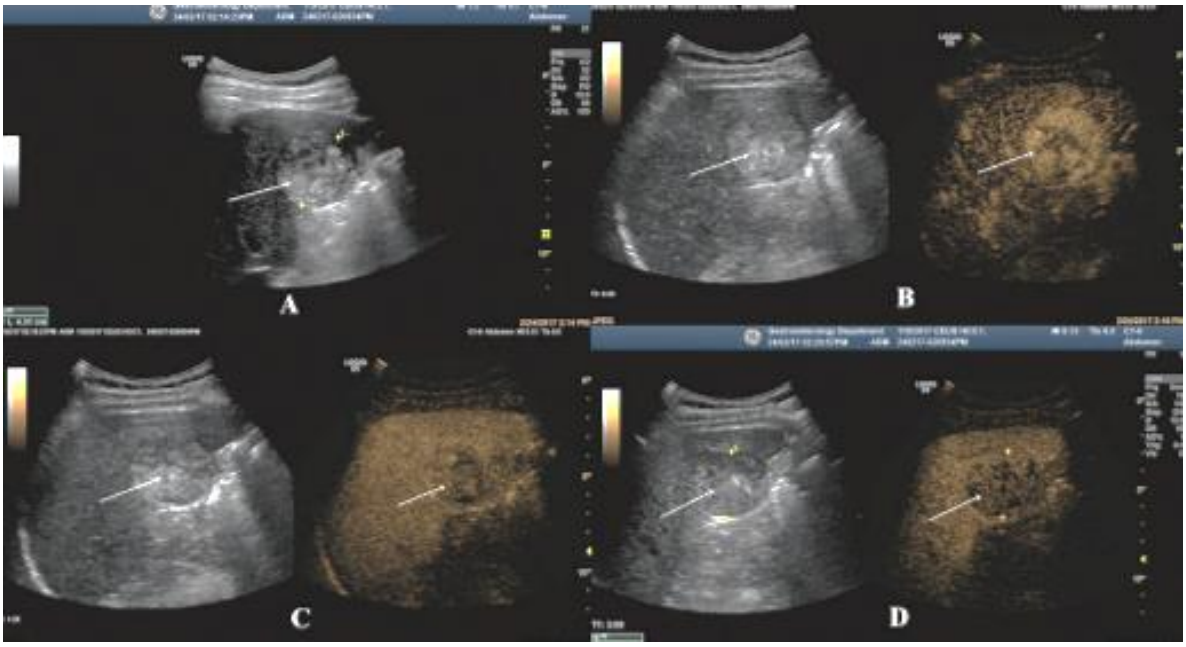


Fig. 3.2. CEUS study in a hepatocellular carcinoma: B Mode (A), hyperenhancing lesion in the arterial phase (B), hypoenhancement in the portal phase (C) and wash out with hypoenhancing lesion in the late phase (D).

Table 3.4. CEUS performance in malignant vs benign lesions

	Sensitivity %	Specificity %	Accuracy %	PPV %	NPV %
Benign lesions	97.8	83.9	89.9	82.2	98.1
Malignant lesions	83.9	97.8	89.9	98.1	82.2

CEUS performance in the most frequent lesions is presented in Table 3.5.

Table 3.5. CEUS performance in focal liver lesions

	Sensitivity %	Specificity %	Accuracy %	PPV %
HCC	76.6	98.4	91.2	96 1
HMG	89.2	99	96.9	96.4
Metastases	90.9	98.4	96.9	93.6
FNH	84	99.5	98.8	89.7
Chokngio camn onia	61.4	99.3	98.2	71.4
Abscess	86.6	99.9	99.6	95.1
Adenoma	56.2	99 9	99.2	90

HCC: hepatocellular carcinoma; HMG: hemangioma; FNH: focal nodular hyperplasia

Regarding HCC – the most frequent malignant lesion in daily practice, CEUS performance is presented in Table 3.6.

Table 3.6. CEUS performance in HCC

	Sensitivity %	Specificity %	Accuracy %	PPV %	NPV %
HCC ≤ 2 cm	56.3	99.6	91.3	97	90.5
HCC >2 cm	78.9	98.1	91.1	96	89

Discussion

During the first 30 sec after the introduction of contrast bolus (arterial phase), some of the lesions show a typical enhancement pattern (FNHs, hemangiomas or HCCs). In metastatic lesions, this phase differentiates hyper- from hypo- vascular ones. During the portal phase (30 - 120 sec following the contrast bolus), the vascular pattern will differentiate between benign (which remain enhanced) and malignant lesions (in which wash out occurs, the lesion becoming hypoenhanced as compared with the surrounding parenchyma). An exception to this pattern is seen in HCCs, especially in the well differentiated ones, in which the wash-out is seen later, usually after 3-4 minutes. In the late phase (starting 120 sec. following the contrast bolus until the "bubbles" are destroyed), malignant lesions will continue to wash out. Thus, a hypoenhanced lesion as compared with the surrounding tissue in the portal and/or late phase means malignancy, while hyper- or isoenhanced lesion in these phases signifies a benign lesion.

CEUS showed good performance for differentiate benign vs malignant lesions (93% Se and 90% Sp) in a large meta-analysis published by Friedrich-Rust, which included 8147 focal liver lesions (Friedrich-Rust et al, 2013). In another meta-analysis (Xie et al, 2011) there were no significant differences between the performance of CEUS and CE-CT/CE-MRI regarding specificity (89% vs. 82% vs 85%) and sensitivity (87% vs. 86% vs 87%) for the diagnosis of malignant liver lesions. The same reliable results were observed in a previous study SRUMB (Sporea et al, 2014). In this study, in an intention to analyze diagnosis analysis (when all cases were taken into consideration), CEUS was conclusive for benign vs. malignant differentiation in 89.3% of cases. When only conclusive cases were taken into consideration, CEUS had 95.7% Se, 96.4% Sp, 98% PPV, 92.6% NPV and 96% accuracy for differentiation between benign vs. malignant lesions.

It must be underlined that not all FLLs are as easy or as difficult to diagnose by CEUS. Easy to diagnose are FNHs (where the arterial centrifugal enhancement in the first 10-15 seconds is typical), hemangioma (with centripetal, progressive, slow,nodular enhancement, sometimes with an unenhancing central thrombotic area) or focal fatty infiltrations (with the same enhancement pattern as the surrounding liver parenchyma in all sequences). Usually, liver metastases show rapid and strong wash-out in the portal phase, so that the final diagnosis can be established in 1-2 minutes. Hepatocellular carcinoma is more difficult to diagnose (76.6% Ac in our cohort), especially well differentiated HCCs, in which the wash-out occurs late or very late (sometimes the contrast microbubbles are destroyed before the wash-out becomes visible). Also, in our study, a low Se was found in small HCCs (≤ 2cm) (56.3%). On the other hand, in the 293 cases where biopsy was performed the main indications were: newly detected FLLs inconclusive in imaging techniques, new lesions found in patients either with oncological background or in patients with chronic liver diseases. When performing CEUS, the clinical context of the patient should be considered. If the patient is known with liver cirrhosis or if

liver elastography performed before CEUS shows high liver stiffness values, a typical arterial enhancement (which appears in approximately 90% of cases) is highly suggestive for HCC, even if the wash-out is present only in approximately 60% of cases (Martie et al, 2012).

Conclusions

In this prospective, multicenter, large study, CEUS proved its utility in correctly assessing the malignant vs. benign character of a FLL. Due to its very good performance, it can be confidently used as a first line imaging method in daily practice, and also, as a point-of-care method.

3.2.4 The role of Contrast-Enhanced Ultrasound in the diagnosis of focal nodular hyperplasia

Background & Aim.

Focal nodular hyperplasia (FNH) is the second most frequent benign liver tumour with a 3% estimated prevalence, being a regenerative nodule as a reaction to a vascular congenital abnormality (Fukukura et al, 1998). Most frequently it is discovered by chance by conventional ultrasound in asymptomatic patients. It is more frequent in women (female: male prevalence 4:1) and in 80-95% of cases as a solitary lesion (Wanless et al, 1989).

Typical for FNH is a fibrous scar which includes an artery larger than usual, originating outside the nodule. This vascular disorder generates a regenerative nodule, which lacks the central terminal hepatic vein and has only capillarized sinusoids derived from the feeding artery. This type of vascularization generates the typical aspect of FNH in contrast imaging.

In ultrasonography FNH often appears as an isoechoic or hypoechoic mass with a hyperechoic central scar relative to the liver parenchyma (D'Onofrio et al, 2015). Colour Doppler ultrasound can reveal the central vessel, originating from the centre to the periphery with a low resistance index and a high flow pattern (Piscaglia et al, 2010). On CEUS FNHs are hyper-vascular, appearing homogeneously hyperenhancing in the arterial phase, with very rapid and centrifugal fill-in. This “spoke-wheel” pattern is essential for differentiating FNHs from other liver nodules (Claudon et al, 2013). Usually, FNHs are hyperenhancing in the portal phase and hyper or isoenhancing in the late phase. The central scar can appear as hypoenhancing in the late phase (Claudon et al, 2013; Piscaglia et al, 2010).

At contrast enhanced-CT, FNH ishyperdense during the hepatic arterial phase and isodense during the portal venous phase. A hyperdense central scar is commonly seen in the late phase (Scialpi et al, 2014).On unenhanced MRI, FNHs appear as isointense on T1-weighted images and iso- to slightly hyperintense on T2-weighted images (Masand, 2018).MRI with hepatobiliary scintigraphy (hepatobiliary contrast media consisting of administration of gadobenate dimeglumine) is considered now the best test to diagnose FNH (Hamad et al, 2021).

Given the invasiveness of the procedure and the benign nature of the lesion, percutaneous liver biopsy is rarely indicated.Surgical resection is also rarely indicated, only in symptomatic patients or in case of suspicion of malignant lesion (Hau et al, 2015).

Due to the high costs and low accessibility of high-performance investigations, CEUS remains a method with high reliability in FNH diagnosis.The aim of this study was to evaluate the performance of CEUS for the assessment of FNH in a large, prospective multicentre study.

Material and method

We performed a multicentre prospective observational study, during a 6-year period (April 2011-June 2017), which included successive CEUS examinations from eight university centres (14 individual departments). My contribution to this study was the examination and analysis of records at the Iasi University Center. The methodology was quite similar to the

previous studies. We included consecutive patients (older than 18 years), with newly discovered FLL during B-mode irrespective of the FLL size. In all patients, B-mode ultrasound was performed followed by CEUS. Also, in all patients a second line imaging method (contrast enhanced CT or MRI) or a biopsy were available, considered as the reference method. The exclusion criteria were the same as the previous studies: contraindications for CEUS; patients who refused to participate; known or easy to diagnose focal liver lesions (cysts, metastasis); patients in whom biopsy or second line sectional imaging methods were unavailable. We used the same dedicated website (http://study.umfcv.ro) for this study. Data were registered online for each individual patient.

In each patient, B-mode US and CEUS were performed with the same high-end ultrasound machine (in Iasi center Hitachi) able to perform low-mechanical index examinations. Convex probes using a low mechanic index (0.09- 0.11) were used. The US scan parameters (ie. focal zone, time gain compensation) were not changed during the CEUS study. We documented for each focal liver lesions the number, size, placement and US aspect on conventional B-mode ultrasound.The contrast agent used was SonoVue® (Bracco SpA, Milan, Italy), a perfluoro gas containing agent, as per standard protocol. Lesions' enhancement patterns were studied in 3 phases which were described before: arterial (10-30 seconds following contrast bolus), portal (30-120 seconds) and late phase (>120 seconds) (Sporea et al, 2017). In each patient, the contrast study duration was at least 5 minutes after bolus injection. To document the study, four video files no longer than 30 seconds each were captured, containing conventional B-mode examination, the arterial phase, the portal phase and the late phase.

CEUS vascular patterns were defined by comparing the focal liver lesions enhancement pattern to the surrounding liver parenchyma. We classified CEUS vascular patterns as we described in 3.2.2 study.

A CEUS diagnosis of FNH was established after the contrast study based on the patterns described in guidelines: rapid "spoke-wheel" enhancement during the arterial phase, hyperenhanced lesion during the venous phase, hyper or isoenhanced lesion in the late phase, sometimes with the visualization of a central hypoechoic scar (Sporea et al, 2017; Dietrich et al, 2020) (Fig. 3.3.). A CEUS examination was considered conclusive if the lesion had a typical enhancement pattern according to national and international guidelines and inconclusive if not. The CEUS diagnosis was compared with the final diagnosis established based on all available imaging and clinical data: contrast enhanced CT, and/or MRI, and/or histology.

Statistical analysis was performed using the GraphPad Prism program, version 7.02 (GraphPad Software, La Jolla, USA). We assessed the accuracy of CEUS for FNH characterization. We calculated Se, Sp, PPV, NPV and accuracy. We included in the statistical analysis all cases reported; we considered the inconclusive CEUS cases as wrongly diagnosed.

Results

During the 6 years study, 2062 focal liver lesions were evaluated by CEUS.

From this cohort, 94/2062 (4.5%) had a typical enhancing pattern for FNH. Contrast enhanced CT/MRI and biopsy diagnosed additional 15 FNH (12 in cases labelled as inconclusive on CEUS and one each labelled by CEUS as haemangioma, adenoma and metastasis). From the 94 cases diagnosed as FNH by CEUS, in nine cases the final diagnosis was different (five of them adenomas, two of them hepatocellular carcinomas, one haemangioma and one focal fatty infiltration). Thus, the final diagnosis was FNH in 100 of the 2062 (4.8%) cases. The mean age was 40.4±13.7 years, 70% were women, and most lesions (61%) were over 36 mm.

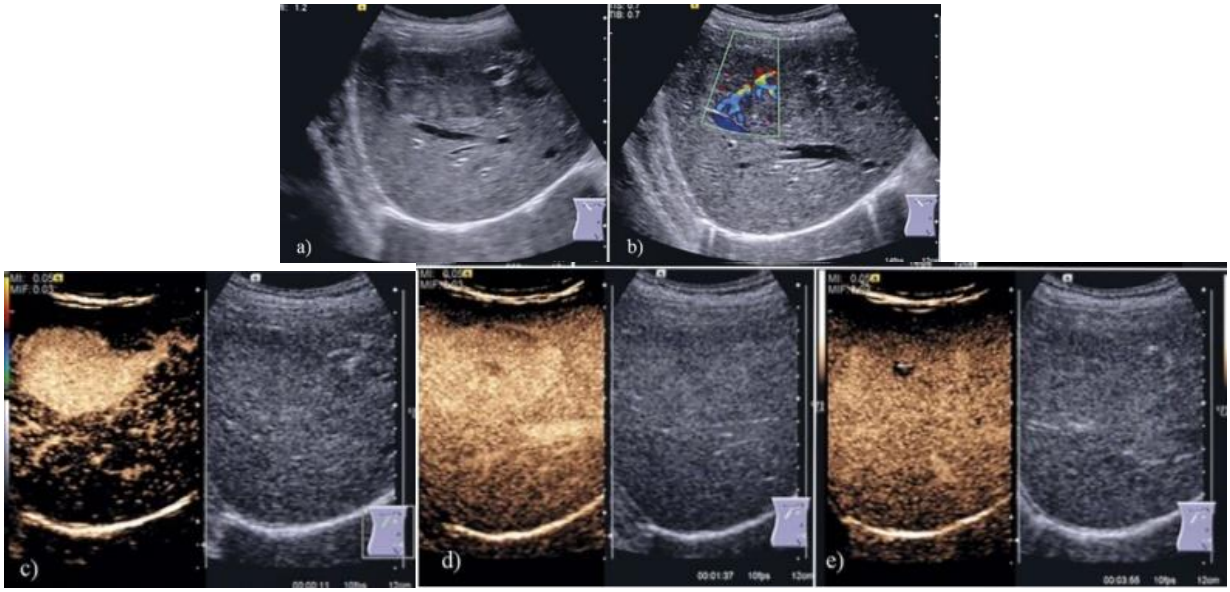


Fig. 3.3.a) Slightly hypoechoic focal liver lesion in the right liver lobe. b) The lesion shows "spoke-wheel" pattern on Doppler US. c) CEUS, arterial phase – the lesion is completely hyperenhanced 11 seconds after bolus injection – excentric feeding artery also visible. d) CEUS, portal phase – the lesion is slightly hyperenhancing with visible central scar. e) CEUS, late phase – the lesion is iso/hyperenhancing with well visible central scar.

The indication for CEUS was in most FNH cases an incidentally found focal liver lesion 98% (98/100), in 1% (1/100) case it was the evaluation of a patient with known oncologic disease and in 1% (1/100) case it was an inconclusive CE-CT scan. At the moment of CEUS examination, none of the patients diagnosed by CEUS with FNH were known with underlying liver disease. However, two of them were later diagnosed with underlying cirrhosis and 2 with chronic hepatitis. CE-MRI was the gold standard in 30% (30/100) cases. In 6% (6/100) patients histology was available – one of them after surgery performed for HCC. By comparing CEUS with the reference method (either CE-CT, CE-MRI or histology) CEUS had 85% Se, 99.5% Sp, 90.4% PPV, 99.2% NPV and 98.8% diagnostic accuracy for the diagnosis of FNH.

Discussion

CEUS is a real-time imaging technique, which, similar to contrast enhanced CT or MRI, shows tissue perfusion, and is able to identify the type of focal liver lesion based on the enhancement pattern in arterial, portal and late vascular phases. The value of CEUS for the differential diagnosis of FLLs was proved in many multicenter studies (Strobel et al, 2008; Tranquart et al, 2008; Sporea et al, 2014). Meta-analyses demonstrated that CEUS accuracy is similar to contrast enhanced CT and MRI (Xie et al, 2011; Friedrich-Rust et al, 2013). Advantages of CEUS as compared with CT and MRI are: the fact that the examination can be done immediately after ultrasonography with a quick result, the absence of radiation exposure (as in CT examination), the absence of side effects (no nephrotoxicity, no iodine exposure), and the lower costs (Sirli et al, 2010). Limitations of CEUS are linked to the limitations of US (poor acoustic window, uncooperative patients) and to the lesion's depth, since lesions located at more than 9 cm from the skin are difficult to examine due to attenuation (Sirli et al, 2020).

In our study, the performance of CEUS to diagnose FNH was very good with excellent Sp (99.5%) and accuracy (98.8%) and a slightly lower Se (85%)(Şirli et al, 2021).Regarding the misdiagnosed cases, most confusions were made with adenomas. Five cases diagnosed by CEUS as FNH were in fact adenomas and one case diagnosed by CEUS as adenoma was in fact FNH. The confusion is probably understandable since both types of lesions show rapid

hyperenhancement in the arterial phase, but in adenoma the fill-in occurs initially at the periphery with very rapid centripetal filling, as opposed to the centrifuge filling seen in FNH. In the DEGUM study the accuracy for FNH was 95.5% (Strobel et al, 2008) with 57.1% Se and 99.3% Sp. In the STIC study, CEUS had 82.5% Se and 94.3% Sp for the diagnosis of FNH (Tranquart et al, 2008). The calculated pooled sensitivity for FNH was 88% in the FriedrichRust et al meta-analysis (Friedrich-Rust et al, 2013) and the Sp can go as high as 100% (Trillaud et al, 2009). In another study that evaluated the accuracy of CEUS performed by two operators for the diagnosis of 85 biopsy proven FNH, CEUS had 80.9% Se, 95.7% Sp, 95.0% PPV, 83.3% NPV and 88.3% accuracy for operator one and 78.7% Se, 93.6% Sp, 92.5% PPV, 81.5% NPV and 86.2% accuracy for operator two. This study concluded that CEUS performed better than contrast enhanced CT for characterizing dynamic centrifugal filling or the "spoke-wheel" sign in small lesions (Wang et al, 2013).

In the Roche et al study (Roche et al, 2015) were included 43 FNH and 20 adenomas, most lesions diagnosed based on the histopathological exam. The conclusion of this study was that CEUS had excellent Sp for diagnosing FNH (100%), but the Se varies according to the lesion's size: 93% for lesions ≤ 35 mm and 7.7% for lesions > 35 mm, the overall Se being 67.4 %. The authors explained this observation by the fact that larger FNH have an increased vascular supply and several feeding arteries and thus, the typical spoke-wheel pattern is not visible. The same observation, that FNHs with signs of centrifugal filling were smaller than those without the sign (3.1±1.5 cm vs. 5.2±3.2 cm, p=0.000) was made in the study by Wang et al (Wang et al, 2013). In our study, the five FNH misdiagnosed as adenomas were 2.5 cm, 6 cm, 6 cm, 3 cm, 7.5 and 3.2 cm in diameter, respectively.

Two focal liver lesions diagnosed by CEUS as FNH proved to be HCC. Both types of lesions have arterial hyperenhancement but in HCC there is a chaotic pattern (Dietrich et al, 2020). Usually, HCCs show mild, late, or very late washout, correlated with the differentiation of the tumour (Liu et al, 2007). In well-differentiated HCCs, the CEUS aspect can be similar to that of FNH but up to 90% of HCCs occur on a background of chronic liver disease with severe fibrosis and cirrhosis (Huang et al, 2020). In our study both HCCs misdiagnosed as FNH were in patients with incidentally discovered focal liver lesions, who were not known with chronic liver disease, but in whom, starting from the HCC, compensated liver cirrhosis was diagnosed.

Conclusions

CEUS is an accurate method to diagnose FNH, the main difficulties occurring in differentiating FNH from adenomas, especially in large lesions.

The main points of my studies in the field of contrast-enhanced ultrasonography are presented in Table 3.6.

Table 3.7. Main points in the contrast-enhanced ultrasonography research field

SCIENTIFIC/CLINICAL RELEVANCE
• CEUS is a non-invasive method, with multiple advantages, extremely useful in characterizing focal liver lesions
• It is a cost-effective method that can be used for the first time in exploring liver lesions
• CEUS accuracy for diagnosing different types of benign and malignant focal liver lesions ranged between 79.8 % and 98.3 %
• The accuracy of CEUS regarding the classification of a liver lesion as benign or malignant is 90%
• CEUS had 85% Se, 99.5% Sp, 90.4% PPV, 99.2% NPV and 98.8% diagnostic accuracy for the diagnosis of FNH.
• For HCC, the accuracy of the diagnosis was 91%, regardless of the size of the tumor

Chapter 4
INTERDISCIPLINARY APPROACH: DIGESTIVE DISEASES, DIABETES, NUTRITION AND METABOLIC DISEASES

4.1. INTRODUCTION

There are multiple connections between digestive diseases and many other specialties. A constant concern over the years has been the study of the various interrelationships between gastrointestinal disorders and diabetes or metabolic disorders. Nutrition is also considered both an etiopathogenic factor and a component of the therapy of digestive diseases. In collaboration with colleagues from the Diabetes, Nutrition and Metabolic Diseases Clinic, we conducted scientific research presented in the form of extensive articles, book chapters, abstracts at national and international conferences, oral presentations. Two papers were awarded - within the National Congress of Diabetes ("Metabolic parameters vary according to different patterns in patients with viral and / or metabolic liver disease") and, respectively, the National Congress of Gastroenterology and Hepatology ("The correlation between circulating periostin levels, the metabolic syndrome and nonalcoholic fatty liver disease among obese patients").Given these many connections between these two specialties: gastroenterology and diabetes, nutrition and metabolic diseases, I will further present the main interdisciplinary research in this field.

