GRIGORE T. POPA UNIVERSITY OF
MEDICINE AND PHARMACY IASI

HABILITATION THESIS

THE RIGHT BALANCE BETWEEN INVASIVE AND NON-INVASIVE IN HEPATO-GASTROENTEROLOGY

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Education is not the learning of facts,
but the training of the mind to think.

Albert EINSTEIN

CONTENTS

ABBREVIATION LIST

AASLD - American Association for the Study of Liver Diseases

AE - adverse event

AFP - alpha fetoprotein

AGA - American Gastroenterological Association

AI - artificial intelligence

ALP - alkaline phosphatase

ALT - alanine aminotransferase

APC - argon plasma coagulation

APSDE - Asian Pacific Society for Digestive Endoscopy

ASGE - American Society for Gastrointestinal Endoscopy

AST - aspartate aminotranspherase

AUGIB - acute upper gastrointestinal bleeding

BMI - body mass index

***C. difficile* -** *Clostridium difficile/ Clostridioides difficile*

C/RL - caudate-right lobe ratio

CAP - controlled attenuation parameter

CBD - common bile duct

CCE - colon capsule endoscopy

CD – Crohn's disease

CDAI - Crohn's disease activity index

CDI - *Clostridium difficile* colitis infection

CECDAI - capsule endoscopy Crohn's disease activity index

CI - confidence interval

CLE - confocal laser endomicroscopy

CNN - convolutional neural network

COVID-19 - Coronavirus Disease 2019

COX - cyclo-oxygenase

CRC - colorectal cancer

CRP - C-reactive protein

CT - computed tomography

DAA - direct-acting antiviral

DBE - double balloon enteroscopy

DXA - dual-energy X-ray absorptiometry

DY - diagnostic yield

EASL - The European Association for the Study of the Liver

ECCO - European Crohn's and Colitis Organisation

EGD - esogastroduodenoscopy

EIM - extraintestinal manifestation

EOT - end of treatment

ERCP - endoscopic retrograde cholangiopancreatography

ESGE - European Society of Gastrointestinal Endoscopy

ESGENA - European Society of Gastroenterology and Endoscopy Nurses and Associates

FAP - familial adenomatous polyposis

FDA - Food and Drug Administration

FIB-4 - fibrosis-4 index

GNT - genotype

GGT - gamma-glutamyl transferase

GI - gatrointestinal

GIST - gastrointestinal stromal tumor

HBIG - hepatitis B immunoglobulin

HBV - hepatitis B virus

HCC - hepatocellular carcinoma

HCV - hepatitis C virus

HCV-RNA - hepatitis C virus ribonucleic acid

HDL-c - high-density lipoprotein cholesterol

HDV - hepatitis D virus

HE - hepatic encephalopathy

HVPG - hepatic venous pressure gradient

IBD - inflammatory bowel disease

IBD-U - inflammatory bowel disease type unclassified

IDA - iron deficiency anemia

IDSA - Infectious Diseases Society of America

IFN - interferon

INR - international normalized ratio

IQR - interquartile range

IQR/M - interquartile range/median ratio

IR - insulin resistance

ITT - intention-to-treat

LB - liver biopsy

LC - liver cirrhosis

LDL-c - low-density lipoprotein cholesterol

LF - liver fibrosis

LI-RADS - Liver Imaging Reporting and Data System

LSM - liver stiffness measurement

LT - liver transplantation

MAFLD - metabolic dysfunction-associated fatty liver disease

MELD - Model of End-Stage Liver Disease

METAVIR - meta-analysis of histological data in viral hepatitis

MR - magnetic resonance

MRI - magnetic resonance imaging

NA - nucleos(t)ide analogue

NAFLD - non-alcoholic fatty liver disease

NAP1 - North-American Pulsed-Field type 1

NASH - non-alcoholic steatohepatitis

NBVV - nonbleeding visible vessel

NET - neuroendocrine tumor

NOAC - non-vitamin K antagonist oral anticoagulant

NSAID - non-steroidal anti-inflammatory drug

NSBBs - nonselective beta-blockers

NVUGIB - non-variceal acute upper gastrointestinal bleeding

OGIB - obscure gastrointestinal bleeding

OMED - Organisation Mondiale d'Endoscopie Digestive

OR - odds ratio

PCC1 - PillCam colon capsule 1

PCC2 - PillCam colon capsule 2

PCR - polymerase chain reaction

PEG - polyethylene glycol

PP - per-protocol

PPE - personal protective equipment

PPI - proton pump inhibitors

PrOD - Paritaprevir/Ritonavir, Ombitasvir and Dasabuvir

PUB - peptic ulcer bleeding

RBV - Ribavirin

SAE - serious adverse event

SARS-CoV-2 - severe acute respiratory syndrome coronavirus 2

SB - small bowel

SBCE - small bowel capsule endoscopy

SBT - small bowel tumor

SD - standard deviation

SMI - skeletal muscle index

SPSS - Statistical Package for Social Sciences

SVR - sustained virological response

SWE - shear-wave elastography

T2DM - type 2 diabetes mellitus

TE - transient elastography

TIPS - transjugular intrahepatic portosystemic shunt

TMA - total muscle area

TNF - tumor necrosis factor

UC - ulcerative colitis

UGIB - upper gastrointestinal bleeding

US - ultrasonography

UTI - urinary tract infection

VB - variceal bleeding

VCTE - vibration-controlled transient elastography

VKA - vitamin K antagonist

WEO - World Endoscopy Organization

WHO - World Health Organization

WtHR - waist-to-height ratio

REZUMAT

Elaborarea acestei teze de abilitare reprezintă, în același timp, o oportunitate și o datorie de a recapitula și trece în revistă realizările profesionale, academice și științifice din perioada postdoctorală. Totodată, alături de sinteza rezultatelor de până acum, voi formula și direcțiile viitoare de dezvoltare, ca o continuare a preocupărilor în domeniile amintite, și extinderea în arii conexe.