4.2. NUTRITION AND DIGESTIVE DISEASES

4.2.1. State of the art

Nutrition can be a cause but also an important component in the management of many diseases. Nutrition is not just about the amount and type of food consumedbut in the modern vision it has multiple roles in prevention, maintaining health and ensuring an optimal quality of life. Both nutrient deficiency (malnutrition) and excess (obesity) are public health problems in many countries. There are numerous guidelines and recommendations on nutrition but each individual reacts differently, genetically determined.Response of an individual to food intake is the result of the interaction of a number of metabolic, genetic, environmental and social factors. Currently, we are talking about both *personalized nutrition* (adapting nutritional recommendations to individualized needs) and *precision nutrition* (finding markers that predict and optimize nutritional intervention). An even more modern approach of nutrition research is the study of the diet-genome interaction, which encompasses the two opposite directions of nutrigenomics (study of the influence diet exerts over the genome, by measuring the cellular or gene response induced by nutritional stimuli) and nutrigenetics (study of the genotype's influence over the cellular or gene response to nutritional stimuli).Current views on personalized nutrition encompass omics technologies (nutrigenomics, transcriptomics, epigenomics, foodomics, metabolomics, metagenomics, etc.), functional food development and challenges related to legal and ethical aspects (Ferguson LR et al, 2016).

The main publications in the field are the followings:

1. Lăcătuşu C, Cijevschi-Prelipcean C, **Mihai C**, Mihai B. Ethics of artificial nutrition. *Rev Rom Bioet* 2014; 12(1): 44-55. IF = 0,462
2. Chirila I, Petrariu FD, Ciortescu I, **Mihai C**, Drug VL. Diet and Irritable Bowel Syndrome.*J Gastrointest Liv Dis* 2012; 21(4): 357-362. IF=1.855
3. Popa RC, Otea A, Gavrilescu O, Dranga M, Bejenariu I, Andronic A, **Mihai C**, Cijevschi Prelipcean C. Good news: coffee in liver disease. *The Medical Surgical Journal* 2020; 124

(1): 14-18.
4. Cijevschi Prelipcean C, Pintilie I, Palaghia M, **Mihai C**. Probioticele si patologia chirurgicala – optiune subevaluata? *Jurnalul de chirurgie* 2013; 9(1): 1-4.
5. **Mihai C**, Cijevschi Prelipcean C, Pintilie I, Nedelciuc O, Jigareanu AO, Dranga M, Mihai B. Nutrition in inflammatory bowel disease. *The Medical-Surgical Journal* 2013;117(3): 662-669.

4.2.2 Nutrition in irritable bowel syndrome

Background & Aim

Irritable Bowel Syndrome (IBS) is a common cause for medical referral and has a clear impact on the patient quality of life and also on the medical system costs. The worldwide prevalence of IBS varies according to the location and design of the study from 1.1% to 22% (Khoshkrood-Mansoori B et al, 2009; Rey E, Talley NJ, 2009). In Romania the prevalence is 14.49% (8.4% man and 17.7% women) with no significant difference between the age groups (Drug VL et al, 2000). Although many patients recognize the impact of specific food in symptom occurrence, very few population-based studies have evaluated the importance of diet in IBS and its role remains uncertain (Eswaran S et al, 2011). The aim of the study was to determine the prevalence of IBS in the general urban population and to evaluate the type of diet associated with IBS symptoms.

Material and methods

The study included a sample of 300 subjects (>18 years old) from a population of 18,000 subjects living in the Pacurari urban area, Iasi, Romania. The sample size and demographic characteristics were estimated to be representative for the general population of the geographic area using Epi Info™ 3.5.2 (CDC) software. The inclusion criteria were: age over 18 years and residency in this urban area, with no exclusion criteria. The selection of subjects was randomized, using a function in Microsoft Excel™ software, from family doctors' patient lists. The family doctors invited the selected subjects by phone for interview and evaluation in their offices.

Two interview-based questionnaires were delivered to all subjects: a Rome III questionnaire (Drossman DA, 2006) for diagnosis of IBS and a food-frequency questionnaire for evaluation of eating habits and frequency of food intake for the last six months. The frequency of food intake questionnaire was developed for use among adults in Romania, but was modified to include more dietary questions. We asked about the main categories of foods consumed in our region, detailing the foods considered of interest to our study. Consumption frequencies were noted: "never or rarely", "monthly", "once a week", "several times a week", "once a day" and "several times a day". We also investigated individual eating habits (including daily breakfast, number of meals and snacks a day, use of home prepared food, meal with family, eating in a hurry as subjective perception).

General medical history (overweight / obesity, diabetes mellitus, hypertension, cancer, cardiovascular, liver, digestive, endocrine, locomotor system, skin, respiratory, neuro-psychiatric diseases and sleep disorders) was also included in the interview together with an objective evaluation of obesity (weight and height were measured by doctors, in their offices).

Age, gender and educational level were studied as demographic factors. Educational level was categorized into three classes: low (no school or elementary school only), medium (high school) and high (college or university). Health-related conditions were investigated: smoking (dichotomized as "current smokers" and "non-smokers"), physical activity (dichotomized as "physically active" if exercise moderate to vigorous at least weekly and "physically inactive" for less) and general well-being (using a 5 point scale "very good – good - acceptable - poor – very poor condition"). Body mass index (BMI) was also calculated.

Descriptive statistics were performed with SPSS 17.0.

Results

During a period of four months (January – April 2011) 300 persons were invited to enroll in the study: 193 subjects (80 males and 113 women) agreed to participate. Participation rate was 64.3%, with no socio-demographic differences between participants and non-participant subjects (for gender, age and educational level, p>0.05). The mean age of the sample was 50.8±16.2 years (range: 20-85).

The prevalence of IBS was 19.17 % (19.47% for females and 18.75 % for males). Evaluation of the age distribution indicated increased prevalence of IBS in subjects above the mean age of sample, with a maximum in the decade 60-69 years (37.5%, p <0.01). Educational level of subjects influenced the prevalence of IBS in the studied population, but not significantly (p=0.066). The trend showed higher prevalence of IBS symptoms toward low educated people (12.5% among high-educated people, 23.0% in medium and 30.3 % in low-educated people). Profession did not reveal any difference between subjects with and without IBS. A history of digestive diseases was more common in subjects with IBS versus non-IBS subjects (29.7% vs. 7.7%, p<0.01). Also, patients with IBS had more commonly cardio-vascular diseases (64.9% vs. 18.6%, p<0.01) including arterial hypertension (75.7 % vs. 31.4%, p<0.01). Obesity (59.5% vs. 24.4%, p<0.01) and diseases of the locomotor system (27% vs. 12.2%, p<0.05) were more common in IBS subjects. History of other diseases, including psychiatric disorders, did not feature often in IBS subjects (p>0.05).

Smoking was not associated with IBS: 13.5 % of IBS subjects were smokers, vs. 29.5% non-IBS subjects (p>0.05). Alcohol (beer, wine or spirits) was also not associated with IBS symptoms (p>0.05). However, 86.5% of IBS subjects and 60.2% of non-IBS subjects were physically inactive (p0.05), acceptable (50% vs. 34.7%, p>0.05), good (36.7% vs. 40.7%, p>0.05). No IBS subjects and 16.9% of non-IBS subjects perceived themselves as in very good condition while no subject perceived himself to be in a very poor condition of well-being. In the sample studied, 49.5% were overweight and 20.8% obese. Presence of obesity was not significantly different in IBS (21.6%) and non-IBS subjects (20.6%) (p>0.05).

Using median as the cut- off point, the IBS subjects ate significantly more frequently the following foods: canned food, processed meat, beef, milk, pulses (legumes), cereals or grain bread /pasta, cafeteria products, fruit compotes (canned or not), herb teas. The difference between IBS and non-IBS subjects was not significant for the consumption of the following type of foods: fish, eggs, fats, vegetables with 5% carbohydrate (lettuce, spinach, tomatoes, peppers), white bread, sugar and sweets, alcoholic beverages and coffee. Using the Spearman correlation test we found an association between IBS and several types of food consumption frequency (Table 4.1).

Table 4.1. Associations of IBS with frequency of food consumption – Spearman test

Food categorie	Correlation coefficient (r)
Canned food	0.279***
Processed meat	0.218**
Potatoes	0.216**
Cereals	0.208**
Grain bread/pasta	0.162*
Fruit compotes	0.337***
Herb teas	0.220**

*p<0.05; **p<0.01; ***p<0.001

Subjective perception of eating in a hurry was more frequent among the IBS patients (41.6 %) than non-IBS subjects (22 %) (p<0.05). Other eating habits (daily breakfast, number

of meals per day, meals with the family or frequent use of home-prepared food) were not significantly different (Table 4.2.).

Table 4.2. Eating habits among IBS and non-IBS subjects

Eating habits	Answers	Non- IBS		IBS		Significance
		No.	%	No.	%	
Breakfast	Not daily	55	35.3	14	37.8	p>0.05
	Daily	101	64.7	23	62.2	
Number of meals and snacks/day	1-2 day	57	42.2	18	51.4	p>0.05
	3/day	48	35.6	14	40.0	
	More than 3/day	30	22 2	3	8.6	
Home cooked food	1 /day or less	65	42.2	16	43.2	p>0.05
	2 /day or more	89	57.8	21	56.8	
Meal with family / day	1 /day or less	101	66.0	22	59.5	p>0.05
	2 /day or more	52	34.0	15	40.5	
Eating in a hurry (subjective perception)	No	117	78.0	21	58.3	p<0.05
	Yes	33	22.0	15	41.7	

Discussions

The prevalence of IBS was 19.47%, higher in women (as most studies have found), but without statistical significance. The prevalence was also increased in older people. Even if the IBS incidence was shown to decrease with age, the prevalence was high in elderly making it an overlooked problem (Agrawal A et al, 2009).

Educational level and profession did not influence the prevalence of IBS. Comparable with other studies, smoking and alcohol was not more common in IBS subjects. In different populations those with higher educational or professional levels were more likely to be physically active and have a healthy diet (Johansson Let al, 1999). Similar with other data, IBS subjects in our study had more commonly gastrointestinal co-morbidities in their past history, but not psychiatric disorders (Talley NJet al, 2001). Higher mean age in IBS subjects may explain the increased prevalence of cardiovascular and locomotor diseases in the IBS subjects.

In our study, certain categories of food (canned food, processed meat, milk, high carbohydrates vegetables, pulses, whole cereals, confectionary, compotes or herb teas) were significantly related to IBS (Chirila I et al, 2012). Food may contribute to symptom onset through several mechanisms including food allergy and intolerance. Also, certain food may alter the composition of the luminal milieu, either directly or indirectly through effects on bacterial metabolism. Finally, IBS symptoms may develop following exposure to food-borne pathogens (Morcos A et al, 2009). A cross-sectional study such as the present one may only reveal association and not causality between the studied elements. A correlation between the studied elements may have several explanations in our case. Frequent consumption of a particular food may positively or negatively influence the presence of disease – for example, canned food, processed meat, milk, or fibre-rich products: 10% carbohydrate vegetables (carrots, onions, beets), pulses (beans, peas, soybeans, lentils), grain cereals/ bread/ pasta or sweet foods: confectionary (cakes, cream, ice-cream), compotes may affect digestive transit, gas production, gut biota and also may cause abdominal discomfort or pain. The presence of IBS may lead, on the other hand, to a specific lifestyle or diet, which may possibly explain the increased use of herb teas in IBS subjects. The relationship between dietary factors and IBS

independent of other potential confounding factors (for example, socioeconomic status) could not be evaluated without a multivariate modelling.

Conclusions

The study, conducted in a general urban population and using Rome III criteria revealed that IBS may be associated with a higher consumption of canned food, processed meat, legumes, whole cereals, confectionary, fruit compotes and herb tea. Further studies are needed to explore the mechanisms that may explain the association.

4.2.3. Nutrition in inflammatory bowel diseases

Background & Aim

The incidence of IBD has increased over the last decades, particularly in developed countries, countries that have adopted habits specific to other geographical areas (Japan, India), or in people that migrated to developed countries and took over new alimentary habits (Windsor JW, Kaplan GG, 2019). This epidemiological trend is primarily accounted for by dietary change. Increased consumption of sugars, fats, low-fiber or high raw diets may cause resistance to degradation, or by incorporation in Peyer plaques macrophages, exacerbation of inflammation in CD in genetically predisposed individuals. Excessive consumption of meat and alcohol favors relapse in UC (the sulfur compounds in these foods intensifies the inflammatory process) (Latella G, 2012). Fats with higher ω-6/ω-3 polyunsaturated fatty acids ratio increase the incidence of CD. European studies have shown that higher linoleic acid levels are associated with increased risk for UC. If ω-3 polyunsaturated fatty acids have anti-inflammatory properties, ω-6 polyunsaturated fatty acids (arachidonic acid, linoleic acid) have proinflammatory properties (Viladomiu et al, 2013).

These considerations led me to being involved in a research group aiming to review the main correlations between nutrition and the pathogenesis of inflammatory bowel disease (Mihai et al, 2013).

Materials and methods

We searched Medline and Scopus databases using the logical string “diet” OR “nutrition” AND “inflammatory bowel disease” to identify these key terms in the title or abstract of English-written articles published between January 2000 and January 2013. Clinical studies or trials, meta-analyses, and systematic reviews focusing on human subjects were selected. After eliminating duplicates, this initial search returned 230 results. We screened all titles and abstracts to select papers that could be considered relevant to the aim of our review. This operation led to a further reduction to only 31 titles. A second search using the same algorithm and replacing the key term of “inflammatory bowel disease” with “CD” OR “UC” issued 11 supplementary relevant papers, which were also included in our analysis (Mihai et al, 2013).

Results

Malnutrition is represented by the discrepancy between body needs and food intake and its components, from macronutrients - carbohydrates, lipids, proteins - to micronutrients - vitamins, minerals. Malnutrition is present in 85% of IBD patients, and weight loss in 80% of CD and 18-62% of UC cases. Malnutrition has negative consequences on cellular and humoral immunity, causes growth and developmental disorders in the child, increases the risk of postoperative complications and, ultimately, decreases the QoL. The factors that determine malnutrition in BII can be divided into 3 categories (Forbes A et al, 2017):gastrointestinal symptoms (anorexia, nausea, vomiting, abdominal pain, diarrhea) - cause restrictive diets, reduced appetite and food intake;food intolerance due to inflammation and surgical resections - leads to nutrient deficiencies; intestinal inflammation, along with fistulas, surgical resections - reduces the absorption surface.

A single indicator is not enough to determine the nutritional status, its assessment requiring nutritional history, physical examination and laboratory data. Subjective global assessment, originally used in oncology, could be a tool for the screening of IBD patients (using the following parameters: history of weight loss, food intake, gastrointestinal symptoms, functional capacity). Serum levels of albumin may be used as a marker of nutritional status. Hypoalbuminemia occurs in 25-80% of CD patients and 25-50% of those with UC and reflects the metabolic response to stress, so that in IBD its level can be normalized by proper nutrition and by the correction of inflammatory process (Mihai et al, 2013).

Clinical nutrition is a supportive component of the IBD therapy, which corrects specific nutritional deficits and determines the induction and maintenance of the remission.

Individual features of oral nutrition must be established following a dietitian advice for a healthy, individualized diet with an elementary, fractioned intake with liquid oral supplements. Compliance with elemental or semi-elemental diets in CD and UC flare is poor due to their cost, taste, smell, texture (Prince et al, 2011).

Resting energy requirements are not increased in CD patients, and the BMI can be used to assess the caloric needs. The energy requirements of IBD patients are strictly dependent of the BMI (Table 4.3).

Table 4.3. Energy requirements of IBD patients according to BMI values (Klein S, 2002)

BMI (kg/m^2)	Energy requirement (kcal/kg body weight)
<15	36-45
15-19	31-35
20-29	26-30
>30	15-25

BMI, body mass index

Patients with IBD have increased protein needs due to intestinal losses and increased catabolism (infection/abscess/required surgery). Protein needs are assessed by body weight and disease status. The recommended daily protein intake for IBD patients without kidney disease is 1-1.5 g/kg, being reduced to 0.8 g/kg body weight for the patients with renal failure not receiving dialysis (Prince et al, 2011). Vitamin and mineral requirements in IBD patients, as well as supportive measures in case of nutritional deficiencies, are found in Table 4.4.

Vitamin B_{12} deficiencyis found in distal ileal CD or in case of gastric resection (intrinsic factor deficiency) or terminal ileum (absorption deficit). Due to these aspects serum methylmalonic acid (S-MMA) concentration is elevated, vitamin B_{12} concentration is decreased, and homocysteine is elevated. The treatment consists in the administration of vitamin B_{12}.

Folic acid deficiencyoccurs either through increased requirements or by decreased intake (the main sources of folic acid – fruits, vegetables – are difficult to tolerate for IBD patients). Supplementation with folic acid 1 mg/day corrects the deficiency and protects against the risk for colorectal cancer. A special mention must be made on increased homocysteine levels that occur in vitamin B_{12} and folate deficiency, with increased risk for thrombosis, atherosclerosis, cardiovascular diseases and stroke (Massironi et al, 2013).

Bone metabolism disorders, usually due to calcium and vitamin D deficiency,increase the risk of fractures in IBD by 40%. The prevalence of osteoporosis is evaluated at 3-58% in UC and 4-50% in CD, due to deficiencies in proteins, vitamin D, and Ca intake, presence of inflammatory cytokines and corticosteroids. A major role is represented by old age, weight

loss, disease status, and treatment with corticosteroids. Supplementation with calcium + vitamin D preparations (1000-15000 mg calcium + vitamin D 400-600 IU/day) is recommended and taking into consideration the fact that Prednisone can induce vitamin D resistance by increasing parathyroid hormone level and calcium losses. In patients with severe malabsorption 2,000-4,000 IU of vitamin D/day need to be administered parenterally, and in osteoporosis, bisphosphonates are preferred (Massironi et al, 2013).

Table 4.4. Vitamin and mineral requirements and treatment of deficiencies in IBD patients

Nutrient	Recommended daily requirements	Signs and symptoms of deficiency	Recommended replacement oral dose
Zinc	15 mg	Dry, flaky skin, palm peeling, diarrhea, mental status changes	50 mg elemental/day
Iron	10-15 mg	Microcytic anemia, fatigue	300 mg 1-3/day
B_{12}	3 mcg	Megaloblastic anemia, ataxia, diarrhea, mental status changes	1000 mcg/day
Folate	400 mcg	Sore mouth, glossitis, diarrhea, inattentiveness, megaloblastic anemia	1 mg/day
Calcium	800-1500 mg	Osteopenia, osteoporosis, tetany	1500-2000 mg/day
Magnesium	400 mg	Nausea, muscle weakness, arrhythmia, confusion, convulsions	150 mg elemental 4x/day
Vitamin D	400 IU	Rickets, osteomalacia, bone pains, muscle weakness, tetany	Variable

Zinc deficiencyoccurs in fistulas and short bowel syndrome. The recommended dose for substitution is 15 μg/kg/day. Iron deficiencysecondary to hemorrhage occurs in 80% of UC patients and in 40% of those with colonic CD. In iron deficiency anemia injectable iron (100-200 mg ferrous sulfate) is preferred because iron administered p.o. is accompanied by gastrointestinal intolerance in 21% of cases intestinal inflammation and increased risk for colorectal cancer. Magnesium deficiencyoccurs in case of fistulas and colectomies, and short bowel syndrome and parenteral administration is preferred: 5% magnesium gluconate which has a high solubility (Eiden, 2003).

Enteral nutrition(EN), used since the 70's and 80's in IBD, may be used as an autonomous therapy or as an adjunctive to drug therapy. Studies on EN showed several characteristic aspects for patients with CD (Gupta et al, 2013): corticosteroid therapy is more effective in obtaining remission compared to EN in adults; EN in children, particularly ileum located, is superior to corticosteroid therapy, being the choice of first-line treatment for reducing the negative effects of corticosteroids on growth and bone metabolism. The studies on EN in UC have shown inconsistent results, most of them not recommending it either in inducing or maintaining remission. Combined therapy is indicated perioperatively in malnourished patients with intestinal stenosis (weight loss > 10%, BMI <18.5 kg/m^2, and albumin below 30 g/l). The response to EN is assessed after 3 to 6 weeks (Levine et al, 2013). EN diminishes inflammation and induces remission without the side effects of 5-amino-salicilyc derivates (5 ASA), steroids, or Azathioprine (AZA). It has also a role in maintaining remission, especially in patients with EN-induced remission. It can cause "mucosal healing" through its local immunstimulatory action (Levine et al, 2013). EN is administered orally, by nasogastric or nasoenteric tubes. If EN should be administered for more than 4 weeks, a percutaneous gastrostomy or jejunostomy is required (in CD patients this procedure increases the risk for fistulas). The main complications are: aspiration, hemorrhage, perforation, nose

bleeding, pneumothorax, tube obstruction, intestinal ischemia and in stoma there is a high risk of infections, fissures or ulcerations (Gupta et al, 2013).

Parenteral nutrition is indicated in the following situations associated with IBD: obstruction, fistula, toxic megacolon, short bowel syndrome, severe malabsorption, loss of fluids and electrolytes, other conditions that make EN impossible/ineffective. CD is one of the main indications for parenteral nutrition at home. The most common complications of parenteral nutrition can be: liver, biliary, catheter sepsis, thrombosis, decreased quality of life (Alanazi et al, 2011).

Conclusion

Nutrition in IBD is a complex problem that requires a good nutritionist-gastroenterologist collaboration and patients with IBD will benefit by the assessment and correction of existing deficits. EN is an adjuvant therapy in inducing and maintaining clinical remission and in mucosal healing. Parenteral nutrition is reserved for severe cases with complications.

4.2.4. Ethical issues in artificial nutrition

Background & Aim

As nutritional approach is not enough, in most cases, to cure a disease by itself, artificial nutrition cannot be considered a curative therapy. Artificial nutrition should rather be seen as an adjunctive treatment, offering the patient supplementary nutritional resources during the course of background curative or palliative therapies. Administration of nutrients using gastric or jejunal feeding tubes, or intravenous catheters, may increase the chances of survival in patients who are not able of oral feeding.

However, these benefits are complemented by a wide range of side effects, which transform the matter of choosing between one or the other of artificial nutrition's specific procedures into a medical, and often an ethical, dilemma. EN may be associated with nausea, vomiting, diarrhoea, esophageal perforations or pulmonary aspiration of the feeding mixtures. Parenteral nutrition induces an increased risk of phlebitis, catheter site infections, dyselectrolytemia, peripheral edema or acute pulmonary edema, if renal failure develops (Slomka, 2003). Both enteral and parenteral nutrition are associated with higher risks for bleeding. They also trigger a wide range of medical and ethical consequences when restraint of a non-compliant patient is brought into discussion in order to avoid his potentially detrimental removal of tubes and catheters needed by artificial nutrition procedures.

Materials and methods

We searched Medline and Scopus databases using the logical string “artificial nutrition” OR “enteral nutrition” OR “parenteral nutrition” AND “ethics” to identify these key terms in the title or abstract of English-written articles published between January 2000 and January 2014. Clinical studies or trials, meta-analyses, and systematic reviews focusing on human subjects were selected. After eliminating duplicates, this initial search returned 609 results. We screened all titles and abstracts to select papers that could be considered relevant to the aim of our review. This operation led to a further reduction to only 47 titles. A second search using the same algorithm and replacing the key term of “artificial nutrition“ with “feeding tube“ OR “nutrition support” and the key term of “ethics” with “ethical issues” OR “patient consent” issued 8 supplementary relevant papers, which were also included in our analysis (Lăcătușu et al, 2014).

Results

Artificial nutrition includes several procedures (Lăcătușu et al, 2014):

- Intravenous fluid administration provides the daily needed fluids for the patient’s proper hydration, but it does not deliver, in most cases, an adequate supply of nutriments;

- Nasogastric tubes inserted through the patient's nasal passages and upper digestive tract into the stomach provide a proper supply of liquids and nutriments. They are mostly used in patients with acute illnesses, since their placement cannot be permanent;

- Hypodermoclysis represents the subcutaneous infusion of less than a liter of fluid per day;

- Percutaneous endoscopic gastrostomy is the procedure of inserting a tube into the patient's stomach through the abdominal wall; this technique is used when it is anticipated that the patient will not be able to feed orally for several weeks. Rarely, the tube may be passed through the abdominal wall and placed directly into the jejunum (jejunostomy). Both techniques are capable to ensure a proper supply of nutriments and liquids.

For a long time, controversies emerged whether artificial nutrition is part of the basic medical care or a medical treatment. The cultural and religious concepts left their marks upon the perspectives from which nutrition is regarded in the daily life and as a part of the medical care. The Catholic Church sees life as a benefit, no matter how severe a person's disabilities may be. Therefore, feeding the patient is considered to be basic care under any circumstances (Monod et al, 2011). Pediatricians claim that in newborns EN is part of the basic care. However, in adults, modern ethics sees artificial nutrition as a medical procedure, so the decision to initiate or withdraw needs to comply with the same ethical rules like transfusion or dialysis (Monod et al, 2011).

Since artificial nutrition is considered a medical treatment, competent adults may accept or not this procedure, like any other medical treatment. The patients' consent is crucial to initiate or continue artificial nutrition, even if refusing the respective techniques may cause their death. The issue of initiating or withdrawing artificial nutrition becomes even more delicate with incompetent patients.

If the patient is not able to understand or express his wish, the physician may have several options. Obviously, if the patient has previously mentioned his wish in writing regarding the therapy he would accept under these circumstances, these wishes need to be obeyed. In some countries, family has legal rights to make decisions or – if the patient orally stated his wish in front of family members, friends or even the physician – these forms of orally expressing his decision may be taken into consideration when making a decision. Some countries consider that the patient may be legally represented by a person (a family member, a friend or a lawyer) previously appointed to represent his interests when he becomes unable to decide. In Romania there are no clear legal provisions concerning either the advance directive or the other persons who might decide on behalf of a patient who became incompetent to make decisions on the possibility of artificial nutrition. Therefore, legal norms and medical practice guidelines in this field are strongly required in our country.

There are no legal differences between withholding and withdrawing artificial nutrition. If the burden of artificial nutrition is greater than its benefit or if it no longer meets its intended goals, then this treatment can be withdrawn with consent of the patient or – in some countries – of the legal representative. If the patient or his legal representative withdraws his initial consent, artificial nutrition can be interrupted. Withdrawing support therapy will be made in the following order: dialysis, vasopressors, blood tests and afterwards the nutritional support and mechanical ventilation. If a patient's diagnosis or prognosis is uncertain, a test period for that nutritional intervention could be useful: a set of therapeutic goals and a period of time in which they should be reached are to be established and the treatment may start. At the end of the initially established time, the efficiency of the artificial nutrition is assessed; if it has not proved to be beneficial, it will be discontinued (Monturo, 2009).

The efficiency of artificial nutrition depends on the patient's overall condition and the reason it is recommended. Artificial nutrition has good results in patients with temporary swallowing or superior gastrointestinal tract diseases and in those with certain time-limited

disabling conditions. We can mention here patients with non-neoplastic upper gastrointestinal tract obstructions, patients who receive treatment which prevents them from eating for more than two weeks, patients with persistent or recurrent intestinal obstructions, patients with post-medication disorders or intestinal resections (Körner et al, 2006). Artificial nutrition might even prolong life, therefore allowing time for a more precise assessment of a patient's recovery chances, if his initial prognosis is uncertain. Therefore, ethically speaking, artificial nutrition is highly recommended in these cases. If a patient is dependent on artificial nutrition for an adequate nutriment supply and enjoys his life, artificial nutrition is clearly useful, not only physiologically, but also in terms of quality of life.

In *elderly persons* with acute diseases, artificial nutrition can reduce complications and mortality. In persons receiving home care assistance, artificial nutrition determines only a minor weight improvement, without any change in mortality.

Around 40% of the patients having suffered a *stroke* have swallowing disorders and cannot eat. In these situations, the nutritional status progressively fails and leads to an unfavorable prognosis. This is the reason why some authors support the early provision of artificial nutrition (insertion of a nasogastric tube or percutaneous endoscopic gastrostomy), although there are no studies to prove the benefits of this therapy. However, even if dysphagia is present in more than a third of the patients when being hospitalized, this percentage decreases in a week to 16%; therefore, enteral nutrition is generally recommended to be postponed for a week. This period of time allows a more accurate assessment of the patients, as well as their recovery from aphasia or dysarthria, so that they can express their own options. It is usually preferred to initially use a nasogastric tube, since it involves low mortality and can be easily removed if deglutition recovers. If the patient does not recover from deglutition disorders and needs long-term enteral nutrition, resorting to percutaneous endoscopic gastrostomy may be necessary. Most authors recommend the invasive methods of enteral nutrition only two to four weeks after the stroke (MacFie, 2005).

The persistent vegetative state is a form of permanent alteration of consciousness where patients are in a state of partial wakefulness and have physiological sleep-wake cycles, but are completely unconscious about themselves or the surrounding world. It is induced by any pathological situation in which the functioning of the cerebral cortex is totally damaged, but the nervous activity of the brainstem is preserved. Providing nutrition support to these patients to provide comfort and reduce suffering has not a scientific basis (Fuhrman, 2008). However, artificial nutrition can definitely prolong life for some patients under a persistent vegetative state, so it might be prudent to provide artificial nutrition when the diagnosis is uncertain.

Population ageing results, among other consequences, in the dramatic increase of *Alzheimer's disease* and *cerebrovascular disease* with cognitive impairment incidences. In mild or moderate forms of dementia patients do not remember having eaten or not. It is then necessary to monitor their meals and even place some snacks within the patients' reach, in order to help themselves and properly maintain their nutritional status (Körner et al, 2006). Eating disorders usually appear in advanced forms of dementia; patients may present deglutition alterations, pulmonary aspiration of feeding material, inability to self-feed, loss of interest in food or even resistance to feeding (MacFie, 2005). All this frequently leads to malnutrition and food supplements may be useful (Körner et al, 2006). Some physicians appeal to EN (usually through percutaneous endoscopic gastrostomy), aiming to improve the patient's nutritional status, prevent or heal pressure sores and prevent aspiration through the trachea and secondary pneumonia. Sometimes, healthcare staff may use EN simply due to the fact that it meets their needs (even if not those of the patient), since feeding a dementia patient by mouth takes more skills and time than tube feeding. However, studies have shown that, in patients with dementia, EN does not reduce the risk of pressure sores or pneumonia, does not induce an improvement of the cognitive abilities or daily performances and does not increase the patients'

comfort, weight or functional status or their survival (Fuhrman, 2008). Overall, EN in patients with dementia has not proved any real long-term benefit. Moreover, the immediate risk of death resulting from the insertion of a feeding tube can be quite high, varying between 4 and 54% (Fuhrman, 2008). The use of a nasogastric tube may induce diarrhea, aspiration syndrome, tube obstruction or its removal by the patient. Percutaneous endoscopic gastrostomy may be associated with discomfort, aspiration syndrome, infections, oral hypersecretion, feeding tube dysfunctions (MacFie, 2005). There are cases when patients with dementia are physically restrained to prevent them from removing the feeding tubes. In order to increase the quality of life, it is recommended that patients with advanced dementia be provided oral feeding. In their case effort should be made to remove dietary restrictions. In the latest studies on dementia, artificial nutrition is considered to have more risks than benefits and not to be initiated; providing comfort and dignity to the patient is more important than the nutritional treatment (MacFie, 2005). Exception should be made for the patients with vascular dementia, who may improve their cognitive functions. This emphasizes the importance of accurate neurological assessment to confirm the diagnosis.

In some forms of *cancer*, patients actually "starve to death" and in these cases artificial nutrition is beneficial. We mention here cancers localized in the cephalic and cervical regions, esophageal neoplasm with secondary local obstruction and ovarian cancer with intermittent small-bowel obstruction. Weight loss is often an inevitable result of antineoplastic therapy, so in such cases nutritional interventions may prevent nutritional and physical impairment (Fuhrman, 2008). If there are doubts about the patient's prognosis, artificial nutrition may be provided for a limited period of time and the decision to continue or withdraw will be made depending on the clinical results. In patients with terminal cancer, malnutrition is responsible for 25% of deaths. Patients with terminal cancer are rarely hungry and, if hunger occurs, it is tempered by small amounts of food. It is recommended to provide appetizing meals, according to the patient's preferences, at an adequate temperature, in a quiet environment, accompanied by comforting music. Patients must not be forced to eat against their will (Fuhrman, 2008). Providing comfort and improving symptoms are, on the other hand, more important than an aggressive nutritional support.

Patients with terminal illnesses, nearing death, lose their appetite and become incapable to self-feed. Until recently, death from malnutrition and/or dehydration was thought to induce supplementary pain to the patient, so that artificial nutrition was recommended by routine. Nowadays it is known that the decrease of liquid and nutrients intake determines an improvement of the symptoms. Most terminal patients often do not feel hungry or thirsty. The starvation ketosis leads to the body's release of endogenous opioids, which are thought to block pain and discomfort (Slomka, 2003). The role of hydration in terminal patients is controversial. More studies have shown that liquids play just a small role in these patients' comfort as long as they are provided rigorous oral hygiene. The sensation of dry mouth may be treated with pieces of ice chips, moistened swabs or lip balms. There is no evidence that medically assisted hydration at the end of life prolongs survival. Water deprivation increases the intern production of endogenous opiates that lead to a euphoric state and seem to be associated with pain reduction, inducing the patient a state of somnolence before death. Moreover, intravenous hydration can exert a negative impact upon the quality of life because of risks of patient's physical restrain, increasing pulmonary secretions and urinary output, bleeding, nausea, vomiting, fecal incontinence and edema.

Conclusion

Artificial nutritional support is a therapy raising numerous ethical issues and which needs to be implemented according to the patient's wishes, diagnosis, prognosis and therapeutic goals. The communication between the patient, his family and the healthcare team is crucial. If the discussion is open and sincere, it will help making decisions to the best interest of the

patient. Patient's autonomy always needs to lie at the foundation of these decisions. Moreover, once artificial nutrition has been initiated, it is necessary to periodically monitor its efficiency and possible side effects, so that the initial decisions may be adjusted consistent with the upcoming reality. In Romania adequate legislation and specific protocols need to be set in order to facilitate the progress of making decisions related to artificial nutrition from the perspective of the risk/benefit balance.

4.3. DIABETES MELLITUS, METABOLIC SYNDROME AND DIGESTIVE DISEASES

4.3.1. State of the art

Diabetes mellitus (DM) is a disease with a growing prevalence, especially in developed countries. This increase is mostly due to the alarmingly outgrowing number of cases of type 2 diabetes, originating in an unhealthy lifestyle and an increased prevalence of obesity (International Diabetes Federation, 2019). According to the most recent available data, more than 463 million persons with diabetes existed worldwide in 2019, and their number is predicted to outrun 700 million in 2045 (International Diabetes Federation, 2019). Given the chronic complications it may induce, diabetes mellitus is a major public health problem.

Complications from diabetes mellitus involve a wide range of internal organs and systems. Some of the best researched forms of digestive disease in diabetes mellitus are due to the diabetic autonomic neuropathy, which concerns the vegetative nervous system.The vegetative (or autonomic) nervous system adds together the enteric, parasympathetic and sympathetic nerve systems. The autonomic neuropathy can bring upon the patients with diabetes mellitus some of the most burdensome symptoms, which severely lower the quality and sometimes the duration of life. On the other hand, autonomic neuropathy goes frequently unrecognized and under-diagnosed. The first forms of autonomic neuropathy to appear seem to concern the vagus nerve, which probably is one of the most vulnerable segments of the vegetative nervous system. Among patients with a duration of diabetes longer than 20 years, up to half of them may display gastrointestinal symptoms (Sandireddy et al, 2014).

The metabolic syndrome is defined as a complex of risk factors for cardiovascular disease: abnormal values of serum glucose levels, high blood pressure, increased triglyceride levels, low HDL-cholesterol levels and abdominal obesity. Even though this association between cardiovascular risk factors was postulated years ago, the pathogenesis of the metabolic syndrome is still unclear; insulin resistance was progressively attributed a more and more important role in the determinism of the metabolic syndrome during the last years. The increasing prevalence of obesity and the sedentary lifestyle induce a continuously increasing prevalence of the metabolic syndrome, which thus becomes a clinical and public health problem. Subjects with metabolic syndrome exhibit atherogenic dyslipidemia, high blood pressure and serum glucose levels, as well as a prothrombotic și proinflammatory status.subjects with metabolic syndrome have a double risk for developing cardiovascular disease in the next 5 to 10 years, when compared with individuals without metabolic syndrome (Alberti et al, 2009). Moreover, the existence of the metabolic syndrome determines a 5-fold increase in the risk for type 2 diabetes (Alberti et al, 2009).

Non-alcoholic fatty liver disease (NAFLD) has become the today's epitome of metabolic chronic liver disease. Defined as the lipid overload in the hepatocytes that is caused by neither excessive alcohol intake (over 20 g/day in women and 30 g/day in men), nor hepatitis B or C viruses, autoimmune hepatitis, drug liver toxicity, or rare gene defects, NAFLD is estimated to be present in up to one-third of the adult population of developed countries (Younossi et al, 2018).Long-term NASH leads not only to liver damage (liver

cirrhosis and HCC) but also to increased mortality from diabetes, cardiovascular or kidney disease.

My preoccupations in this research field were materialized in the following papers:

1. Mihai BM, **Mihai C**, Cijevschi-Prelipcean C, Grigorescu ED, Dranga M, Drug V, Sporea I, Lăcătușu CM.Bidirectional Relationship between Gastric Emptying and Plasma Glucose Control in Normoglycemic Individuals and Diabetic Patients. *Journal of Diabetes Research* 2018; ID 1736959, 9 pages. IF = 2,29
2. **Mihai C**, Mihai B, Trifan A, Stanciu C, Gheorghe L, Diculescu M, Curescu M, Brisc C, Goldis A, Bataga S, Sandulescu L, Rogoveanu I, Seicean A, Cijevschi Prelipcean C. Metabolic syndrome and genotype 1 virus C compensated liver cirrhosis in the era of directly acting antiviral therapy. *Hepatitis monthly* 2017 17(7):e58022. IF = 1.67
3. **Mihai C**, Chiosa AM, Savin A, Badea M, Ungureanu I, Cucuteanu B, Mihai B, Drug V, Trifan A, Stanciu C, Prelipcean Cijevschi C. Steatosis in Patients with Genotype 1b Virus C Compensated Liver Cirrhosis. Proceedings xxxvith national congress of gastroenterology, hepatology and digestive endoscopy. Cluj Napoca 8-11 iunie 2016. Editor: Dan Dumitrascu. FILO diritto editure. www.gastro2016.medical-congresses.ro.: 252-258.
4. Cijevschi Prelipcean C, Dranga M, **Mihai C**. Reconsidering statins – current view. *The Medical Surgical Journal* 2019; 123 (1): 27-32.
5. Mihai B, **Mihai C**, Cijevschi Prelipcean C, Lacatusu C. Rare types of diabetes mellitus. *Rev medico-chirurgicala a soc de medici si naturalisti* 2012; 116(3): 700-707.
6. Lăcătuşu C, Mihai B, Graur M, **Mihai C**, Dranga M, Cijevschi-Prelipcean C. Hepatita cronică cu virus C, steatoza şi insulinorezistenţa – implicaţii clinice. *Rev medico-chirurgicala a soc de medici si naturalisti* 2011; 115(2): 306-315.
7. Mihai B, Lacatusu C, Graur M, Cijevschi Prelipcean C, **Mihai C**. Tratamentul medicamentos al polineuropatiei diabetice periferice. *Rev medico-chirurgicala a soc de med si nat* 2010; 114 (2): 332-341.
8. Lacatusu C, Mihai B, Graur M, **Mihai C**, Cijevschi Prelipcean C, Zbranca E. Obezitatea, sindromul metabolic si steatohepatita nonalcoolica – rolul insulinorezistentei. *Romanian Journal of Endocrinology and Metabolism* 2009; 8 (2): 7 – 17.
9. Cijevschi Prelipcean C, **Mihai C**, Stefanescu G, Irimia AM, Zbranca E. Sindromul ovarelor polichistice, insulinorezistenta si steatohepatita nonalcoolica. *Romanian Journal of Endocrinology and Metabolism* 2009; 8 (2): 67 – 72.
10. Lăcătuşu C, Mihai B, Cijevschi-Prelipcean C, **Mihai C**, Graur M.Adipocitokinele şi steatohepatita nonalcoolică. *Revista Medico – Chirurgicala* 2008: 112 (4): 882 – 889.

4.3.2. Relationship between Gastric Emptying and Plasma Glucose Control

Background & Aim

Gastric emptying and glycemic control pathways are closely interrelated processes. Gastric chyme is transferred into the duodenum with velocities depending on its solid or liquid state, as well as on its caloric and nutritional composition. Once nutrients enter the intestine, the secretion of incretins (hormonal products of intestinal cells) is stimulated. Among incretins, glucagon-like peptide-1 (GLP-1) has multiple glycemic-regulatory effects that include delayed gastric emptying, thus triggering a feedback loop lowering postprandial serum glucose levels. Glycemic values also influence gastric emptying; hyperglycemia slows it down, and hypoglycemia accelerates it, both limiting glycemic fluctuations.

In clinical medicine, gastroparesis is frequently encountered in association with DM, as about one-third of all cases of gastroparesis originate from complicated diabetes. Prevalence of

gastroparesis is about 5% in type 1 diabetes patients and about 1% in type 2 DM. As gastroparesis is often associated with delayed gastric emptying, most research papers analyzed the velocity of gastric emptying, which is reduced in 30 to 50% of cases with long-lasting diabetes (Camilleri et al, 2013). In most cases, patients with overt gastroparesis have a long duration of insulin-dependent DM and a poor glycaemic control for at least several years. Usual gastroparetic symptoms may include nausea, vomiting, bloating, early satiety and epigastric pain. 80% of the patients presenting with gastroparesis are predisposed to the dysfunction of the small intestine, expressed as bacterial overgrowth in the small bowel or as pathogenic interactions between host and gut microbiota (Camilleri et al, 2013).

Materials and methods

We searched Medline and Scopus databases using the logical string "diabetes" OR "diabetes mellitus" AND "gastric emptying" to identify these key terms in the title or abstract of English-written articles published between January 2000 and January 2018. Clinical studies or trials, meta-analyses, and systematic reviews focusing on human subjects were selected. After eliminating duplicates, this initial search returned 794 results. We screened all titles and abstracts to select papers that could be considered relevant to the aim of our review. This operation led to a further reduction to only 52 titles. A second search using the same algorithm and replacing the key term of "gastric emptying" with "gastroparesis" OR "gastric motility disorders" and the key term of "diabetes" with "glucose disorders" OR "hyperglycemia" issued 14 supplementary relevant papers, which were also included in our analysis (Mihai et al, 2018).

Results

Analysis of the mechanisms involved in the physiologic control of gastric emptying

When foods enter the stomach, the proximal gastric region initially relaxes in order to "accommodate" the ingested nourishments. They subsequently reach the distal areas of the stomach and, by means of antral contractions, are ground and mixed with the gastric hydrochloric acid secretion. Gastric chyme (Greek: khymos = juice), a semifluid mass, is thus formed. When the resulting particles are less than 1–2mm in diameter, they go through the pylorus into the duodenum. Their transit is backed by antral contractions and relaxation of the pyloric sphincter. In normal conditions, the rate of gastric emptying may vary between 1 to 4 kcal/min (Marathe et al, 2013), depending on the composition of gastric content (solids or liquids) and the macronutrient type. As a result of their high caloric content, lipids are evacuated more slowly from the stomach than carbohydrates or proteins (Tappenden, 2017). Digestion of solids begins after feeding with a lag time of 20 to 40 minutes, required for their grounding into 1–2mm diameter particles, and then, a quasilinear gastric emptying begins. Therefore, their evacuation from the stomach begins approximately 40 minutes after food intake and may last for a few hours. Evacuation of liquids is immediate (without any lag phase) and usually mono-exponential. The more nutritionally dense the liquids are, the slower the gastric emptying becomes (Phillips et al, 2015). In fact, caloric content of foods may exert a greater influence on gastric emptying than previously thought. Some recent results suggest not only liquids of equal energetic densities are evacuated from the stomach with similar speeds, but meals with the same caloric content given with equal amounts of water have nearly identical gastric emptying curves, no matter if their initial form was solid or liquid (Okabe et al, 2017).

Nearly thirty years ago, an intermittent transpyloric flux was described, with antral contractions and pyloric relaxation turning up at approximately 20-second intervals, while duodenal flux was continuous and uniform. At that moment, the mechanical function of the duodenal bulb was thought to be the only factor to transform this flux from intermittent to continuous. The mechanism is in fact far more complex. When nutrients reach the intestine, they become a signal stimulating the blood release of intestinal hormones known as the incretin system. K cells from the upper intestine (duodenum) secrete glucose-dependent insulinotropic peptide; in the distal segment of the intestine, L cells secrete GLP-1. Both GLP-1 and glucose-

dependent insulinotropic peptide have glucose-dependent insulin secretion effects; GLP-1 inhibits glucagon secretion, and glucose-dependent insulinotropic peptide exerts glucagonotropic actions (Phillips et al, 2015; Wu et al, 2016). While it was demonstrated that glucose-dependent insulinotropic peptide has no effect on gastric emptying, GLP-1 induces an inhibitory feedback effect, delaying gastric emptying. Recent data also suggest a relation between glycemic values and stomach emptying (Wu et al, 2016). High velocity gastric emptying allows nutrients to reach the intestine more rapidly, thus increasing postprandial glycemia; on the other hand, hyperglycemia delays stomach emptying, so the nutrients are propelled more slowly for absorption at the intestinal level (Plummer et al, 2015). Hypoglycemia induces reverse effects, by accelerating gastric emptying and increasing the nutrient absorption speed, thus allowing for a prompter correction of glycemic levels (Marathe et al, 2013). The ability to increase gastric emptying was found to persist in healthy individuals even after repeated hypoglycemic episodes (Wu et al, 2016). This is in contrast to other hypoglycemia-induced reactions, such as the clinical signs induced by adrenergic response, which are subdued by impaired hormonal counter-regulation and tend to fade out during recurrent hypoglycemia.

As mentioned before, gastric emptying establishes a bidirectional relationship with glycemic levels: glycemia influences gastric emptying, while the latter may also influence the value of postprandial glycemia. Postprandial serum levels of glucose are essential in diabetic patients. Postprandial hyperglycemia is associated with increased oxidative stress and thus directly involved in the pathogenesis of chronic micro- and macrovascular complications of DM.

Several research teams found a direct relationship between the rate of gastric emptying and postprandial serum glucose levels. In patients with type 1 DM, altered rates of gastric emptying may impair efforts to adjust doses of prandial insulin according to the amounts of ingested nutrients. The most difficult problem in patients with type 1 DM is not the issue of too high or too low speeds of gastric emptying but its unpredictability. Most authors found that gastric emptying is increased even after recurrent episodes of hypoglycemia, not only in healthy individuals but also in diabetic patients (Kar et al, 2016). Evaluation of gastric emptying in insulin-treated diabetic persons with frequent hypoglycemia found it to be delayed in most situations; in fact, such individuals exhibit discrepancies between the action of prandial insulin and the rates of gastric emptying. Hereupon, the evaluation of diabetic patients with frequent hypoglycemia should best include the assessment of gastric emptying; the "gastric hypoglycemia" (by delayed gastric emptying) proves to be an important concept in the management of DM (Parthasarathy et al, 2017).

Analysis of the effects of nutrients on gastric emptying and plasma glucose levels

Effects exerted by the main nutrients or other food components on both these aspects can hardly be separated from other perspectives, given that both gastric emptying and postprandial glycemic values imply the coexistence of meals and therefore nutrients. Adding sources rich in proteins to carbohydrate-based meals determines a 20% to 30% reduction in postprandial glucose levels. Besides the stimulation of insulin secretion (driven by direct stimulatory effects, but also indirectly, through an increased incretin response), underlying pathways include delayed gastric emptying under the influence of the same incretin hormones. Whey or soy proteins seem to give the best responses, but favorable effects on postprandial glycemic levels were also reported for rice, pea, and oat proteins. The structure of whey and soy proteins is rich in branched-chain amino acids, allowing faster digestion and absorption times, and therefore a quicker insulin release from pancreatic beta cells (Giezenaar et al, 2017).

In contrast with whole-structure proteins, intragastric administration of isolated amino acids such as lysine, leucine, or isoleucine does not seem to influence gastric emptying, even though it may reduce postprandial glycemic levels, most probably by direct stimulation of insulin secretion (Mano et al, 2018). Other three amino acids, histidine, glutamate, and aspartate, were

reported to increase both postprandial glycemic levels, velocity of gastric emptying, and GLP-1 serum concentrations (Mano et al, 2018). L-Tryptophan isomeric form was found to significantly delay gastric emptying, even though the effect on GLP-1 secretion was minimal.

Classical nutrition information considers that meals with high lipid content reduce the velocity of gastric emptying. Recent data seem to confirm this theory by showing, for example, that high-fat meals may worsen symptoms of diabetic gastroparesis when compared to low-fat ones (Homko et al, 2015). More than the absolute lipid load, the degree of emulsification and the lipid droplet size seem to influence gastric emptying. Fine emulsions of olive oil in water slowed gastric emptying more than a coarse emulsion or a nonemulsified mixture of olive oil and water. Dietary fiber-rich foods are also able to reduce postprandial glycemia, with soluble fiber exerting the most pronounced effect. Most common explanations usually refer to an unmediated ability of soluble fiber to delay glucose absorption (Homko et al, 2015). However, the exact magnitude and conditionality of the effect fibers may exert on gastric emptying are still debatable.

Analysis of the effects of GLP-1 receptor agonists on gastric emptyingand plasma glucose levels

GLP-1 receptor agonists are classified based on their half-life; the short-acting agents are designated as prandial agonists (exenatide BID, lixisenatide), while long-acting agents are considered nonprandial agonists (exenatide QW, liraglutide, dulaglutide). Their glycemic effects differ, as prandial agonists mostly influence postprandial glycemia and nonprandial agonists exert a greater effect on fasting glycemia (Meier et al, 2015). As nonprandial GLP-1 receptor agonists have longer half-lives and prolonged action, the gastric emptying effect is reduced by tachyphylaxis and their influence on postprandial glycemia is thus diminished by comparison with prandial agonists. However, effects of short-acting and long-acting GLP-1 receptor agonists on postprandial glycemia are not always different, since semaglutide, a longer-duration GLP-1 receptor agonist seems able to lower postprandial glycemia and the velocity of gastric emptying in obese subjects.

Novel therapeutic guidelines for type 2 diabetes recommend the association of prandial GLP-1 receptor agonists to basal insulin; benefits of such pharmacologic combinations bring together the predominant effect of basal insulin on fasting glycemia and the effect of prandial GLP-1 receptor agonists on postprandial glycemia, based on their ability to inhibit gastric emptying (Albèr et al, 2017). This association between basal insulin and prandial GLP-1 receptor agonists is preferred today to the classical intensification of basal insulin therapy by adding prandial insulins, as it offers advantages of both a lower risk for hypoglycemia and a reduced weight gain (Albèr et al, 2017).

The effect of GLP-1 receptor agonists in diabetic patients with autonomic neuropathy and delayed gastric emptying, even though less studied, is usually feared to be deleterious in clinical practice by inducing or aggravating digestive intolerance, and therefore, their administration is intuitively avoided by most physicians in the case of patients with diabetic gastroparesis. Even if no detrimental effects would occur, choosing short-acting GLP-1 receptor agonist therapies in patients with diabetic gastroparesis seems an illogical and useless option, since their benefits are based on delay of gastric emptying. Patients with type 2 diabetes and slow gastric emptying at baseline may benefit more from treatment with long-acting GLP-1 receptor agonists, while in those with preserved gastric emptying, short-acting GLP-1 receptor agonists may be preferable.

Conclusion

Gastric emptying and glycemic control exert an ongoing influence upon each other. Normal rates of gastric emptying, of 1 to 4 kcal/min, correspond to the best balance between intestinal propulsion and absorption of macronutrients (especially carbohydrates), incretin hormone secretion, and postprandial glycemic levels. Contrary to this equilibrium state, higher

rates of gastric emptying may induce postprandial hyperglycemia but also represent a compensatory mechanism intervening when hypoglycemia occurs, while slower gastric emptying limits postprandial glycemic excursions or even acts as compensator under hyperglycemic conditions. In diabetic patients, fluctuations in gastric emptying are induced by complex pathophysiological pathways; these fluctuations may have a highly variable, unpredictable time pattern and limited correlations with the severity of clinical manifestations but strongly associate with variations in postprandial glycemic levels. Increased knowledge of this relation between gastric emptying and postprandial glycemic values allowed therapies targeting both factors to be updated, including diets with modified content or incretin-based medications. Future research and development will probably expand the range of both types of interventions, with diets based on reconsidered meal content or sequence and more GLP-1 receptor agonists approved for clinical use (*Mihai et al, 2018*).

4.3.3. Metabolic syndrome and antiviral therapy in virus C advanced liver disease

Background & Aim

There are many pathogenic mechanisms involved in the development of hepatic insulin-resistance in patients with hepatic C virus (HCV), both through a direct viral effect and through inflammatory cytokines: the inhibition of insulin receptor substrate, and the activation of suppressor of cytokine signalign with inhibition of phosphatidylinositol 3 kinase-transducer, which has a key role in insulin metabolism. As far as the lipid metabolism is concerned, HCV relies on the host lipid metabolism to enter the hepatocytes and replicate, consequently impacting on the lipid metabolism itself. The hepatic accumulation of lipids is caused both by virus (viral steatosis in genotype 3 virus) and other associated metabolic factors in other genotypes (metabolic steatosis). They have multiple mechanisms: increased availability of lipogenic substrate, increased de novo lipogenesis, decreased oxidation of fatty substrates, and decreased export of fatty substrates from the hepatocyte into the bloodstream. Insulin-resistance and hepatic steatosis are the negative risk factors in HCV infection, leading to accelerated progression of hepatic fibrosis, risk of hepatocarcinoma, and in mortality through cardiovascular complications (Kralj et al, 2016).

In the recent years, HCV therapy has been revolutionized through the development of directly acting antiviral therapy (DAA), an efficient, quick, and well tolerated therapy with the potential to cure HCV infection. Whilst metabolic parameters were the negative prognostic risk factors in peginterferon and ribavirin therapy, they do not seem to affect the sustained viral response (SVR) in DAA. Genotype 3 virus (associated with hepatitis virus-induced steatosis) is an exception, as it is now the most difficult type to treat. There is no consensus in this regard, with some data suggesting that certain metabolic parameters (for examples, the low levels of LDL-cholesterol) prior to treatment have a negative impact on SVR even in DAA therapy (Lim, 2014).

I initiated a project evaluating the association of MS in patients with HCV liver cirrhosis (compensated, genotype 1b) and changes in MS parameters after SVR, following a 12-week therapy with paritaprevir, ritonavir, ombitasvir, dasabuvir, and ribavirin (PrOD+R) (Mihai et al, 2017).

Materials and methods

A multicenter retrospective study included 809 patients diagnosed with compensated HCV cirrhosis (Child class A), all 1b genotype treated for 12 weeks with DAA (PrOD+R) regimen (according to the protocol practiced in Romania), and achieved SVR. Clinical characteristics and laboratory data were collected from medical records. The diagnosis of HCV liver cirrhosis was based on clinical, biological, and imaging parameters, as well as non-invasive methods (FibroMax) of assessing liver fibrosis. FibroMax is a non-invasive method of determining the activity, alcoholic and non-alcoholic steatosis and liver fibrosis through an

algorithm which measures the following markers: α2-macroglobulin, haptoglobin, apolipoprotein A1, γ-glutamyltransferase, alanine and aspartate aminotransferase, total bilirubin, fasting glucose, cholesterol and triglycerides. Treatment took place for 12 weeks from 01 December, 2015 to 20 June, 2016, with an undetectable viral load at 12 weeks from completion of therapy (SVR). Patients with decompensated liver cirrhosis, hepatocarcinoma, co-infections with hepatitis B or HIV, chronic alcohol users prior the treatment were excluded from the study.

MS was defined according to the definition of the International Diabetes Federation (Alberti et al, 2009) as fulfilling 3 of the 5 criteria: abdominal circumference >90 cm in men and >80 cm in women, fasting plasma glucose >100mg/dl (or a diagnosis of T2DM), triglycerides >150mg/dl (or therapy with fibrates), HDL-cholesterol < 40mg/dl in men and < 50mg/dl in women (or hypolipemiant therapy), blood pressure>130/85mmHg (or treated arterial hypertension). All patients were assessed prior to starting DAA therapy and at the time of SVR (24 weeks after the commencement). The severe adverse events at SVR visit were also noted: liver decompensation (ascites, hepato-portal encephalopathy, upper digestive bleeding), hepatocarcinoma and death.

The results were collected in a central database and analyzed with SPSS 18.0. Statistical analysis used both descriptive and analytical methods with a significance level of 95% (CI 95%).

Results

The studied cohort included 438 females (54.1%) and 371 males (45.9%), with an age range of 34 to 79 years and the mean age of 59.21±8.72 years.

The descriptive MS parameters according to the gender are presented in Table 4.5.

Table 4.5. Descriptive indicators of metabolic syndrome parameters according to the gender

Parameter	Gender	N	Mean ± Std. Deviation	Std. Error	95% Confidence Interval for Mean		Min	Max
					Lower Bound	Upper Bound		
AC, cm	Male	371	90.02 ± 9.43	0.49	89.06	90.98	67	132
	Female	438	81.52 ± 10.62	0.51	80.52	82.52	58	163
TG, mg/dL	Male	371	131.51 ± 58.64	3.04	125.52	137.49	38	610
	Female	438	125.89 ± 45.35	2.17	121.63	130.15	40	410
HDL, mg/dL	Male	371	54.65 ± 5.67	0.29	54.07	55.23	31	68
	Female	438	54.88 ± 5.51	0.26	54.37	55.40	33	68
SBP, mmHg	Male	371	125.08 ± 12.30	0.64	123.82	126.33	91	159
	Female	438	124.60 ± 12.43	0.59	123.43	125.77	90	170
DBP, mmHg	Male	371	75.22 ± 9.49	0.49	74.25	76.19	60	95
	Female	438	76.24 ± 9.11	0.44	75.38	77.09	60	110
FPG, mg/dL	Male	371	108.53 ± 30.44	1.58	105.43	111.64	61	291
	Female	438	108.67 ± 33.18	1.59	105.55	111.78	63	310

Abbreviations: AC, abdominal circumference; DBP, diastolic blood pressure; FPG, fasting plasma glucose; HDL, high-density lipoproteins; SBP, systolic blood pressure; TG, triglycerides.

Based on the MS criteria, the commonest parameters were abnormal glycemia (54.1%), followed by visceral obesity (38.6%), raisedtryglicerides (26.1%), high blood pressure (12.1%) and a low HDL-cholesterol (4.6%).

Out of the 809 patients, 105 (13%) demonstrated 3 out of the 5 criteria for MS. 59% of patients were females, with a female to male ratio of 1.4:1. Age ranged between 37 to 79 years;

the median age (61 years) was close to that of the study's cohort (59.75±8.28 years), with the peak of frequency being around 60 years. The mean age was lower in men (55.72) compared to women (62.55 years), p=0.001.

We identified the following results based on the diagnostic criteria for MS:

- Five diagnostic criteria were present in 3 patients (0.4%);

- Four diagnostic criteria were present in 3.5% of the patients as follows: 16 patients with abdominal obesity, hypertriglyceridemia, low HDL-cholesterol values, and high fasting plasma glucose levels, 7 patients with abdominal obesity, hypertriglyceridemia, high blood pressure, and high fasting plasma glucose levels, 5 patients with abdominal obesity, low HDL-cholesterol values, high blood pressure, and high fasting plasma glucose levels, and 1 patient with hypertriglyceridemia, low HDL-cholesterol values, high blood pressure, and high fasting plasma glucose levels;

- Three diagnostic criteria were present in 9.1% of the patients: 37 patients with abdominal obesity, hypertriglyceridemia and high fasting plasma glucose levels, 6 patients with abdominal obesity, low HDL-cholesterol values and high fasting plasma glucose levels; 13 patients with abdominal obesity, high blood pressure and high fasting plasma glucose levels, 3 patients with hypertriglyceridemia, high blood pressure, and high fasting plasma glucose levels, 4 patients with abdominal obesity, hypertriglyceridemia and high blood pressure, and 8 patients with abdominal obesity, hypertriglyceridemia and high blood pressure.

The most frequent association in MS was abdominal obesity, hypertriglyceridemia, and high fasting plasma glucose levels (37 patients; 35.2%).

The re-assessment of MS parameters after SVR showed favorable changes which were statistically significant: a significantly lower serum triglyceride level (182.32 vs. 153.50 mg/dL, p=0.001), lower systolic blood pressure (130.57 vs. 124.85 mmHg; p=0.001), lower diastolic blood pressure (80.26 vs. 78.42 mmHg; p=0.001), and lower glycemic levels (130.06 vs. 120.71 mg/dL; p=0.001), as well as a significant rise in HDL-cholesterol levels (48.61 vs. 50.50 mg/dL; p=0.003). Abdominal circumference was the only parameter, which did not change after SVR (Table 4.6).

Table 4.6. Changes in metabolic syndrome markers after the achievement of sustained viral response

Marker	Initial		SVR		p value
	Mean ± SD	Standard Error	Mean ± SD	Standard Error	
Triglycerides, mg/dL	182.32 ± 76.34	7.45	153.50 ± 63.38	6.19	0.001
HDL, mg/dL	48.61 ± 8.86	0.86	50.50 ± 7.80	0.76	0.003
SBP, mm Hg	130.57 ± 13.58	1.33	124.85 ± 12.97	1.27	0.001
DBP, mm Hg	80.26 ± 8.83	0.86	78.42 ± 8.77	0.86	0.001
Glycaemia, mg/dL	130.06 ± 42.44	4.14	120.71 ± 34.70	3.39	0.001

Abbreviations: DBP, diastolic blood pressure; SBP, systolic blood pressure; SVR, sustained viral response.

Following the changes sustained after SVR, 26.7% of the patients no longer fulfilled the minimum 3 criteria for MS (Table 4.7). The most significant improvements from a frequency perspective were noted in the reduction of blood pressure and triglyceride levels.

In the studied cohort, there were 23 severe adverse events (2.84%): 8 decompensations of liver disease, 12 hepatocarcinomas, and 3 deaths. No correlation was found between the presence of MS and the risk of severe adverse events (Table 4.8). Instead, it was noted that 37.5% of the patients who decompensated, 66.7% of the ones who developed hepatocarcinoma, and 100% of those that died had abnormal glycemic levels (Mihai et al, 2017).

Table 4.7. Proportion of changes in metabolic syndrome parameters after achieving a sustained viral response[a]

MeS Criteria	Initial	SVR	p Value
Abdominal obesity	100 (95.2)	100 (95.2)	1.000
↑ Blood pressure	42 (40.0)	23 (21.9)	0.001
↑ Glycaemia	91 (96.7)	84 (80.0)	0.034
↓ HDL-cholesterol	37 (35.2)	26 (24.8)	0.024
↑ Triglycerides	79 (75.2)	55 (52.4)	0.001
MeS	105 (100.0)	77 (73.3)	

Abbreviation: MeS, metabolic syndrome; high (↑) or low (↓) values according to MeS definition.[a]Values are expressed as No. (%).

Table 4.8. Metabolic syndrome and severe adverse events[a]

Adverse Events	No MeS (N = 704)	MeS (N = 105)	MeS Criteria					MeS	p value
			↑AC	↑ TG	↓HDL	↑BP	↑FPG		
LCD (N = 8) (1.0%)	7 (1.0)	1 (1.0)	1 (12.5)	1 (12.5)	1 (12.5)	1 (12.5)	3 (37.5)	1 (12.5)	0.968
HCC (N = 12) (1.5%)	9 (1.3)	3 (2.9)	7 (58.3)	4 (33.3)	1 (8.3)	1 (8.3)	8 (66.7)	3 (33.3)	0.212
Death (N = 3) (0.4%)	3 (0.4)	0 (0.0)	0 (0.0)	1 (33.3)	0 (0.0)	1 (33.3)	3 (100)	0 (0.0)	0.503

Abbreviations: AC, abdominal circumference; BP, blood pressure; FPG, fasting plasma glucose; HCC, hepatocarcinoma; HDL, high-density lipoproteins; LCD, liver cirrhosis decompensation; MeS, metabolic syndrome; TG, triglycerides; high (↑) or low (↓) values according to MeS definition.[a]Values are expressed as No. (%).

Discussion

Multiple definitions of MS exist, but all include obesity, insulin resistance/hyperglycemia, dyslipidemia, and arterial hypertension as the inclusion criteria. MS leads to a rise in mortality, especially as a result of cardiovascular causes. The hepatic manifestation of MS is steatosis, in the context of NAFLD. Similarly, HCV infection induces hepatic steatosis through direct mechanisms (valid especially in genotype 3) and is associated with multiple metabolic abnormalities: "hepatitis C-associated dysmetabolic syndrome". In our cohort, the prevalence of MS was 13%, with a female-to-male ratio of 1.4:1, lower than estimated in the general population of Romania 38.5% (Popa et al, 2016). In the published literature, the presence of MS in patients with HCV varied, 12.4% in Europe and 35% in the United States (Banks et al, 2016). These differences are explained by the different definitions of MS used, extremely different cohort studies on patients with HCV infection and variations in the prevalence of MS in the populations investigated.Many authors consider that, despite insulin resistance being the common aetiological link, the prevalence of MeS in HCV infection is similar to that in the general population (Adinolfi et al, 2016).

Visceral obesity is one of the features of hepatitis C-associated dysmetabolic syndrome, observed in 38.6% of the studied patients. It is demonstrated that insulin-resistance has a hepatic origin, as well as a peripheral one, with a hypothesis stating that HCV could infect fatty tissue. The association of obesity-hyperglycemia-hypertriglyceridemia was the most frequent occurrence in MS. Currently, it is known that HCV infection is associated with insulin-resistance, type 2 DM, and liver steatosis. In a recent meta-analysis, DM is considered as the second most common extra-hepatic manifestation of HCV (after depression), found in 15% of the patients. The high proportion of patients with raised glycemic values could be explained by the advanced stage of liver fibrosis in the cohort studies, knowing that in HCV infection, T2DM correlates with the degree of hepatic fibrosis (Younossi et al, 2016).

The interaction of HCV with lipid metabolism is a complex one, which is incompletely understood. Hepatitis C-associated dysmetabolic syndrome, in particular, features the associations of hepatic steatosis with those of hypocholesterolemia and reduced triglyceride levels. The virus enters the hepatocyte by binding to LDL receptors. The low levels of LDL are caused by geranylgeranyl-diphosphate in viral replication, which is a substrate for the synthesis of cholesterol. The high levels of LDL compete with LDL receptors and reduce viral replication, whilst the high levels of HDL support the entry of the virus into the hepatocyte. In the hepatocyte, HCV interferes with the lipid metabolism of the host to replicate and assemble leading to hepatic steatosis. The main mechanisms through which HCV leads to accumulation of triglycerides in the hepatocyte are the activation of lipogenesis, its impact on mitochondrial lipid oxidation, the lowering of microsomal triglyceride transfer protein activity, and the reduced activity of peroxisome proliferator activating receptor (PPAR). The release of the virus from hepatocytes occurs by means of the very low-density lipoprotein (VLDL). In the studied group of patients, hypertriglyceridemia was found in 26.08% of patients, whilst low HDL was encountered in only 5% of the patients. It was explained by the particular features of lipid metabolism associated with HCV (Zhu et al, 2014).

Whilst there are a number of controversies, some evidence may suggest an involvement of HCV in the development of cardiovascular complication, insulin resistance, hepatic steatosis, and type 2 DM as a common link (Lonardo et al, 2016). In our study, hypertension was observed in 12.1% of the patients.

Our data showed that all MS parameters, with the exception of abdominal circumference. had a significant improvement after SVR. The most significant improvements were noted in the reduction of blood pressure and triglyceride levels. Consequently, almost one-third of the patients no longer featured the diagnostic criteria for MS. Moreover, our data confirmed that the significant drop in glycemic levels (both in terms of mean value and the percentage of patients with hyperglycemia) after SVR is attained using DAA therapy. The reversibility of hypocholesterolemia, hypotriglyceridemia, and hepatic steatosis is demonstrated after the attainment of SVR in therapy regimens based on interferon. In our study, a rise in HDL-cholesterol was noted and a drop in triglyceride levels after attainment of SVR in patients treated with PrOD+R. The changes in lipid metabolism, which quickly appear after viral clearance, highlight once again the direct effect of HCV in lipid homeostasis (Meissner et al, 2015).

The effects of viral eradication on cardiovascular risk are controversial. In the current study, there was a significant reduction in mean blood pressure post SVR, as well as a lower proportion of patients with hypertension. Some suggest that the attainment of SVR leads to a rise in blood lipids and consequently an increase in cardiovascular risk. In the current study, the improvement of arterial blood pressure after SVR emphasized the direct link between HCV and cardiovascular complications.

Conclusion

The role of HCV in the complexity of metabolic abnormalities remains a highly interesting topic for future research. Patients with HCV liver cirrhosis (compensated genotype 1b) frequently display features of MS (high glycaemia, visceral obesity), but not MS itself. Glycemic abnormalities are associated with a higher risk of hepatic decompensation, hepatocarcinoma, and death. The attainment of SVR through PrOD+R led to the short-term improvements in MS parameters.

The main points regarding the association between digestive diseases and nutrition, diabetes and metabolic diseases are presented in Table 4.9.

Table 4.9. Main points in researches regarding the interaction between digestive diseases and nutrition, diabetes and metabolic diseases

SCIENTIFIC/CLINICAL RELEVANCE
• Nutrition is both an etiological factor and an integral part of the treatment of digestive diseases; although nutrion research is extremely difficult, the results obtained are extremely important in understanding the pathophysiology of diseases and in personalized medicine.
• Diabetes mellitus is a disease with a growing prevalenceworldwide. There are many interconnections between diabetes and digestive diseases, from gastroparesis to non-alcoholic fatty liver disease and digestive cancers.
• It is a bidirectional relationship between gastric emptying and plasma glucose control. Increased knowledge of this relation will allowe therapies targeting both factors to be updated, including diets with modified content or incretin-based medications.
• Through effective therapies for chronic viral hepatitis, the metabolic fatty liver disease remains the main liver disease, with increased morbidity and mortality, both hepatic and cardiovascular.
• On short term follow-up metabolic syndrome parameters improve after obtaining sustained viral response with direct-acting antivirals in patients with virus C chronic advanced liver disease.

SECTION II

FORTHCOMING PROJECTS AND DEVELOPMENT IN THE SCIENTIFIC FIELD

1. PERSPECTIVES IN THE PROFESSIONAL ACTIVITY

Medicine is more than a simple profession, it is a vocation in which continuous learning and self-improvement are the premises of a successful activity.The professional training of the mentor means competent professional training for students, residents, PhD students.

I was lucky enough to practice gastroenterology - in my opinion the most varied, complex and spectacular part of internal medicine.In modern medicine, things are very dynamic, so I want a permanent up-dating of the knowledge acquired through individual study, exchanges of experience, participation in scientific events.I also want to connect to the news present in related specialties (diabetes and metabolic diseases, cardiology, surgery, oncology, etc.) in order to better understand and manage complex cases.

Beyond its clinical valences, modern gastroenterology also involves the acquisition and performance of investigations such as ultrasonography and digestive endoscopy.Starting from the expertise and recognition already acquired in the field of ultrasonography, I will maintain and develop my knowledge in this field.I will continue to practice contrast – enhancement ultrasound, a non-invasive technique, extremely useful in clinical practice.In this area I will try to focus beyond the applications in the focal liver lesions diagnosis to other areas of interest, such as the evaluation of IBD activity or the follow-up of post-treatment hepatocellular carcinoma.Starting from the fact that the metabolic fatty liver has become the main liver disease, I will focus on the non-invasive exploration of this disease, through elastometry techniques.

IBD has an increasing incidence and prevalence in recent years.It affects the young population, active so that it involves huge costs for the medical and social system. Despite therapeutic advances, there are many difficult, complex cases that require interdisciplinary approach. There are the necessary premises, so that, together with the other colleagues with interest in the field, I want to set up a Center of Excellence in the field of IBD. This center will serve the entire population of the region of Moldova and will promote excellence in diagnosis and treatment, as well as education, training, research.

2. PERSPECTIVES IN THE ACADEMIC ACTIVITY

In the didactic activity, the acquisition of the habilitation title represents an achievement but also a great responsibility.I will continue my activity of teaching and guiding students and residents, being aware of the need to be able to initiate and lead research projects, to consolidatethe research team. I will be concerned with continuous self-improvement, with the acquisition of all the novelties in my field of activity. I will constantly update teaching materials and courses, in accordance with national and international guidelines and protocols in a permanent dynamic process.

I want to create a core of work, consisting of students and residents with an interest in research in the field of gastroenterology, with the outline of research topics and directions; they could be used later in the elaboration of bachelor's or doctoral theses and could be constituted as a way of selection of future university staff. Future PhD students must be capable, high-performance, honest people who can adapt to teamwork. The young PhD students must have

the opportunity to increase their professional autonomy, to learn gradual, to draft original papers, to publish in performant journals, to add value to the research team I am part of.

In the last 2 years, the SARS CoV 2 pandemic has forced the acquisition of new teaching techniques, focused on online education.In addition to the known disadvantages, online education also has many advantages: flexibility and self-paced learning, better time management, improved virtual communication and collaboration, a broader, global perspective and new technical skills. All these can be used as new tools in carrying out future teaching and research projects.

Maintaining and creating new links between research teams from different specialties, national and international collaboration in multicenter studies will add value and visibility to future projects. The access of funding sources to finance the projects is of vital importance for my scientific development plans. My entire professional, didactic and research activity as well as my membership in "Grigore T. Popa" University of Medicine and Pharmacy - a university that promotes excellence in education and research - is the framework for future achievments.

3. FUTURE RESEARCH PROJECTS

Scientifically, I propose to continue the current research directions as well as to approach new ones.

3. 1. Research in the field of inflammatory bowel disease

3.1.1. Disease clearance in IBD

In recent decades, with the development of increasingly effective therapies, therapeutic targets in IBD have changed.Beyond clinical remission, endoscopic healing has proven important in maintaining remission, decreasing the number of surgeries and hospitalizations, increasing the QoL.The association of clinical remission with endoscopic one defines the notion of "deep remission". The terms are not yet fully accepted; for example, endoscopic healing means for some the improvement / normalization of the endoscopic appearance, while the FDA considers both endoscopic and histological remission. Recently, Daneseintroduced in UC the concept of "disease clearance" which includes clinical remission, endoscopic and histological healing (Danese et al, 2020).Data from clinical trials report histological cures between 19.5% and 43.9% (Danese et al, 2020). Older studies have shown that, in the real world, achieving this composite goal (clinical, endoscopic and histological remission) rarely occurs, both in UC and CD (Schoepfer et al, 2012; Tursi et al, 2014). However, new biologic therapies entered into clinical practice have encouraging results with endoscopic and histological remission in almost half of UC patients (Ma et al, 2019). In CD an important element of healing is transmural healing, evaluated by cross-sectional imaging (ultrasound,contrast-enhanced computed tomography, and magnetic, resonance enterography). However, in the concept "treat to target" the new consensus STRIDE (Selecting Therapeutic Targets inInflammatory Bowel Disease), considers histological healing in UC and transmural healing in CD as measures of remission depth and not therapeutic targets (Turner et al, 2021).

In this direction of research there are multiple gaps: standardization and validation of the concept of disease clearance, possible even beyond histological and transmural healing to molecular mechanisms; the influence of these parameters on the evolution and prognosis of the disease; the influence of different types of therapies and therapeutic strategies on mucosal and transmural healing.

Benefiting from a database built in over 20 years, we can study these issues in our cohort of IBD patients.

3.1.2. New biomarkers in inflammatory bowel disease

Perceived as a condition of developed countries, now there is a global burden of IBD worldwide. This rise in lower and middle income countries seems to be driven by exposure to environmental risk factors. There is a huge need to find non-invasive markers for diagnosis, prognosis and therapeutic management in IBD patients.Recent studies have shown that the tools of precision medicine will be able to be implemented in clinical practice in the near future.Significant progress has been made by studying genomic data such as genome, transcriptome, proteome, metabolome, and microbiome.

Genetic, Environmental and Microbial (GEM) project demonstrated that altered gut microbiota is associated with fecal proteolytic activity before clinical onset of UC (Galipeau et al, 2021). The PREDICTS study demonstrated that a panel of serum antibodies and proteins can predict CD 5 years before onset (Turpin et al, 2020). The RISK cohort developed risk prediction models based on gene-expression data combined with microbial markers in colonic biopsies of children with CD (Kugathasan et al, 2017). PROFILE trial used the molecular CD8T-cell signature in blood to predict CD outcome (Parkes et al, 2018). Variants in the HLA-DQA1*05 allele are associated with risk of immunogenicity in CD patients treated with anti – TNF (Sazonovs et al, 2020). The microbial, metabolomic, and proteomic profile can predict relapse or surgical recurrence in IBD patients (Borren et al, 2020; Sokol et al, 2020). Oncostatin M, a stromal activator in IBD, has proved the role in determining nonresponse to anti-TNF therapy (Noor et al, 2021). MicroRNAs (miRNAs) are a class of small, non-coding, negative regulators of gene expression. These markers can be easy detected in whole blood, serum, faeces and intestinal tissues. They have proven useful in assessing both inflammation and response to treatment (Dragoni et al, 2021). Further research on the intestinal microbiota, using genetic sequencing of 165 RNA genes would be of real scientific interest.There are also attempts in the literature to find composite markers. For example, scores based on the activity of multiple cytokines may assess the response to infliximab or vedolizumab treatment (Bertani et al, 2020). The monitor test (the mucosal healing index) includes 13 serum proteins (carcinoembryonic antigen-related cell adhesion molecule, vascular cell adhesion molecule, CRP, serum amyloid A, angiopoietin-1, angiopoietin-2, matrix metalloproteinase (MMP)-1, MMP-2, MMP-3, MMP-9, extracellular matrix metalloproteinase inducer, transforming growth factor, and IL-7) predicts mucosal healing in patients with CD (Sandborn et al, 2018).

Many challenges persist in the application of precision medicine in IBD in order to integrate of all multi-omic data obtained from numerous researches. Artificial intelligence has an important role to achieve and interpret this “Big data” in IBD. There is already a software that facilitates the integration and analysis of multi-omic data at several levels (iCluster148) (de Suza et al, 2017).

Provided that the research funds are obtained, I intend to continue the research in identifying new biomarkers or combinations thereof that will contribute to a personalized medicine in IBD.

3.1.3. Obesity, diabetes mellitus, metabolic syndrome and inflammatory bowel disease

The association of obesity with IBD is another controversial topic. Classically, patients with IBD are considered underweight due to malnutrition. However, with the increasing prevalence of obesity in the general population and the emergence of innovative therapies that control and maintain remission in IBD, the prevalence of obesity can reach 40% of patients with IBD (Singh et al, 2017).Obesity promotes the appearance of IBD by disrupting the intestinal microbiota, insulin resistance and altering visceral adipose tissue - leads to increased inflammatory status in the intestinal mucosa. The impact of obesity on the prognosis and management of IBD patients is not fully known. Obesity increases thromboembolic risk, the risk of surgery in UC, the perianal damage and the need for hospitalization in CD (Seminerio et

al, 2015). The response to biologic therapy (Infliximab, Adalimumab, Vedolizumab) is diminished in obese patients who have low serum drug levels and more frequently lack or loss of response to treatment However, Hu's (Hu et al, 2017) meta-analysis demonstrates that obese patients with IBD have a better evolution compared to non-obese patients, with a lower probability of hospitalization, surgery, corticosteroid therapy.

Large population studies show an increased risk of type 2 DM in patients with IBD, independent of corticosteroid use (Jess et al, 2020). The connection between DM and IBD is bidirectional. Hyperglycemia and insulin resistance cause oxidative stress, chronic inflammation, altered cell junctions, increased intestinal barrier permeability and bacterial translocation (25). On the other hand, TNFα - a key cytokine in the inflammatory cascade of IBD - increases insulin resistance (Thaiss et al, 2018). There are few studies that prospectively follow the evolution of IBD in patients with DM. Published data suggest increased inflammatory activity, increased resource requirements, decreased QoL, increased risk of complications, infections, and higher mortality in diabetic patients with IBD (Kumar et al, 2020).

Both metabolic syndrome and IBD have an increasing incidence and prevalence, as a consequence of lifestyle changes, with the widespread adoption of the "Western" type. The association of IBD with metabolic syndrome is not accidental, as there are common etiopathogenic links between the two diseases: inflammation, abnormal immune response, disorders in the endocrine function of adipose tissue, intestinal dysbiosis (Michalak et al, 2016).

Starting from these contradictory data from the literature, I propose to study, both retrospectively and prospectively, the complex interrelationship between IBD and weight status, lipid profile, glycemic disorders.

3.1.4. Thromboembolic events in inflammatory bowel disease patients

A prevalence of 1-7% of thromboembolism in IBD is estimated, 2-3 times higher compared to the general population, similar in UC and CD. The most common are deep vein thrombosis in the lower limbs and pulmonary embolism, but other locations are possible: cerebral, portal vein, mesenteric vein, retinal veins (Alkim et al, 2017). Cases of arterial thrombosis (cardiac, cerebral, mesenteric) have also been reported, especially in young patients in the postoperative period, as well as cases of migratory thrombophlebitis. There are studies that have identified a 20% higher risk of cardiovascular events in IBD compared to the general population. The data are still controversial, with a recent meta-analysis indicating a modest risk of ischemic heart disease in patients with UC and CD compared to the general population, regardless of the presence of obesity (Feng et al, 2017).

The risk of thrombosis is 6 times higher in patients with hospitalized IBD compared to those not hospitalized. Procoagulatory pathophysiological mechanisms are complex and include interactions between platelets, coagulation factors, the fibrinolytic system, inflammatory cytokines, and vascular endothelium (Lagrange et al, 2020).Routine investigation of thrombophilic factors is not required in all patients with IBD; this will only be done on those with a family or personal history of thromboembolic events.Although prophylactic anticoagulant treatment in hospitalized patients with active BII is recommended by all scientific societies, there are multiple studies demonstrating its underutilization in current clinical practice.The optimal duration of anticoagulant treatment and the use of new oral anticoagulants require further study.Based on these data, I propose to initiate a prospective multicenter study to evaluate the risk factors and the optimal treatment for thromboembolism in IBD patients.

3. 2. Research in the field of ultrasonography

3.2.1. Ultrasound in inflammatory bowel disease

Ultrasound can assess the IBD activity and the response to treatment. Bowel ultrasonography has been demonstrated to be an accurate monitoring modality in the context of different therapeutic agents in CD and was also found to be helpful in monitoring UC disease course and treatment response (Noor et al, 2021). It is an affordable, cheap, repeatable method and can be used like a point - of –care test. Intestinal ultrasound significantly impacts disease management in the follow-up of IBD patients and has the potential to reduce the need for additional endoscopy and MRI.

In *transabdominal ultrasound* of the bowel the signs of inflammation are: bowel wall thickening, loss of normal bowel stratification with associated lack of compressibility by the transducer, hyperemia (disease activity correlates with increased vascularity), inflammation of mesenteric and perienteric fat and lymphoadenopaty (Bettenworth D et al, 2019). In METRIC trial the sensitivity for the presence if inflammation was similar (more than 90%) for ultrasound and MRI (Taylor et al, 2018). Transabdominal ultrasound is also useful in detection of CD complications (strictures, abscesses, fistulas) as well as UC monitoring.

Color Doppler imaging is an important technique that has been used to detect vascular signals from blood vessels in the bowel wall and the intensity of fat inflammation. The Limberg score is a semi-quantitative index, developed to assess CD activity. Doppler ultrasonography can be used in the assessment of abdominal aortic and superior mesenteric artery flow as an adjunctive method in the evaluation of CD activity (Mitselos et al, 2021).

Small intestine contrast ultrasonography(the ingestion of a polyethylene glycol solution, which results in bowel distention and better delineation of the wall layers)has been demonstrated not only to improve image quality and diagnostic performance, but also to facilitate the discrimination between fibrosis and inflammation (Kumar et al, 2015). Small intestine contrast ultrasonography detects postsurgical recurrence in CD patients with a sensitivity and specificity of 99% and 74%, respectively (Rispo et al, 2018).

Contrast-enhanced ultrasound (CEUS) detects hypervascularity and hyperperfusion in intestinal segments using maximum peak intensity or wash-in slope coefficient. CEUS improves the assessment of disease activity and allows the accurate prediction of postsurgical recurrence(Wilkens et al, 2018).

Ultrasound elastography is another tool able to successfully differentiate fibrotic from non-fibrotic stenosis in CD. There are two predominant types of elastography: shear wave elastography, which uses acoustic radiation force impulse, and real-time elastography, also known as strain elastography, which measures the mechanical properties of the tissue to determine the degree of stiffness (Lu et al, 2019). Despite some encouraging resultsthe elastographic parameters for the bowel are not yet standardized (Gabbiadini et al, 2021).

In our clinic all these ultrasound-derived imaging are available. The experience gained so far and the large number of patients with IBD are assets to study the role of ultrasonography in the management of IBD patients .

3.2.2. Point of care ultrasound in hepatology: "one stop shop"

Ultrasonography is a technique used for over 50 years, but it remains an extremely valuable tool in clinical practice.It is a non-invasive, accessible, reproducible and repeatable method of real value in chronic diffuse and circumscribed chronic liver diseases.There is virtually no diagnosis of hepatic steatosis, chronic hepatitis, liver cirrhosis in the absence of ultrasonography. Diagnosis of focal liver nodules is another challenge of daily practice. In recent years, CEUS has proven to be a handy tool, extremely useful in differentiating focal liver lesions.

Elastographic methods derived from ultrasonography have an essential contribution in the evaluation of liver diseases.According to the latest consensus of portal hypertension (Baveno VII, 2021), both liver and splenic stiffness, measured by elastometry, have predictive value for the occurrence and progression of portal hypertension.Transient Elastography (FibroScan) is the oldest and the most used ultrasound-based elastographic method.. It has a high accuracy in determining liver fibrosis, the higher the fibrosis is more advanced (Tsochatzis et al, 2011). The national and international guidelines of the scientific ultrasound societes have described the cut-off values of fibrosis in each etiology of liver diseases (Dietrich et al, 2017; Ferraioli et al, 2018). CAP (Controlled Attenuation Parameter)quantifies heopathic steatosis by exploring the posterior attenuation of the ultrasound beam. CAP implemented into the FibroScan device has a good value for the evaluation of MAFLD patients.

Point - or 2D share waveselastography implemented in an ultrasound system, available on multiple ultrasound devices, have also been shown to be effective tools for assessing liver fibrosis in clinical practice.There is currently a trend to develop new ultrasonographic devices for quantifying steatosis (attenuation imaging, ultrasound-guided attenuation parameter, attenuation, speed of sound estimation) and multiparametric integration (Multiparametric Ultrasound) (Sporea, Bende 2021). In this way, by a simple ultrasound examination we will be able to quantify steatosis, inflammation and fibrosis.

Each patient with liver disease will perform abdominal ultrasound, Doppler ultrasound, liver and splenic elastometry in the same session.In case of presence of a focal liver lesion, CEUS will be performed. This concept of "one stop shop" (Sporea, 2016) opens the way for numerous clinical research, diagnosis, prognosis and monitoring in the field of chronic liver disease.

3.3. Research in the field of the diabetes and metabolic syndrome associated with digestive diseases

3.3.1. Complex interrelation between NAFLD and type 2 DM

Non-alcoholic fatty liver disease (NAFLD) is an emergent problem due to pandemic increase of obesity and DM all over the world. In the last years the term “NAFLD” tends to be replaced with “metabolic associated fatty liver disease” (MAFLD) due to metabolic alterations in these patients (Eslam et al, 2020). DM is one of the leading causes of hepatic steatosis, causing an accelerated progression of fibrosis. In a recent meta-analysis, the prevalence of NAFLD in patients with diabetes was 55%, with severe fibrosis in 17% of cases (Younossi et al, 2019). A large body of clinical evidence now suggests that NAFLD may precede and/or promote the development of type 2 DM, hypertension and cardiovascular diseases, in parallel with the severity of liver fibrosis. Newer antidiabetic drugs (selective PPAR-γ modulators, glucagon-like peptide-1 receptor agonists and sodium-glucoseco-transporter-2 inhibitors) alone or in combination and acting alone or with potent statin therapy which is recommended in T2DM, might contribute substantially to NAFLD/NASH amelioration, possibly reducing not only liver-specific but also cardiovascular morbidity.

Based on these considerations, and from the good interdisciplinary collaboration we have had so far, I propose a research project in which patients with prediabetes or diabetes will be evaluated both in terms of NAFLD (serological and imaging markers) and cardiovascular involvement. This group of patients will be followed prospectively, following the NAFLD and cardiovascular involvement under different types of DM treatment.

3.3.2. Artificial intelligence and NAFLD

As we have shown in the chapter on IBD, AI has found its applicability in various fields of medicine.As we knowthe gold standard for the diagnosis and staging of liver fibrosis and NAFLD is liver biopsy. However liver biopsy is an invasive investigation, which involves risks

and possible complications, difficult to accept by the patient. As a result, a multitude of biological and imaging parameters (CAP, elastography, MRI), alone or in combination, fight for their place in estimating the severity of steatosis and fibrosis in patients with NAFLD.In a recent meta-analysis, AI-assisted models had good performance in the assessment of liver fibrosis and steatosis. For the diagnosis of liver fibrosis, the pooled sensitivity, specificity, PPV, NPV and diagnostic odds ratio were 0.78 (0.71–0.85), 0.89 (0.81–0.94), 0.72 (0.58–0.83), 0.92 (0.88–0.94) and 31.58 (11.84–84.25); 0.97 (0.76–1.00), 0.91 (0.78–0.97), 0.95 (0.87–0.98), 0.93 (0.80–0.98) and 191.52 (38.82–944.81), respectively, for the diagnosis of liver steatosis (Decharatanachart et al, 2021). Validations of AI performances are warranted before implementing these AI-assisted systems in clinical practice.

3.3.3. Intestinal microbiota, metabolic syndrome and metabolic fatty liver

Intestinal microbiota becomed the new frontier of medicine.There is a two-way relationship between the gut microbiota and obesity. The "western style microbiota" , with high quantities of *Firmicutes* and low quantities of *Bacteroides*, inhibits the activation of proteinkinase, and the expression of protein 4 angiopoietin-like – both being factors of adiposities induced by hunger (Graham et al, 2015). On the other hand obesity is characterized by the little degree of inflammation, with rising levels of inflammatory cytokines, and microbiota alteration. In type 2 DM it is also a modified microbiome with low *Faecalobacterium prausnitzii*and *Firmicutes*; an indirect correlation between insulin resistance and butyrate producing microbiome has been observed (Brunkwall L, Orho-Melander M, 2017).

Nonalcoholic fatty liver disease is characterized by an intestinal bacterial overgrowth, especially of *Enterobacteriaceae*, with the production of endotoxins and an increased intestinal permeability. The endotoxins penetrate the portal vein and decrease fasting-induced adipose factor secretion, promoting de novo fatty acid synthesis and triglyceride production. Intestinal microbiota (*Escherichia coli*) produces ethanol which changes the intestinal barrier permeability, increases the passage of endotoxins from the intestinal lumen to the portal blood, and stimulates hepatic inflammation (Duarte et al, 2019). Another toxic product of the microbiota metabolismis trimethylamine N-oxidewhich increases liver fat deposition, inflammatory and oxidative lesions and decreases glucose metabolism.

Recent studies have shown that the use of prebiotics and probiotics can be an alternative therapy in the treatment of NAFLD (Khan et al, 2021).Despite these promising results, future studies are necessary to understand the full role of microbiota in gut-liver axis, NAFLD development and progression. Additionally, further data is needed to demonstrate the role of probiotics/synbiotics as a novel pharmacologic approaches to NAFLD.

3.4. NEW RESEARCH DIRECTIONS

In addition to the old and new research directions, described in this habilitation thesis, I also consider other new research opportunities. Over the course of over 20 years I have addressed various other topics: chronic viral hepatitis, Terahertz spectroscopy, portal encephalopathy, pancreatitis, viral chronic hepatitis, foreign bodies in the upper digestive tract). The experience gained so far, the creation of a research team, the interdisciplinary collaboration, as well as the scientific opportunities offered by the membership of "Grigore T. Popa" University of Medicine and Pharmacy are all arguments for a permanent academic and scientific development.

SECTION III

REFERENCES

- Aberra, F.N.; Lewis, J.D.; Hass, D. et al. Corticosteroids and immunomodulators, postoperative infectious complication risk in inflammatory bowel disease. *Gastroenterology* 2003; 125, 320–327.
- Adinolfi LE, Rinaldi L, Guerrera B, et al. NAFLD and NASH in HCV Infection: Prevalence and Significance in Hepatic and Extrahepatic Manifestations. *Int J Mol Sci* 2016; 25:**17**(6).
- Agrawal A, Khan MH, Whorwell PJ. Irritable bowel syndrome in the elderly: An overlooked problem? *Dig Liver Dis* 2009;41:721-724.
- Alanazi NH, Alsharif MM, Rasool G et al. Nutrition in inflammatory bowel disease. *JPEN J Parenter Enteral Nutr* 2011; 35(5): 571-580.
- Albèr A, Brønden A, Knop FK. Short-acting glucagon-like peptide-1 receptor agonists as add-on to insulin therapy in type 1 diabetes: a review. *Diabetes Obes Metab* 2017; 19(7): 915-925.
- Alberti KG, Eckel RH, Grundy SM, et al. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. *Circulation* 2009; 120(16): 1640-1645.
- Albrecht T, Blomley M, Bolondi L, et al. Guidelines for the use of contrast agents in ultrasound. *Ultraschall Med* 2004;25:249-256.
- Alkim H, Koksal AR, Boga S, et al. Etiopathogenesis, Prevention, and Treatment of Thromboembolism in Inflammatory Bowel Disease. *Clin Appl Thromb Hemost* 2017;23(6):501-510.
- An YK, Prince D, Gardiner F et al. *Med J Aust.* 2019; 211(10):461-467.
- Andoh A, Imaeda H, Aomatsu T et al. Comparison of the fecal microbiota profiles between ulcerative colitis and Crohn's disease using terminal restriction fragment length polymorphism analysis. *J Gastroenterol* 2011; 46: 479–486.
- Antonelli E, Bassotti G, Tramontana M et al. Dermatological Manifestations in Inflammatory Bowel Diseases. *J Clin Med.* 2021; 19;10(2):364.
- Aomatsu T, Imaeda H, Fujimoto T et al. Terminal restriction fragment length polymorphism analysis of the gut microbiota profiles of pediatric patients with inflammatory bowel disease. *Digestion* 2012; 86: 129–135.
- Azramezani Kopi T, Shahrokh S, Mirzaei S et al. The role of serum calprotectin as a novel biomarker in inflammatory bowel diseases: a review study. *Gastroenterol Hepatol Bed Bench*. 2019;12(3):183-189.
- Banks DE, Bogler Y, Bhuket T et al. Significant disparities in risks of diabetes mellitus and metabolic syndrome among chronic hepatitis C virus patients in the U.S. *Diabetes Metab Syndr*. 2016.
- Barreiro-de Acosta M, Vallejo N, de la Iglesia D et al. Evaluation of the Risk of Relapse in Ulcerative Colitis According to the Degree of Mucosal Healing (Mayo 0 vs 1): A Longitudinal Cohort Study. *J Crohns Colitis.* 2016; 10(1):13-9.

- Baxt WG. Application of artificial neural networks to clinical medicine. *Lancet* 1995; 46(8983):1135-1138.
- Ben-Horin, S.; Mao, R.; Chen, M. Optimizing biologic treatment in IBD: Objective measures, but when, how and how often? *BMC Gastroenterol.* 2015; 15, 178.
- Bennebroek Evertsz' F, Bockting CL, Stokkers PC, et al. The effectiveness of cognitive behavioral therapy on the quality of life of patients with inflammatory bowel disease: multicenter design and study protocol (KL!C-study). *BMC Psychiatry* 2012; 12: 227.
- Bernstein CN, Blanchard JF, Rawsthorne P, Yu N. The prevalence of extraintestinal diseases in inflammatory bowel disease: a population-based study. *Am J Gastroenterol*, 2001; 96(4):1116–1122.
- Bertani L, Baglietto L, Antonioli L et al. Assessment of serum cytokines predicts clinical and endoscopic outcomes to vedolizumab in ulcerative colitis patients. *Br J Clin Pharmacol*. 2020;86(7):1296–305.
- Bettenworth D, Bokemeyer A, Baker M, et al. Stenosis Therapy and Anti-Fibrotic Research (STAR) Consortium. Assessment of Crohn's disease-associated small bowel strictures and fibrosis on cross-sectional imaging: a systematic review. *Gut* 2019;68(6):1115-1126.
- Biasci, D.; Jc, L.; Nm, N. A blood-based prognostic biomarker in IBD. *Gut* 2019; 68, 1386–1395.
- Bohm, N. Diagnosissis and management of iron deficiency anemia in inflammatory bowel disease. *Am. J. Manag. Care* 2021; 27 (Suppl. S11), S211–S218.
- Borren NZ, Plichta D, Joshi AD et al. Multi-omics profiling in patients with quiescent inflammatory bowel disease identifies biomarkers predicting relapse. *Inflamm. Bowel Dis.* 2020;26:1524–1532.
- Breban M, Beaufrère M, Glatigny S. The microbiome in spondyloarthritis. *Best Pract Res Clin Rheumatol* 2019; 33(6):101495.
- Breban M, Tap J, Leboime A et al. Faecal microbiota study reveals specific dysbiosis in spondyloarthritis. *Ann Rheum Dis* 2017; 76: 1614–1622.
- Bromke MA, Neubauer K, Kempiński R, Krzystek-Korpacka M. Faecal Calprotectin in Assessment of Mucosal Healing in Adults with Inflammatory Bowel Disease: A Meta-Analysis. *J Clin Med.* 2021; 10(10):2203.
- Brunkwall L, Orho-Melander M. The gut microbiome as a target for prevention and treatment of hyperglycaemia in type 2 diabetes: from current human evidence to future possibilities. *Diabetologia* 2017; 60(6): 943-51.
- Bryant RV, Winer S, Travis SP, Riddell RH. Systematic review: histological remission in inflammatory bowel disease. Is 'complete' remission the new treatment paradigm? An IOIBD initiative. *J Crohns Colitis.* 2014;8(12):1582-1597.
- Burisch J, Munkholm P. The epidemiology of inflammatory bowel disease. *Scand JGastroenterol*. 2015;50:942–51.
- Cabassa P, Bipat S, Longaretti L et al. Liver metastases: Sulphur hexafluoride-enhanced ultrasonography for lesion detection: a systematic review. *Ultrasound Med Biol* 2010; 36: 1561–1567.
- Camilleri M, Parkman HP, Shafi MA et al. Clinical guideline: management of gastroparesis. *Am J Gastroenterol* 2013; 108(1): 18-37.
- Cardoneanu A, Cijevschi Prelipcean C, Danciu M, Mihai C, Dranga M, Gavrilescu O, Rezus E. Looking beyond gut inflammation in inflammatory bowel disease. *Romanian Journal of Morphology & Embriology* 2018; 59(4): 1097-1105.
- Cardoneanu A, Mihai C, Rezus E, Burlui A, Popa I, Prelipcean CC. Gut Microbiota Changes in Inflammatory Bowel Diseases and Ankylosing Spondylitis. *J GastrointestLiver Dis* 2021; 30 (1): 46-54.

- Carleton NM, Thakkar S. How to Approach and Interpret Studies on AI in Gastroenterology. *Gastroenterology*. 2020;159(2):428-432.
- Casellas, F. Influence of inflammatory bowel disease on different dimensions of quality of life. *European Journal of Gastroenterology and Hepatology* 2001; 13:567- 572.
- Casellas F, Arenas JI, Baudet JS et al. Impairment of health-related quality of life in patients with inflammatory bowel disease: a Spanish multicenter study. *Inflamm Bowel Dis*. 2005;11(5):488-96.
- Chateau T, Feakins R, Marchal-Bressenot A et al. Histological remission in ulcerative colitis: under the microscope is the cure. *Am J Gastroenterol*. 2020;115(2):179- 189.
- Chen P, Zhou G, Lin J et al. Serum Biomarkers for Inflammatory Bowel Disease. *Front Med (Lausanne)*2020; 7:123.
- Chirila, I; Petrariu, FD; Ciortescu, I et al. Diet and Irritable Bowel Syndrome, *J of Gastrointes and Liver Dis*, 2012; 21(4): 357-362.
- Christensen B, Erlich J, Gibson PR et al. Histologic healing is more strongly associated with clinical outcomes in ileal Crohn's disease than endoscopic healing. *Clin Gastroenterol Hepatol*. 2020;18(11):2518.e1-2525.e1.
- Claudon M, Cosgrove D, Albrecht T et al. Guidelines and good clinical practice recommendations for contrast enhanced ultrasound (CEUS) - update 2008. *Ultraschall Med* 2008;29:28-44.
- Claudon M, Dietrich CF, Choi BI et al. Guidelines and good clinical practice recommendations for Contrast Enhanced Ultrasound (CEUS) in the liver - update 2012: A WFUMB-EFSUMB initiative in cooperation with representatives of AFSUMB, AIUM, ASUM, FLAUS and ICUS. *Ultrasound Med Biol* 2013;39:187-210.
- Compston JE, Horton LW, Laker MF et al. Bone disease after jejunoileal bypass for obesity. *Lancet* 1978; 2: 1-4.
- Costello ME, Ciccia F, Willner D et al. Intestinal dysbiosis in ankylosing spondylitis. *Arthritis Rheumatol* 2015; 67:686–691.
- Dai Y, Chen MH, Fan ZH et al. Diagnosis of small hepatic nodules detected by surveillance ultrasound in patients with cirrhosis: Comparison between contrast-enhanced ultrasound and contrast-enhanced helical computed tomography. *Hepatol Res* 2008; 38:281-290.
- Danese S, Roda G, Peyrin-Biroulet L. Evolving therapeutic goals in ulcerative colitis: towards disease clearance. *Nat Rev Gastroenterol Hepatol* 2020;17(1):1-2.
- Decharatanachart P, Chaiteerakij R, Tiyarattanachai T, Treeprasertsuk S. Application of artificial intelligence in chronic liver diseases: a systematic review and meta-analysis. *BMC Gastroenterol* 2021 6;21(1):10.
- D'Haens G, Ferrante M, Vermeire S et al. Fecal calprotectin is a surrogate marker for endoscopic lesions in inflammatory bowel disease. *Inflamm Bowel Dis.* 2012;18(12):2218-24.
- Dietrich CF, Bamber J, Berzigotti A, et al. EFSUMB Guidelines and Recommendations on the Clinical Use of Liver Ultrasound Elastography, update 2017. *Ultraschall Med* 2017; 38: 349-72.
- Dietrich CF, Nolsøe CP, Barr RG, et al. Guidelines and Good Clinical Practice Recommendations for Contrast-Enhanced Ultrasound (CEUS) in the Liver-Update 2020 WFUMB in Cooperation with EFSUMB, AFSUMB, AIUM, and FLAUS. *Ultrasound Med Biol.* 2020;46(10):2579-2604.
- D'Incà R, Dal Pont E, Di Leo V et al. Can calprotectin predict relapse risk in inflammatory bowel disease? *Am J Gastroenterol.* 2008; 103(8):2007-14.
- D'Onofrio M, Crosara S, De Robertis R et al. Contrast-Enhanced Ultrasound of Focal Liver Lesions. *AJR Am J Roentgenol.* 2015;205(1):W56-66.

- Dragoni G, Innocenti T, Galli A. Biomarkers of Inflammation in Inflammatory Bowel Disease: How Long before Abandoning Single-Marker Approaches? *Dig Dis.* 2021; 39(3):190-203.
- Dranga M, Boiculese LV, Popa IV, et al. Anemia in Crohn's Disease — The Unseen Face of Inflammatory Bowel Disease. *Medicina* 2021; 57: 1046.
- Dranga M, Mihai C, Mihai BM, et al. Fecal calprotectine – can we differentiate IBD from IBS? Proceedings. Meeting of the Romanian Society of Neurogastroenterology with Rome IV Regional Central – East European Meeting, Neurogastro 2017: 82-86.
- Dranga M, Mihai C, Drug V et al. Can We Recommend a Rapid Test in Assessing Activity in Ulcerative Colitis? *Turk J Gastroenterol*. 2016; 27(2):149-55.
- Dranga M, Mihai C, Gavrilescu O, et al. The Role of Combining Biochemical Markers in Assessing the Endoscopic Activity in Ulcerative Colitis. *Revista de Chimie* 2018; 69 (5):1268-1271.
- Drossman DA. The functional gastrointestinal disorders and the Rome III process. *Gastroenterology* 2006;130:1377-1390.
- Drossman DA, Patrick DL, Mitchell CM et al. Health-related quality of life in inflammatory bowel disease. Functional status and patient worries and concerns. *Dig Dis Sci.* 1989; 34(9):1379-86.
- Drug VL, Oprea L, Bunescu D, et al. The epidemiology of irritable bowel syndrome in an urban general population. *Rom J Gastroenterol* 2000;9:83-86.
- Duarte SMB, Stefano JT, Oliveira CP. Microbiota and nonalcoholic fatty liver disease/nonalcoholic steatohepatitis (NAFLD/NASH). *Ann Hepatol* 2019;18(3):416-421.
- Duboc H, Rajca S, Rainteau D et al. Connecting dysbiosis, bile-acid dysmetabolism and gut inflammation in inflammatory bowel diseases. *Gut* 2017; 62(4):531-9.
- Dulai PS, Peyrin-Biroulet L, Danese S, et al. Approaches to integrating biomarkers into clinical trials and care pathways as targets for the treatment of inflammatory bowel diseases. *Gastroenterology* 2019;157(4):1032.e1-1043.e1.
- Dumitrescu G, Mihai C, Dranga M, Cijevschi Prelipcean C. Bone mineral density in patients with inflammatory bowel disease from north-eastern Romania. *The Medical-Surgical Journal* 2013; 117 (1): 23-28.
- Dumitrescu G, Mihai C, Dranga M, Prelipcean CC. Serum 25-hydroxyvitamin D concentration and inflammatory bowel disease characteristics in Romania.*World J Gastroenterol* 2014;20(9): 2392-6.
- Eccleston C, Fisher E, Law E, et al. Psychological interventions for parents of children and adolescents with chronic illness. *Cochrane Database Syst Rev* 2012; 4: CD009660.
- Eiden K.A. Nutritional Considerations in Inflammatory Bowel Disease. Nutrition issues in gastroenterology. *Pract Gastroenterol* 2003; 5: 33-54.
- Eslam M, Sanyal AJ, George J. MAFLD: A Consensus-Driven Proposed Nomenclature for Metabolic Associated Fatty Liver Disease. *Gastroenterology* 2020; 158(7): 1999-2014.e1.
- Eswaran S, Tack J, Chey WD. Food: the forgotten factor in the irritable bowel syndrome. *Gastroenterol Clin North Am* 2011;40:141- 162.
- Faller M, Gasser B, Massard G et al. Pulmonary migratory infiltrates and pachypleuritis in a patient with Crohn's disease. *Respiration* 2000; 67: 459–463.
- Feng W, Chen G, Cai D, et al. Infammatory bowel disease and risk of ischemic heart disease: an updated meta-analysis of cohort studies. *J Am Heart Assoc* 2017;6:5892.
- Fengming Y, Jianbing W. Biomarkers of inflammatory bowel disease. *Dis Markers.* 2014.

- Ferguson LR, De Caterina R, Görman U, et al. Guide and Position of the International Society of Nutrigenetics/Nutrigenomics on Personalised Nutrition: Part 1 - Fields of Precision Nutrition. *J Nutrigenet Nutrigenomics.* 2016;9(1):12-27.
- Ferraioli G, Wong VW, Castera L, et al. Liver Ultrasound Elastography: An Update to the World Federation for Ultrasound in Medicine and Biology Guidelines and Recommendations. *Ultrasound Med Biol* 2018; 44(12): 2419-40.
- Filmann, N.; Math, D.; Rey, J. et al. Prevalence of Anemia in Inflammatory Bowel Diseases in European Countries: A Systematic Review and Individual Patient Data Meta-analysis. *Inflamm. Bowel Dis.* 2014; 20, 936–945.
- Forbes A, Escher J, Hébuterne X, et al. ESPEN guideline: Clinical nutrition in inflammatory bowel disease. *Clin Nutr.* 2017;36 (2):321-347.
- Forner A, Vilana R, Ayuso C et al. Diagnosis of hepatic nodules 20 mm or smaller in cirrhosis: Prospective validation of the noninvasive diagnostic criteria for hepatocellular carcinoma. *Hepatology* 2008; 47: 97–104.
- Fragoulis GE, Liava C, Daoussis D et al. Inflammatory bowel diseases and spondyloarthropathies: From pathogenesis to treatment. *World J Gastroenterol* 2019; 25(18): 2162-2176.
- Fragoulis GE, Siebert S, McInnes IB. Therapeutic Targeting of IL-17 and IL-23 Cytokines in Immune Mediated Diseases. *Annu Rev Med* 2016; 67: 337-353.
- Frank DN, St Amand AL, Feldman RA et al. Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. *Proc Natl Acad Sci USA* 2007; 104(34):13780–13785.
- Freeman K, Willis BH, Fraser H et al. Faecal calprotectin to detect inflammatory bowel disease: a systematic review and exploratory meta-analysis of test accuracy. *BMJ Open.* 2019;9(3):e027428.
- Friedrich-Rust M, Klopffleisch T, Nierhoff J et al. Contrast-Enhanced Ultrasound for the differentiation of benign and malignant focal liver lesions: a meta-analysis. *Liver Int* 2013;33:739-755.
- Fuhrman MP. Nutrition support at the end of life: a critical decision. *Today's Dietitian* 2008; 10(9): 68-73.
- Fujimoto T, Imaeda H, Takahashi K et al. Decreased abundance of Faecalibacterium prausnitzii in the gut microbiota of Crohn's disease. *J Gastroenterol Hepatol.* 2013; 28(4):613-9.
- Fukukura Y, Nakashima O, Kusaba A et al. Angioarchitecture and blood circulation in focal nodular hyperplasia of the liver. *J Hepatol* 1998;29:470-475.
- Fuller-Thomson E, Sulman J. Depression and inflammatory bowel disease: findings from two nationally representative Canadian surveys. *Inflamm Bowel Dis.* 2006;12(8):697-707.
- Gabbiadini R, Zacharopoulou E, Furfaro F, et al. Application of Ultrasound Elastography for Assessing Intestinal Fibrosis in Inflammatory Bowel Disease: Fiction or Reality? *Curr Drug Targets* 2021;22(3):347-355.
- Galipeau HJ, Caminero A, Turpin W, et al.; CCC Genetics, Environmental, Microbial Project Research Consortium. Novel fecal biomarkers that precede clinical diagnosis of ulcerative colitis. *Gastroenterology.* 2021; 160:1532–1545.
- Gasche C. Complications of inflammatory bowel disease. *Hepatogastroenterol* 2000; 47: 49-56.
- Gavrilescu O, Cijevschi Prelipcean C, Dranga M, et al. The specialized educational and psychological counseling in inflammatory bowel disease patients – a target or a challenge? *Turk J Gastroenterol* 2020; 31(11): 760-6.

- Gavrilescu O, Dranga M, Cardoneanu A, et al. Irritable bowel disease versus Crohn's disease – impact on quality of life. ISI Proceedings. Meeting of the Romanian Society of Neurogastroenterology with Rome IV Regional Central – East European Meeting, Neurogastro 2017: 87-91.
- Gavrilescu O, Dranga M, Soponaru C, et al. Quality of life and fecal calprotectin in inflammatory bowel diseases. The Medical-Surgical Journal 2018; 122(2): 283-288.
- Gavrilescu O, Mihai C, Anton Paduraru DT, et al. Impact of Inflammatory Bowel Disease on Quality of Life. *Revista de cercetare si interventie sociala* 2015; 50: 80-95.
- Gevers D, Kugathasan S, Denson LA et al. The treatment-naive microbiome in new-onset Crohn's disease. *Cell Host Microbe* 2014; 15: 382–392.
- Giezenaar C, Trahair LG, Luscombe-Marsh ND et al. Effects of randomized whey-protein loads on energy intake, appetite, gastric emptying, and plasma gut-hormone concentrations in older men and women. *Am J Clin Nutr* 2017; 106(3): 865-877.
- Girgis HZ, Mitchell BR, Dassopoulos T, et al. An intelligent system to detect Crohn's disease inflammation in Wireless Capsule Endoscopy videos. 2010; 1373–1376.
- Gisbert, J.P.; Bermejo, F.; Pajares et al. Oral and intravenous iron treatment in inflammatory bowel disease: Hematological response and quality of life improvement. *Inflamm. Bowel Dis.* 2009; 15, 1485–1491.
- Goodhand JR, Wahed M, Rampton DS. Management of stress in inflammatory bowel disease: a therapeutic option? *Expert Rev Gastroenterol Hepatol* 2009; 3: 661-79.
- Graham C, Mullen A, Whelan K. Obesity and the gastrointestinal microbiota: a review of associations and mechanisms. *Nutr Rev* 2015; 73(6): 376-85.
- Guagnozzi, D.; Lucendo, A.J. Anemia in inflammatory bowel disease: A neglected issue with relevant effects. *World J. Gastroenterol.* 2014; 20, 3542–3551.
- Gubatan J, Levitte S, Patel A et al. Artificial intelligence applications in inflammatory bowel disease: Emerging technologies and future directions. *World J Gastroenterol*. 2021;27(17):1920-1935.
- Gupta K, Noble A, Kachelries KE et al. A novel enteral nutrition protocol for the treatment of pediatric Crohn's disease. *Inflamm Bowel Dis* 2013; 19 (7): 1374-1378.
- Guidi, L.; Marzo, M.; Andrisani, G. et al. Faecal calprotectin assay after induction with anti-Tumour Necrosis Factor α agents in inflammatory bowel disease: Prediction of clinical response and mucosal healing at one year. *Dig. Liver Dis.* 2014; 46, 974–979.
- Guillo L, D'Amico F, Serrero M et al. Assessment of extraintestinal manifestations in inflammatory bowel diseases: A systematic review and a proposed guide for clinical trials. *United European Gastroenterol J.* 2020; 8(9):1013-1030.
- Hamad S, Willyard CE, Mukherjee S. Focal Nodular Hyperplasia. In: StatPearls [Internet]. Treasure Island (FL): *StatPearls Publishing*; 2021.
- Hardalaç, F.; Ba¸sarano ˘glu, M.; Yüksel, M. et al.The rate of mucosal healing by azathioprine therapy and prediction by artificial systems. *Turk. J. Gastroenterol*. 2015; 26, 315–321.
- Hassan AR, Haque MA. Computer-aided gastrointestinal hemorrhage detection in wireless capsule endoscopy videos. *Comput Methods Programs Biomed* 2015;122:341–353.
- Hau HM, Atanasov G, Tautenhahn HM et al. The value of liver resection for focal nodular hyperplasia: resection yes or no? *Eur J Med Res.* 2015;20:86.
- Hedin CRH, Vavricka SR, Stagg AJ et al. The pathogenesis of extraintestinal manifestations: implications for IBD research, diagnosis, and therapy. *J Crohns Colitis* 2019; 13:541–554.

- Hibi T, Sakuraba A, Watanabe M et al. C-reactive protein is an indicator of serum infliximab level in predicting loss of response in patients with Crohn's disease. *J Gastroenterol*. 2014;49(2):254-62.
- Holdam, A.S.; Bager, P.; Dahlerup, J.F. Biological therapy increases the health-related quality of life in patients with inflammatory bowel disease in a clinical setting. *Scand. J. Gastroenterol.* 2016; 51,706–711.
- Homko CJ, Duffy F, Friedenberg FK et al. Effect of dietary fat and food consistency on gastroparesis symptoms in patients with gastroparesis. *Neurogastroenterol Motil* 2015; 27(4): 501-508.
- Hong W, Chen X, Jin S, et al. Use of an artificial neural network to predict persistent organ failure in patients with acute pancreatitis. *Clinics (Sao Paulo)* 2013;68:27–31.
- Huang Q, Pan F, Li W et al. Differential Diagnosis of Atypical Hepatocellular Carcinoma in Contrast-Enhanced Ultrasound Using Spatio-Temporal Diagnostic Semantics. IEEE J *Biomed Health Inform* 2020;24:2860-2869.
- Hujoel IA, Murphree DH, Van Dyke CT, et al. Machine Learning in Detection of Undiagnosed Celiac Disease. *Clin Gastroenterol Hepatol* 2018;16:1354-1355.e1.
- Hu Q, Ren J, Li G, et al. The impact of obesity on the clinical course of inflammatory bowel disease: a meta-analysis. *Med Sci Monit* 2017;23:2599-2606.
- International Diabetes Federation. *IDF Diabetes Atlas*, Ninth Edition 2019.
- Irvine EJ. Quality of Life in inflammatory bowel disease: biases and other factors affecting scores. *Scand J Gastroenterol Suppl.* 1995; 208:136-40.
- Irving PM, Pasi KJ, Rampton DS. Thrombosis and inflammatory bowel disease. *Clin Gastroenterol Hepatol* 2005; 3(7):617-28.
- Isaacs KL. How prevalent are extraintestinal manifestations at the initial diagnosis of IBD? *Inflamm Bowel Dis,* 2008; 14(Suppl 2):S198–S199.
- Jess T, Jensen BW, Andersson M et al. Inflammatory Bowel Diseases Increase Risk of Type 2 Diabetes in a Nationwide Cohort Study. *Clin Gastroenterol Hepatol* 2020;18(4):881-888.
- Jo PC, Jang HJ, Burns PN et al. Integration of Contrast-enhanced US into a Multimodality Approach to Imaging of Nodules in a Cirrhotic Liver: How I Do It. *Radiology*. 2017;282(2):317-331.
- Johansson L, Thelle DS, Solvoll K et al. Healthy dietary habits in relation to social determinants and lifestyle factors. *Br J Nutr* 1999;81:211-220.
- Jones J, Loftus EV Jr, Panaccione R et al. Relationships between disease activity and serum and fecal biomarkers in patients with Crohn's disease. *Clin Gastroenterol Hepatol*. 2008;6(11):1218-24.
- Jovanovic P, Salkic NN, Zerem E. Artificial neural network predicts the need for therapeutic ERCP in patients with suspected choledocholithiasis. *Gastrointest Endosc* 2014;80:260–268.
- Kalafateli M, Triantos C, Theocharis G et al. Health-related quality of life in patients with inflammatory bowel disease: a single-center experience. *Ann Gastroenterol.* 2013; 26(3):243-248.
- Kar P, Jones KL, Plummer MP, et al. Antecedent hypoglycemia does not attenuate the acceleration of gastric emptying by hypoglycemia. *J Clin Endocrinol Metab* 2017; 102(11): 3953-3960.
- Khaki-Khatibi F, Qujeq D, Kashifard M, et al. Calprotectin in inflammatory bowel disease. *Clin Chim Acta*. 2020;510:556-565.
- Khan A, Ding Z, Ishaq M, et al. Understanding the Effects of Gut Microbiota Dysbiosis on Nonalcoholic Fatty Liver Disease and the Possible Probiotics Role: Recent Updates. *Int J Biol Sci* 2021;17(3):818-833.

- Khan I, Ullah N, Zha L et al. Alteration of Gut Microbiota in Inflammatory Bowel Disease (IBD): Cause or Consequence? IBD Treatment Targeting the Gut Microbiome. *Pathogens* 2019; 8(3):126.
- Khoshkrood-Mansoori B, Pourhoseingholi MA, Safaee A, et al. Irritable bowel syndrome: a population based study. *J Gastrointestin Liver Dis* 2009; 18:413-418.
- Kim KB, Kim HW, Lee JS, Yoon SM. Inflammatory Bowel Disease and Vitamin D. *Korean J Gastroenterol.* 2020; 76(6):275-281.
- Klein S. A primer of nutritional support for gastroenterologists. *Gastroenterology* 2002; 122(6): 1677-1687.
- Knoll RL, Forslund K, Kultima JR et al. Gut microbiota differs between children with Inflammatory Bowel Disease and healthy siblings in taxonomic and functional composition: a metagenomic analysis. *Am J Physiol Gastrointest Liver Physiol* 2017; 312(4): G327-G339.
- Knowles, S.R.; Graff, L.A.; Wilding, H. et al. A. Quality of life in inflammatory bowel disease: A systematic review and meta-analyses—Part I. *Inflamm. Bowel Dis.* 2018; 24, 742–751.
- Knowles, S.R.; Keefer, L.; Wilding, H. et al. A. Quality of Life in Inflammatory Bowel Disease: A Systematic Review and Meta-analyses—Part II. *Inflamm. Bowel Dis*. 2018; 24, 966–976.
- Knowles SR, Monshat K, Castle DJ. The efficacy and methodological challenges of psychotherapy for adults with inflammatory bowel disease: a review. *Inflamm Bowel Dis.* 2013;19:2704–15.
- Konerman MA, Lu D, Zhang Y, et al. Assessing risk of fibrosis progression and liverrelated clinical outcomes among patients with both early stage and advanced chronic hepatitis C. *PLoS ONE* 2017;12:e0187344.
- Kothari, M.M.; Nguyen, D.L.; Parekh, N.K. Strategies for overcoming anti-tumor necrosis factor drug antibodies in inflammatory bowel disease: Case series and review of literature. *World J. Gastrointest. Pharmacol. Ther.* 2017; 8, 155–161.
- Körner U, Bondolfi A, Bühler E et al. Ethical and legal aspects of enteral nutrition. *Clin Nutr* 2006; 25(2): 196-202.
- Kralj D, Virovic Jukic L, Stojsavljevic S et al. Hepatitis C virus, insulin resistance, and steatosis. *J Clin Trans Hepatol* 2016; 4(1): 66-75.
- Kristensen SL, Ahlehoff O, Lindhardsen J et al. Disease activity in inflammatory bowel disease is associated with increased risk of myocardial infarction, stroke and cardiovascular death--a Danish nationwide cohort study. *PLoS One* 2013; 8(2):e56944.
- Kugathasan S, Denson LA, Walters TD, et al. Prediction of complicated disease course for children newly diagnosed with Crohn's disease: a multicentre inception cohort study. *Lancet.* 2017;389:1710–1718.
- Kumar A, Teslova T, Taub E et al.Comorbid Diabetes in Inflammatory Bowel Disease Predicts Adverse Disease-Related Outcomes and Infectious Complications. *Dig Dis Sci* 2020.
- Kumar S, Hakim A, Alexakis C, et al. Small intestinal contrast ultrasonography for the detection of small bowel complications in Crohn's disease:correlation with intraoperative findings and magnetic resonance enterography. *J Gastroenterol Hepatol* 2015;30:86–91.
- Lagrange J, Lacolley P, Wahl D et al. Shedding Light on Hemostasis in Patients With Inflammatory Bowel Diseases. *Clin Gastroenterol Hepatol* 2020;20:S1542-3565.
- Lamb, C.A.; Kennedy, N.A.; Raine, T. et al. British Society of Gastroenterology consensus guidelines on the management of inflammatory bowel disease in adults. *Gut* 2019; 68 (Suppl. S3), s1–s106.

- Lakatos PL, Szalay F, Tulassay Z et al. Hungarian IBD Study Group. Clinical presentation of Crohn's disease. Association between familial disease, smoking, disease phenotype, extraintestinal manifestations and need for surgery. *Hepatogastroenterology,* 2005; 52(63):817–822.
- Latella G. Impact of environmental and dietary factors on the course of inflammatory bowel disease. *World J Gastroenterol* 2012; 18(29): 3814-3822.
- Lăcătuşu C, Cijevschi-Prelipcean C, Mihai C, Mihai B. Ethics of artificial nutrition.*Revista Română de Bioetică* 2014; 12(1): 44-55.
- Le Berre C, Sandborn WJ, Aridhi S et al. Application of Artificial Intelligence to Gastroenterology and Hepatology. *Gastroenterology.* 2020;158(1):76-94.e2.
- LeCun Y, Bengio Y, Hinton G. Deep learning. *Nature.* 2015;521(7553):436-44.
- Lee J, An JY, Choi MG, et al. Deep Learning–Based Survival Analysis Identified Associations Between Molecular Subtype and Optimal Adjuvant Treatment of Patients With Gastric Cancer. *JCO Clinical Cancer Informatics* 2018:1–14.
- Leslie WD, Miller N, Rogala L, Bernstein CN. Vitamin D status and bone density in recently diagnosed inflammatory bowel disease: the Manitoba IBD Cohort Study. *Am J Gastroenterol* 2008; 103: 1451-1459.
- Levine A, Wine E. Effects of enteral nutrition on Crohn's disease: clues to the impact of diet on disease pathogenesis. *Inflamm Bowel Dis* 2013; 19(6): 1322-1329.
- Levine JS, Burakoff R. Extraintestinal manifestations of inflammatory bowel disease. *Gastroenterol Hepatol (NY),* 2011; 7(4):235–241.
- Lim T. Metabolic syndrome in chronic hepatitis C infection: does it still matter in the era of directly acting antiviral therapy? *Hepat Med* 2014; 6: 113-118.
- Lin JF, Chen JM, Zuo JH et al. Meta-analysis: fecal calprotectin for assessment of inflammatory bowel disease activity. *Inflamm Bowel Dis.* 2014;20(8):1407-15.
- Liu D-Y, Gan T, Rao N-N, et al. Identification of lesion images from gastrointestinal endoscope based on feature extraction of combinational methods with and without learning process. *Medical Image Analysis* 2016; 32:281–294.
- Liu GJ, Xu HX, Lu MD et al. Correlation between enhancement pattern of hepatocellular carcinoma on realtime contrast-enhanced ultrasound and tumour cellular differentiation on histopathology. *Br J Radiol* 2007;80:321- 330.
- Liu K, Kaffes AJ. Iron deficiency anaemia: a review of diagnosis, investigation and management. *Eur J Gastroenterol Hepatol.* 2012;24(2):109-16.
- Liu LP, Dong BW, Yu XL et al. Evaluation of focal fatty infiltration of the liver using color Doppler and contrast-enhanced sonography. *J Clin Ultrasound* 2008; 36:560-566.
- Liu X, Li NS, Lv LS et al. A comparison of the performances of an artificial neural network and a regression model for GFR estimation. *Am J Kidney Dis* 2013; 62(6): 1109-1115.
- Liu X, Zeng B, Zhang J et al. Role of the gut microbiome in modulating arthritis progression in mice. *Sci Rep* 2016; 6: 30594.
- Lix LM, Graff LA, Walker JR et al. Longitudinal study of quality of life and psychological functioning for active, fluctuating, and inactive disease patterns in inflammatory bowel disease. *Inflamm Bowel Dis.* 2008; 14(11):1575-84.
- Lonardo A, Ballestri S, Guaraldi G, et al. Fatty liver is associated with an increased risk of diabetes and cardiovascular disease - Evidence from three different disease models: NAFLD, HCV and HIV. *World J Gastroenterol* 2016; 22(44): 9674-9693.
- Lonnfors S, Vermeire S, Greco M et al. IBD and health-related quality of life-discovering the true impact. *J Crohns Colitis.* 2014;8:1281–6.

- Lupu, A.; Diculescu, M.; Diaconescu, R et al. Prevalence of anemia and iron deficiency in Romanian patients with inflammatory bowel disease: A prospective multicenter study. *J. Gastrointestin Liver Dis.* 2015; 24, 15–20.
- Ma C, Lee JK, Mitra AR, et al. Systematic review with meta-analysis: efficacy and safety of oral Janus kinase inhibitors for inflammatory bowel disease. *Aliment Pharmacol Ther* 2019;50(1):5-23.
- Maaser C, Sturm A, Vavricka SR, et al. ECCO-ESGAR guideline for diagnostic assessment in IBD part 1: initial diagnosis, monitoring of known IBD, detection of complications. *J Crohns Colitis.* 2019;13(2):144-164.
- MacFie J. Ethics and nutrition. In: Gibney MJ, Marinos E, Ljungqvist O, Dowsett J, editors. *Clinical Nutrition.* London: Blackwell Science Ltd., 2005, 132-145.
- Machiels K, Joossens M, Sabino J et al. A decrease of the butyrate-producing species Roseburia hominis and Faecalibacterium prausnitzii defines dysbiosis in patients with ulcerative colitis. *Gut.* 2014; 63(8):1275-83.
- Maeda, Y.; Kudo, S.; Ogata, N. et al. Can artificial intelligence help to detect dysplasia in patients with ulcerative colitis? *Endoscopy* 2020.
- Magro, F.; Gionchetti, P.; Eliakim, R. et al. Third European Evidence-based Consensus on Diagnosis and Management of Ulcerative Colitis. Part 1: Definitions, Diagnosis, Extra-intestinal Manifestations, Pregnancy, Cancer Surveillance, Surgery, and Ileo-anal Pouch Disorders. *J. Crohns Colitis* 2017; 11, 649–670.
- Mano F, Ikeda K, Joo E et al. Effects of three major amino acids found in Japanese broth on glucose metabolism and gastric emptying. *Nutrition* 2018; 46: 153-158.
- Marathe CS, Rayner CK, Jones KL, Horowitz M. Relationships between gastric emptying, postprandial glycemia, and incretin hormones. *Diabetes Care* 2013; 36(5): 1396-1405.
- Marineaţă A, Rezuş E, Mihai C, Cijevschi Prelipcean C. Extra-intestinal manifestations and complications in inflammatory bowel disease. *The Medical-Surgical Journal* 2014;118(2): 279-288.
- Martie A, Sporea I, Sirli R, Popescu A, Danila M. How often hepatocellular carcinoma has a typical pattern in contrast enhanced ultrasound? *Maedica (Buchar)* 2012;7:236-240.
- Marzano A.V., Ishak R.S., Saibeni S. et al. Autoinflammatory skin disorders in inflammatory bowel diseases, pyoderma gangrenosum and Sweet's syndrome: A comprehensive review and disease classification criteria. *Clin. Rev. Allergy Immunol.* 2013; 45:202–210.
- Masand PM. Magnetic resonance imaging features of common focal liver lesions in children. *Pediatr Radiol.* 2018;48(9):1234-1244.
- Massironi S, Rossi RE, Cavalcoli FA et al. Nutritional deficiencies in inflammatory bowel disease: Therapeutic approaches. *Clin Nutr* 2013; 14(13): 98-108.
- Matsuoka K, Kanai T. The gut microbiota and inflammatory bowel disease. *Semin Immunopathol* 2015; 37:47–55.
- Matzkies FG, Targan SR, Berel D et al. Markers of intestinal inflammation in patients with ankylosing spondylitis: a pilot study. *Arthritis Res Ther* 2012; 29:14(6): R261.
- McCombie AM, Mulder RT, Gearry RB. Psychotherapy for inflammatory bowel disease: a review and update. *J Crohns Colitis* 2013; 7: 935-49.
- Meier JJ, Rosenstock J, Hincelin-Méry A et al. Contrasting effects of lixisenatide and liraglutide on postprandial glycemic control, gastric emptying, and safety parameters in patients with type 2 diabetes on optimized insulin glargine with or without metformin: a randomized, open-label trial. *Diabetes Care* 2015; 38(7): 1263-1273.

- Meissner EG, Lee YJ, Osinusi A, et al. Effect of sofosbuvir and ribavirin treatment on peripheral and hepatic lipid metabolism in chronic hepatitis C virus, genotype 1-infected patients. *Hepatology* 2015; 61(3): 790-801.
- Menees SB, Powell C, Kurlander J, et al. A meta-analysis of the utility of C-reactive protein, erythrocyte sedimentation rate, fecal calprotectin, and fecal lactoferrin to exclude inflammatory bowel disease in adults with IBS. *Am J Gastroenterol.* 2015;110(3):444-54.
- Michalak A, Mosińska P, Fichna J. Common links between metabolic syndrome and inflammatory bowel disease: Current overview and future perspectives. *Pharmacol Rep* 2016;68(4):837-846.
- Mihai BM, Mihai C, Cijevschi-Prelipcean C et al. Bidirectional relationship between gastric emptying and plasma glucose control in normoglycemic individuals and diabetic patients. *J Diabetes Res* 2018: 1736959.
- Mihai C, Cijevschi Prelipcean C, Dranga M, et al. Correlations between inflammatory biomarkers and activity in inflammatory bowel diseases. *Revista de Chimie* 2018; 69 (3):710-713.
- Mihai C, Cijevschi Prelipcean C, Pintilie I et al. Nutrition in inflammatory bowel diseases. *Rev Med Chir Soc Med Nat* 2013; 117(3): 662-669.
- Mihai C, Mihai B, Trifan A, et al. Metabolic syndrome and genotype 1 virus C compensated liver cirrhosis in the era of directly acting antiviral therapy. *Hepat Mon* 2017; 17(7): e58022.
- Mikocka-Walus A, Bampton P, Hetzel D et al. Cognitive-behavioural therapy for inflammatory bowel disease: 24-month data from a randomised controlled trial. *Int J Behav Med.* 2017;24:127–35.
- Mikocka-Walus AA, Turnbull DA, Andrews JM et al. Psychological problems in gastroenterology outpatients: A South Australian experience. Psychological co-morbidity in IBD, IBS and hepatitis C. *Clin Pract Epidemiol Ment Health.* 2008;4:15.
- Mintz R, Feller ER, Bahr RL, Shah SA. Ocular manifestations of inflammatory bowel disease. *Inflamm Bowel Dis* 2004; 10: 135-139.
- Miranda-García P, Chaparro M, Gisbert JP. Correlation between serological biomarkers and endoscopic activity in patients with inflammatory bowel disease. *Gastroenterol Hepatol.* 2016;39(8):508-15.
- Mitselos IV, Fousekis FS, Lamouri C, et al. Current noninvasive modalities in Crohn's disease monitoring. *Ann Gastroenterol* 2021;34(6):770-780.
- Monod S, Chiolero R, Büla C, Benaroyo L. Ethical issues in nutrition support of severely disabled elderly persons: a guide for health professionals. *J Parenter Enteral Nutr* 2011; 35(3): 295-302.
- Monturo C. The artificial nutrition debate: still an issue… after all these years. *Nutr Clin Pract* 2009; 24(2): 206-213.
- Morcos A, Dinan T, Quigley EM. Irritable bowel syndrome: Role of food in pathogenesis and management. *J Dig Dis* 2009;10:237- 246.
- Moss, A.C. Optimizing the use of biological therapy in patients with inflammatory bowel disease. *Gastroenterol. Rep.* 2015; 3, 63–68.
- Nemni R, Fazio R, Corbo M et al. Peripheral neuropathy associated with Crohn's disease. *Neurology* 1987; 37: 1414-1417.
- Ng SC, Shi HY, Hamidi N. Worldwide incidence and prevalence of inflammatory bowel disease in the 21st century: a systematic review of population-based studies. *Lancet*2018;390:2769–78.

- Nigam G.B., Bhandare A.P., Antoniou G.A., Limdi J.K. Systematic review and meta-analysis of dermatological reactions in patients with inflammatory bowel disease treated with anti-tumour necrosis factor therapy. *Eur. J. Gastroenterol. Hepatol.* 2020.
- Nishida A, Inoue R, Inatomi O et al. Gut microbiota in the pathogenesis of inflammatory bowel disease. *Clin J Gastroenterol* 2018; 11(1):1-10.
- Noor NM, Sousa P, Paul S, Roblin X. Early Diagnosis, Early Stratification, and Early Intervention to Deliver Precision Medicine in IBD. *Inflamm Bowel Dis* 2021; 4:izab228.
- Okabe T, Terashima H, Sakamoto A. What is the manner of gastric emptying after ingestion of liquids with differences in the volume under uniform glucose-based energy content? *Clin Nutr* 2017; 36(5): 1283-1287.
- Orchard TR, Thiyagaraja S, Welsh KI et al. Clinical phenotype is related to HLA genotype in the peripheral arthropathies of inflammatory bowel disease. *Gastroenterology* 2000; 118: 274-278.
- Orchard TR, Wordsworth BP, Jewell DP. Peripheral arthropathies in inflammatory bowel disease:theirarticulardistributiondistributionandnaturalhistory.*Gut* 1998;42:387-391.
- Otilia Gavrilescu, Catalina Mihai, Dana Teodora Anton Paduraru, Stefana Moisa et al. Impact of Inflammatory Bowel Disease on Quality of Life. *Revista de cercetare si interventie sociala*, 2015; Vol. 50: 80-95.
- Ott C, Schölmerich J. Extraintestinal manifestations and complications in IBD. *Nat Rev Gastroenterol Hepatol.* 2013; 10(10):585-95.
- Ott C, Takses A, Obermeier F et al. Smoking increases the risk of extraintestinal manifestations in Crohn's disease. *World J Gastroenterol*, 2014; 20(34):12269– 12276.
- Park, S.C.; Jeen, Y.T. Current and emerging biologics for ulcerative colitis. *Gut Liver* 2015; 9, 8–27.
- Parkes M, Noor NM, Dowling F, et al. Predicting outcomes for Crohn's disease using a molecular biomarker (PROFILE): protocol for a multicentre, randomised, biomarker-stratified trial. *BMJ Open.* 2018;8:e026767.
- Parthasarathy G, Kudva YC, Low PA et al. Relationship between gastric emptying and diurnal glycemic control in type 1 diabetes mellitus: a randomized trial. *J Clin Endocrinol Metab* 2017; 102(2): 398-406.
- Pascal V, Pozuelo M, Borruel N et al. A microbial signature for Crohn's disease. *Gut* 2017; 66(5):813-822.
- Peng J-H, Fang Y-J, Li C-X, et al. A scoring system based on artificial neural network for predicting 10-year survival in stage II A colon cancer patients after radical surgery. *Oncotarget* 2016;7:22939–22947.
- Phillips LK, Deane AM, Jones KL et al. Gastric emptying and glycaemia in health and diabetes mellitus. *Nat Rev Endocrinol* 2015; 11(2): 112-128.
- Piscaglia F, Bolondi L. Italian Society for Ultrasound in Medicine and Biology (SIUMB) Study Group on Ultrasound Contrast Agents. The safety of Sonovue in abdominal applications: retrospective analysis of 23188 investigations. *Ultrasound Med Biol* 2006; 32: 1369 – 1375.
- Piscaglia F, Lencioni R, Sagrini E et al. Characterization of focal liver lesions with contrast-enhanced ultrasound. *Ultrasound Med Biol* 2010;36:531-550.
- Pizzi LT, Weston CM, Goldfarb NI et al. Impact of chronic conditions on quality of life in patients with inflammatory bowel disease. *Inflamm Bowel Dis.* 2006;12(1):47-52.
- Plummer MP, Jones KL, Cousins CE et al. Hyperglycemia potentiates the slowing of gastric emptying induced by exogenous GLP-1. *Diabetes Care* 2015; 38(6): 1123-1129.
- Popa IV, Burlacu A, Gavrilescu O, Dranga M, Prelipcean Cijevschi C, Mihai C. A new approach to predict ulcerative colitis activity through standard clinical-biological

parameters using a robust neural network model. *Neural Computing & Applications* 2021: 10.1007/s00521-021-06055-x.

- Popa IV, Burlacu A, Mihai C, Cijevschi Prelipcean C. A Machine Learning Model Accurately Predicts Ulcerative Colitis Activity at One Year in Patients Treated with Anti-Tumour Necrosis Factor α Agents. *Medicina* 2020; 56: 628.
- Popa IV, Diculescu M, Mihai C, et al. Developing a Neural Network Model for a Non-invasive Prediction of Histologic Activity in Inflammatory Bowel Diseases. *Turkish J Gastroenterol* 2021; 32(3): 276-286
- Popa IV, Diculescu M, Mihai C et al. COVID-19 and Inflammatory Bowel Diseases: Risk Assessment, Shared Molecular Pathways, and Therapeutic Challenges. *Gastroenterology Research and Practice* 2020, 1–7.
- Popa IV, Dranga M, Gavrilescu O, et al. Artificial neural networks - a new approach in non-invasive monitoring of inflammatory bowel diseases. *The Medical-Surgical Journal* 2017; 121(4): 695-700.
- Popa S, Moţa M, Popa A, et al. Prevalence of overweight/obesity, abdominal obesity and metabolic syndrome and atypical cardiometabolic phenotypes in the adult Romanian population: PREDATORR study.*J Endocrinol Invest.* 2016;39(9):1045-53.
- Prince A, Whelan K, Moosa A et al. Nutritional problems in inflammatory bowel disease: the patient perspective. *J Crohns Colitis* 2011; 5(5): 443-450.
- Rehman A, Rausch P, Wang J et al. Geographical patterns of the standing and active human gut microbiome in health and IBD. *Gut* 2016; 65(2):238-48.
- Rey E, Talley NJ. Irritable bowel syndrome: novel views on the epidemiology and potential risk factors. *Dig Liver Dis* 2009;41:772- 780.
- Ricanek P, Brackmann S, Perminow Get al. IBSEN II Study Group. Evaluation of disease activity in IBD at the time of diagnosis by the use of clinical, biochemical, and fecal markers. *Scand J Gastroenterol.* 2011;46(9):1081-91.
- Rispo A, Imperatore N, Testa A, et al. Diagnostic accuracy of ultrasonography in the detection of postsurgical recurrence in Crohn's disease:a systematic review with meta-analysis. *Inflamm Bowel Dis* 2018;24:977–988.
- Roche V, Pigneur F, Tselikas L et al. Differentiation of focal nodular hyperplasia from hepatocellular adenomas with low-mechanical-index contrast-enhanced sonography (CEUS): effect of size on diagnostic confidence. *Eur Radiol* 2015;25:186-195.
- Rodrigues BL, Mazzaro MC, Nagasako CK et al. Assessment of disease activity in inflammatory bowel diseases: Non-invasive biomarkers and endoscopic scores. *World J Gastrointest Endosc.*2020; 12(12):504-520.
- Rogler G, Singh A, Kavanaugh A, Rubin DT. Extraintestinal Manifestations of Inflammatory Bowel Disease: Current Concepts, Treatment, and Implications for Disease Management. *Gastroenterology.* 2021; 161(4):1118-1132.
- Rokkas T, Portincasa P, Koutroubakis IE. Fecal calprotectin in assessing inflammatory bowel disease endoscopic activity: a diagnostic accuracy meta-analysis. J *Gastrointestin Liver Dis.* 2018;27(3):299-306.
- Romberg-Camps MJ, Bol Y, Dagnelie PC et al. Fatigue and health-related quality of life in inflammatory bowel disease: results from a population-based study in the Netherlands: the IBD-South Limburg cohort. *Inflamm Bowel Dis.* 2010;16(12):2137-47.
- Rubin, D.T. When Should Therapy for Inflammatory Bowel Disease Be Stopped? *Gastroenterol. Hepatol.* 2015; 11, 400–402.
- Sajid, M.S.; Tonsi, A.; Baig, M.K. Health related quality of life measurement. *Int. J. Health Care Qual. Assur*. 2008; 21, 365–373.

- Salem F, Kindt N, Marchesi JR et al. Gut microbiome in chronic rheumatic and inflammatory bowel diseases: Similarities and differences. *United European Gastroenterology Journal* 2019; 7(8): 1008–1032.
- Salvarani C, Fornaciari G, Beltrami M, Macchioni PL. Musculoskeletal manifestations in inflammatory bowel disease. *Eur J Intern Med* 2000; 11: 210-214.
- Sandborn WJ, Abreu MT, Dubinsky MC. A noninvasive method to assess mucosal healing in patients∗ with Crohn's disease. *Gastroenterol Hepatol.* 2018; 1–12.
- Sandireddy R, Yerra VG, Areti A et al. Neuroinflammation and oxidative stress in diabetic neuropathy: futuristic strategies based on these targets. *Int J Endocrinol* 2014; 2014: 674987.
- Sazonovs A, Kennedy NA, Moutsianas L, et al.; PANTS Consortium. HLA-DQA1*05 carriage associated with development of anti-drug antibodies to infliximab and adalimumab in patients with Crohn's disease. *Gastroenterology* 2020;158:189–199.
- Săftoiu A, Vilmann P, Dietrich CF, et al. Quantitative contrast-enhanced harmonic EUS in differential diagnosis of focal pancreatic masses (with videos). *Gastrointest Endosc* 2015;82:59–69.
- Schoepfer AM, Vavricka S, Zahnd-Straumann N et al. Monitoring inflammatory bowel disease activity: clinical activity is judged to be more relevant than endoscopic severity or biomarkers. *J Crohns Colitis* 2012;6(4):412-8.
- Schoultz M, Atherton I, Watson A. Mindfulness-based cognitive therapy for inflammatory bowel disease patients: findings from an exploratory pilot randomised controlled trial. *Trials* 2015; 16: 379.
- Scialpi M, Pierotti L, Gravante S et al. Split-bolus versus triphasic multidetector-row computed tomography technique in the diagnosis of hepatic focal nodular hyperplasia: a case report. *J Med Case Rep.* 2014;8:425.
- Seminerio JL, Koutroubakis IE, Ramos-Rivers C, et al. Impact of obesity on the management and clinical course of patients with inflammatory bowel disease. *Inflamm Bowel Dis* 2015; 21: 2857-2863.
- Seyed Tabib NS, Madgwick M, Sudhakar P et al. Big data in IBD: big progress for clinical practice. *Gut.* 2020;69(8):1520-1532.
- Siffledeen JS, Fedorak RN, Siminoski K, et al. Bones and Crohn's risk factors associated with low bone mineral density in patients with Crohn's disease. *Inflamm Bowel Dis* 2004; 10: 220-228.
- Simrén M, Axelsson J, Gillberg R et al. Quality of life in inflammatory bowel disease in remission: the impact of IBS-like symptoms and associated psychological factors. *Am J Gastroenterol*. 2002;97(2):389-96.
- Singeap AM, Girleanu I, Diculescu M, et al. Risk Factors for Extraintestinal Manifestations in Inflammatory Bowel Diseases - Data from the Romanian National Registry. *J Gastrointestin Liver Dis.* 2021; 30(3):346-357.
- Singh S, Dulai PS, Zarrinpar A, et al. Obesity in IBD: Epidemiology, pathogenesis, disease course and treatment outcomes. *Nat Rev Gastroenterol Hepatol* 2017; 14: 110-121.
- Sinonquel P, Eelbode T, Bossuyt P, et al. Artificial intelligence and its impact on quality improvement in upper and lower gastrointestinal endoscopy. *Dig Endosc* 2021; 33(2):242-253.
- Sipponen T, Kolho KL. Fecal calprotectin in diagnosis and clinical assessment of inflammatory bowel disease. *Scand J Gastroenterol* 2015; 50: 74-80.
- Sirli R, Sporea I, Martie A et al. Contrast enhanced ultrasound in focal liver lesions--a cost efficiency study. *Med Ultrason* 2010;12:280-285.

- Slomka J. Withholding nutrition at the end of life: clinical and ethical issues. *Clev Clin J Med* 2003; 70(6): 548-555.
- Sokol H, Brot L, Stefanescu C, et al.; REMIND Study Group Investigators. Prominence of ileal mucosa-associated microbiota to predict postoperative endoscopic recurrence in Crohn's disease. *Gut.* 2020;69:462–472.
- Sporea I, Badea R, Brisc C et al. Romanian National Guidelines on Contrast Enhanced Ultrasound in clinical practice. *Med Ultrason.* 2017;19(4):401-415.
- Sporea I, Badea R, Martie A et al. Contrast Enhanced Ultrasound for the evaluation of focal liver lesions in daily practice. A multicentre study. *Med Ultrason* 2012; 14: 95 – 100.
- Sporea I. Ultrasound: "one stop shop" in hepatology. *Med Ultrason* 2016;18(2):143-4.
- Sporea I, Badea R, Popescu A et al.Contrast-enhanced ultrasound (CEUS) for the evaluation of focal liver lesions - a prospective multicenter study of its usefulness in clinical practice.*Ultraschall Med.* 2014; 35(3):259-66.
- Sporea I, Bende F. Where are we Now with Ultrasound-based Liver Elastography? In: What is New in Gastroenterology and Hepatology; Eds: Sporea I, Popescu A. Bentham Books 2021: 237-254.
- Stapersma L, van den Brink G, van der Ende J et al. Psychological Outcomes of a Cognitive Behavioral Therapy for Youth with Inflammatory Bowel Disease: Results of the HAPPY-IBD Randomized Controlled Trial at 6- and 12-Month Follow-Up. *J Clin Psychol Med Settings.* 2020;27(3):490-506.
- Stebbings S, Munro K, Simon MA et al. Comparison of the faecal microflora of patients with ankylosing spondylitis and controls using molecular methods of analysis. *Rheumatology* 2002; 41:1395–1401.
- Stoll ML, Weiss PF, Weiss JE et al. Age and fecal microbial strain-specific differences in patients with spondyloarthritis. *Arthritis Res Ther* 2018; 20: 14.
- Strobel D, Seitz K, Blank W et al. Contrast-enhanced ultrasound for the characterization of focal liver lesions--diagnostic accuracy in clinical practice (DEGUM multicenter trial). *Ultraschall Med* 2008; 29:499-505.
- Suk Lee Y, Kim N.H, Hyuk Son J. et al. Type 2 autoimmune pancreatitis with Crohn's disease. *Intern Med.* 2018; 57: 2957-2962.
- de Souza HSP, Fiocchi C, Iliopoulos D. The IBD interactome: an integrated view of aetiology, pathogenesis and therapy. *Nat Rev Gastroenterol Hepatol* 2017; 14(12): 739-49.
- Şirli R, Sporea I, Popescu A, et al. Contrast-enhanced ultrasound for the assessment of focal nodular hyperplasia - results of a multicentre study. *Med Ultrason.* 2021; 23(2):140-146.
- Takenaka, K.; Ohtsuka, K.; Fujii, T. et al. Development and Validation of a Deep Neural Network for Accurate Evaluation of Endoscopic Images From Patients With Ulcerative Colitis. *Gastroenterology* 2020; 158, 2150–2157.
- Talley NJ, Howell S, Poulton R. The irritable bowel syndrome and psychiatric disorders in the community: is there a link? *Am J Gastroenterol* 2001;96:1072-1079.
- Tang C, Fang K, Guo Y et al. Safety of Sulfur Hexafluoride Microbubbles in Sonography of Abdominal and Superficial Organs: Retrospective Analysis of 30,222 Cases. *J Ultrasound Med.* 2017;36(3):531-538.
- Tappenden KA. Intake: digestion, absorption, transport, and excretion of nutrients. In: Mahan LK, Raymond JL, editors. *Krause's Food & the Nutrition Care Process.* St. Louis: *Elsevier, 14th edition,* 2017; 2-16.
- Taylor SA, Mallett S, Bhatnagar G, et al.; METRIC study investigators. Diagnostic accuracy of magnetic resonance enterography and small bowel ultrasound for the extent

and activity of newly diagnosed and relapsed Crohn's disease (METRIC): a multicentre trial. *Lancet Gastroenterol Hepatol*. 2018;3:548–558.

- Thaiss CA, Levy M, Grosheva I, et al. Hyperglycemia drives intestinal barrier dysfunction and risk for enteric infection. *Science* 2018;359(6382):1376-1383.
- Timmer A, Preiss JC, Motschall E, et al. Psychological interventions for treatment of inflammatory bowel disease. *Cochrane Database Syst Rev* 2011; 16: CD006913.
- Tkalcić M, Hauser G, Stimac D. Differences in the health-related quality of life, affective status, and personality between irritable bowel syndrome and inflammatory bowel disease patients. *Eur J Gastroenterol Hepatol.* 2010;22(7):862-7.
- Tranquart F, Correas JM, Ladam MV et al. Real-time contrast-enhancedultrasound in the evaluation of focal liver lesions: diagnostic efficacy and economical issues from a French multicentric study. *J Radiol* 2009; 90: 109 – 122.
- Tranquart F LGA, Correas JM, Ladam Marcus V et al. Role of contrast-enhanced ultrasound in the blinded assessment of focal lesions in comparison with MDCT and CEMRI: Results from a multicentre clinical trial. *Eur J Cancer Suppl* 2008; 6:9-15.
- Trillaud H, Bruel JM, Valette PJ, et al. Characterization of focal liver lesions with SonoVue-enhanced sonography: international multicenter-study in comparison to CT and MRI. *World J Gastroenterol* 2009;15:3748-3756.
- Tsochatzis EA, Gurusamy KS, Ntaoula S, et al. Elastography for the diagnosis of severity of fibrosis in chronic liver disease: a meta-analysis of diagnostic accuracy. *J Hepatol* 2011; 54(4): 650-9.
- Turner D, Ricciuto A, Lewis A, et al; International Organization for the Study of IBD. STRIDE-II: An Update on the Selecting Therapeutic Targets in Inflammatory Bowel Disease (STRIDE) Initiative of the International Organization for the Study of IBD (IOIBD): Determining Therapeutic Goals for Treat-to-Target strategies in IBD. *Gastroenterology* 2021;160(5):1570-1583.
- Turpin W, Lee SH, Raygoza Garay JA, et al.; Crohn's and Colitis Canada Genetic Environmental Microbial Project Research Consortium; CCC GEM Project recruitment site directors include Maria Abreu. Increased intestinal permeability is associated with later development of Crohn's disease. *Gastroenterology*. 2020;159:2092–2100.e5.
- Tursi A, Elisei W, Picchio M, et al. Effectiveness and safety of infliximab and adalimumab for ambulatory Crohn's disease patients in primary gastroenterology centres. *Eur J Intern Med* 2014;25:485–490.
- van der Linden S, Valkenburg HA, Cats A. Evaluation of diagnostic criteria for ankylosing spondylitis. A proposal for modification of the New York criteria. *Arthritis Rheum*, 1984; 27(4):361–368.
- Van Praet L, Van den Bosch FE, Jacques P et al. Microscopic gut inflammation in axial spondyloarthritis: a multiparametric predictive model. *Ann Rheum Dis* 2013; 72(3):414–7.
- Varela E, Manichanh C, Gallart M et al. Colonisation by Faecalibacterium prausnitzii and maintenance of clinical remission in patients with ulcerative colitis. *Aliment Pharmacol Ther* 2013; 38(2): 151-61.
- Vavricka SR, Brun L, Ballabeni P et al. Frequency and risk factors for extraintestinal manifestations in the Swiss inflammatory bowel disease cohort. *Am J Gastroenterol*, 2011; 106(1):110– 119.
- Vavricka SR, Rogler G, Gantenbein C, et al. Chronological Order of Appearance of Extraintestinal Manifestations Relative to the Time of IBD Diagnosis in the Swiss Inflammatory Bowel Disease Cohort. *Inflamm Bowel Dis.* 2015; 21(8):1794-800.
- Vermeire S, Van Assche G, Rutgeerts P. Laboratory markers in IBD: useful, magic, or unnecessary toys? *Gut.* 2006;55(3):426-31.

- Vidal A, Gómez-Gil E, Sans M et al. Health-related quality of life in inflammatory bowel disease patients: the role of psychopathology and personality. *Inflamm Bowel Dis.* 2008;14(7):977-83.
- Vidal NO, Brandstrom H, Jonsson KB, Ohlsson C. Osteoprotegerin mRNA is expressed in primaryhumanosteoblast-likecells:down-regulationbyglucocorticoids.*JEndocrinol* 1998;159:191-195.
- Vieira A, Fang CB, Rolim EG, et al. Inflammatory bowel disease activity assessed by fecal calprotectin and lactoferrin: correlation with laboratory parameters, clinical, endoscopic and histological indexes. *BMC Res Notes.* 2009;2:221.
- Viladomiu M, Hontecillas R, Yuan L, et al. Nutritional protective mechanisms against gut inflammation. *J Nutr Biochem* 2013; 24(6):929-939.
- Von Roon AC, Karamountzos L, Purkayastha S, et al. Diagnostic precision of fecal calprotectin for inflammatory bowel disease and colorectal malignancy. *Am J Gastroenterol.* 2007; 102(4):803-13.
- Von Wietersheim, J.; Kessler, H. Psychotherapy with chronic inflammatory bowel disease patients: A review. *Inflamm. Bowel Dis.* 2006; 12, 1175–1184.
- Vrakas S, Mountzouris CK, Michalopoulos G et al. Intestinal Bacteria Composition and Translocation of Bacteria in Inflammatory Bowel Disease. *PLoS ONE* 2017; 12(1): e0170034.
- Waljee, A.K.; Chaisidhivej, N.; Saini, S.D.; Higgins, P.D.R. De-escalation of IBD Therapy: When, Who, and How? *Crohns Colitis 360*2019; 1, otz008.
- Waljee AK, Lipson R, Wiitala WL, et al. Predicting Hospitalization and Outpatient Corticosteroid Use in Inflammatory Bowel Disease Patients Using Machine Learning. *Inflamm Bowel Dis* 2017;24:45–53.
- Waljee, A.K.; Liu, B.; Sauder, K. et al. Predicting corticosteroid-free endoscopic remission with vedolizumab in ulcerative colitis. *Aliment. Pharmacol. Ther.* 2018; 47, 763–772.
- Waljee AK, Sauder K, Patel A, et al. Machine Learning Algorithms for Objective Remission and Clinical Outcomes with Thiopurines. *J Crohns Colitis* 2017;11:801–810.
- Walsh A., Kormilitzin A., Hinds C. et al. Defining Faecal Calprotectin Thresholds as a Surrogate for Endoscopic and Histological Disease Activity in Ulcerative Colitis—A Prospective Analysis. *J. Crohn's Colitis.* 2019; 13:424–430.
- Wang D, Wang Q, Shan F et al. Identification of the risk for liver fibrosis on CHB patients using an artificial neural network based on routine and serum markers. *BMC Infect Dis* 2010; 5:12456-1362.
- Wang K, Lu X, Zhou H et al. Deep learning Radiomics of shear wave elastography significantly improved diagnostic performance for assessing liver fibrosis in chronic hepatitis B: a prospective multicentre study. *Gut* 2019; 68:729–741.
- Wang P, Berzin TM, Glissen Brown JR et al. Real-time automatic detection system increases colonoscopic polyp and adenoma detection rates: a prospective randomised controlled study. *Gut.* 2019;68(10):1813-1819.
- Wang W, Chen L, Zhou R et al. Increased proportions of Bifidobacterium and the Lactobacillus group and loss of butyrate-producing bacteria in inflammatory bowel disease. *J Clin Microbiol* 2014; 52(2):398-406.
- Wang W, Chen LD, Lu MD et al. Contrast-enhanced ultrasound features of histologically proven focal nodular hyperplasia: diagnostic performance compared with contrastenhanced CT. *Eur Radiol* 2013;23:2546-2554.

- Wanless IR, Albrecht S, Bilbao J et al. Multiple focal nodular hyperplasia of the liver associated with vascular malformations of various organs and neoplasia of the brain: a new syndrome. *Mod Pathol* 1989;2:456-462.
- Wei R, Wang J, Wang X, et al. Clinical prediction of HBV and HCV related hepatic fibrosis using machine learning. *EBioMedicine* 2018; 35:124–132.
- Wei Z, Wang W, Bradfield J, et al. Large sample size, wide variant spectrum, and advanced machine-learning technique boost risk prediction for inflammatory bowel disease. *Am J Hum Genet* 2013;92:1008–1012.
- Wells, C.W.; Lewis, S.; Barton, J.R.; Corbett, S. Effects of changes in hemoglobin level on quality of life and cognitive function in inflammatory bowel disease patients. *Inflamm. Bowel Dis.* 2006; 12, 123–130.
- Wen C, Zheng Z, Shao T et al. Quantitative metagenomics reveals unique gut microbiome biomarkers in ankylosing spondylitis. *Genome Biol* 2017; 18(1):142.
- Wilkens R, Hagemann-Madsen RH, Peters DA, et al. Validity of contrast-enhanced ultrasonography and dynamic contrast-enhanced MR enterography in the assessment of transmural activity and fibrosis in Crohn's disease. *J Crohns Colitis*2018;12:48–56.
- Willing BP, Dicksved J, Halfvarson J et al. A Pyrosequencing Study in Twins Shows That Gastrointestinal Microbial Profiles Vary With Inflammatory Bowel Disease Phenotypes. *Gastroenterology* 2010; 139:1844–1854.
- Wills ES, Jonkers DM, Savelkoul PH et al. Fecal Microbial Composition of Ulcerative Colitis and Crohn's Disease Patients in Remission and Subsequent Exacerbation. *PLoS ONE* 2014; 9 (3): e90981.
- Wilson, A.; Reyes, E.; Ofman, J. Prevalence and outcomes of anemia in inflammatory bowel disease: A systematic review of the literature. *Am. J. Med.* 2004; 116 (Suppl. S7A), 44S–49S.
- Windsor JW, Kaplan GG. Evolving Epidemiology of IBD. *Curr Gastroenterol Rep* 2019;21(8):40.
- Wong GL-H, Ma AJ, Deng H, et al. Machine learning model to predict recurrent ulcer bleeding in patients with history of idiopathic gastroduodenal ulcer bleeding. *Aliment Pharmacol Ther* 2019; 49:912–918.
- Wu T, Rayner CK, Horowitz M. Inter-regulation of gastric emptying and incretin hormone secretion: implications for postprandial glycemic control. *Biomark Med* 2016; 10(11): 1167-1179.
- Xie L, Guang Y, Ding H et al. Diagnostic value of contrast-enhanced ultrasound, computed tomography and magnetic resonance imaging for focal liver lesions: a meta-analysis. *Ultrasound Med Biol* 2011; 37: 854–861.
- Yang Y, Chen H, Wang D, et al. Diagnosis of pancreatic carcinoma based on combined measurement of multiple serum tumor markers using artificial neural network analysis. *Chin Med J* 2014;127:1891–1896.
- Yarur AJ, Czul F, Levy C. Hepatobiliary manifestations of inflammatory bowel disease. *Inflamm Bowel Dis.* 2014; 20(9):1655-67.
- Yip TC-F, Ma AJ, Wong VW-S, et al. Laboratory parameter-based machine learning model for excluding non-alcoholic fatty liver disease (NAFLD) in the general population. *Aliment Pharmacol Ther* 2017; 46:447–456.
- Yoon JY, Park SJ, Hong SP et al. Correlations of C-reactive protein levels and erythrocyte sedimentation rates with endoscopic activity indices in patients with ulcerative colitis. *Dig Dis Sci.* 2014;59(4):829-37.
- Younossi Z, Park H, Henry L et al. Extrahepatic manifestations of hepatitis c: a meta-analysis of prevalence, quality of life, and economic burden. *Gastroenterology* 2016; 150(7):1599-1608.

- Younossi Z, Anstee QM, Marietti M, et al. Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention. *Nat Rev Gastroenterol Hepatol*. 2018;15(1):11-20.
- Younossi ZM, Golabi P, de Avila L, et al. The global epidemiology of NAFLD and NASH in patients with type 2 diabetes: A systematic review and meta-analysis. *J Hepatol* 2019;71(4):793-801.
- Zarringhalam, K.; Enayetallah, A.; Reddy, P.; Ziemek, D. Robust clinical outcome prediction based on Bayesian analysis of transcriptional profiles and prior causal networks. *Bioinformatics* 2014; 30, i69–i77.
- Zhang X, Zhang D, Jia H et al. The oral and gut microbiomes are perturbed in rheumatoid arthritis and partly normalized after treatment. *Nat Med.* 2015; 21(8):895-905.
- Zheng MH, Shi KQ, Lin XF et al. A model to predict 3-month mortality risk of acute-on-chronic hepatitis B liver failure using artificial neural network. *J Viral Hepat* 2013; 20(4): 248-255.
- Zhou, Y.; Ren, W.; Irvine, E.J.; Yang, D. Assessing health-related quality of life in patients with inflammatory bowel disease in Zhejiang, China. *J. Clin. Nurs.* 2010; 19, 79–88.
- Zhu YZ, Qian XJ, Zhao P, Qi ZT. How hepatitis C virus invades hepatocytes: the mystery of viral entry. *World J Gastroenterol* 2014; 20(13):3457-3467.
- Zippi M, Corrado C, Pica R et al. Extraintestinal manifestations in a large series of Italian inflammatory bowel disease patients. *World J Gastroenterol*, 2014; 20(46):17463–17467.
- Zittan E, Kelly OB, Kirsch R, et al. Low fecal calprotectin correlates with histological remission and mucosal healing in ulcerative colitis and colonic Crohn's disease. *Inflamm Bowel Dis*. 2016; 22(3):623- 630.