Structura lucrării respectă recomandările Consiliului Național de Atestare a Titlurilor, Diplomelor și Certificatelor Universitare (CNATDCU), fiind compusă din trei secțiuni:

- Secțiunea I - Realizările pe plan academic, profesional și științific din perioada postdoctorală;
- Secțiunea II - Direcții de dezvoltare și proiecte de cercetare viitoare;
- Secțiunea III - Referințe bibliografice.

Secțiunea întâi a tezei de abilitare prezintă o sinteză a rezultatelor din domeniile academic, științific și medical obținute pe parcursul celor 14 ani de activitate, până la poziția actuală, de conferențiar universitar.

Am evidențiat principalele elemente ale activității clinice si academice, precum și realizările științifice, concretizate în lucrări publicate și premiate.

Recunoașterea activității științifice est dovedită de:

- Indicele Hirsch – 11
- 444 citări și un factor de impact cumulat pentru articolele în care sunt autor principal de 24.927

Prezentarea activității științifice este structurată în trei capitole, dedicate realizărilor în plan științific din principala arie de interes – explorarea tubului digestiv prin metoda capsulei endoscopice, dar și din arii adiacente și din domenii conexe de preocupări, întregind abordarea pacientului aparținând de profilul complex al gastroenterologiei și hepatologiei.

O provocare actuală a medicinei o reprezintă găsirea echilibrului ideal între metodele invazive și, respectiv, non-invazive sau minim invazive. În domeniul hepatogastroenterologiei, aceste tipuri diferite de metode pot fi îmbinate benefic, dar pot impune și alegere riguroasă, prin competitivitate legată de avantaje și/sau limite sau riscuri.

Pe parcursul activității profesionale și de cercetare am urmărit să contribui prin elemente personale la această temă a echilibrului perfect între investigațiile invazive și non-invazive sau minim invazive.

Primul capitol ilustrează preocupările din domeniul explorării cu ajutorul capsulei endoscopice a tractului gastrointestinal. Metodă inovativă, non-invazivă de vizualizare a tubului digestiv, a fost introdusă relativ recent în practica clinică. Sunt prezentate tehnica, avantajele și limitele capsulei endoscopice, pentru fiecare versiune din cele trei existente (de esofag, intestin subțire și colon). Accentul este pus pe prezentarea capsulei endoscopice de intestin subțire, ca metodă recunoscută la momentul actual ca primă linie de investigație în suspiciunea de patologie a intestinului subțire. De asemenea, sunt prezentate rezultatele personale provenind din aplicarea nemijlocită a acestei metode în practica clinică: impactul clinic al explorării cu ajutorul capsulei endoscopice a hemoragiei digestive de cauză neprecizată, cauze neobișnuite de hemoragie digestivă obscură, corelații vizuale morfopatologice ale tumorilor de intestin subțire evidențiate prin explorarea cu ajutorul capsulei endoscopice, precum și factori de influență a ratei de vizualizare integrală a intestinului subțire în boala Crohn. Primul capitol se încheie cu prezentarea rezultatelor privind utilizarea capsulei endoscopice în explorarea colonului, ca alternativă a colonoscopiei în

diagnosticul neoplaziilor de colon, precum și în explorarea bolilor inflamatorii colonice neclasificate.

Al doilea capitol este dedicat prezentării contribuțiilor personale din domeniul hepatologiei, arie conexă de interes, parte intrinsecă a abordării holistice a pacientului cu suferință digestivă. Pe primele planuri de preocupare se situează: screeningul hepatitelor virale – în acest sens voi prezenta rezultatele unui studiu de prevalență si factori de risc pentru hepatita cronică virală C, tratamentul antiviral și monitorizarea post-terapie a pacienților – cu accent pe urmărirea „remodelării" hepatice, documentate prin aportul explorărilor non-invazive reprezentate de examinarea computer tomografică și evaluarea prin elastometrie și parametrul de atenuare controlată a steatozei, precum și studiul bolii ficatului gras non-alcoolic, cu incidență în creștere pe plan mondial și semnificație tot mai amplă prin comorbidități și potențial evolutiv.

Al treilea capitol evidențiază aspecte de abordare a pacienților cu suferințe hepato-gastrointestinale aflați în situații particulare. Primul studiu prezintă rezultate privind factorii de risc ai pacienților cu boli inflamatorii intestinale care asociază manifestări extradigestive, iar următoarele, în conjunctura recentă specială a pandemiei SARS-COV-2, prezintă: impactul acesteia asupra infecției cu *Clostridium difficile*, infecția ca factor precipitant al encefalopatiei hepatice și, deasemenea, o analiză a particularităților de efectuare în pandemie a investigațiilor endoscopice specifice pacienților cu afecțiuni gastroenterologice, necesare diagnosticului si terapiei. Ultima parte a acestui capitol este dedicat prezentării, în sinteză, a rezultatelor obținute din studiul particularităților de abordare a patologiei hepato-gastroenterologice la pacientul vârstnic – hemoragia digestivă, bolile inflamatorii, tratamentul antiviral al cirozei hepatice virale C.

Secțiunea a doua prezintă principalele direcții de dezvoltare viitoare a activității didactice, de cercetare științifică și clinice. Pe plan didactic, dezideratul este de a implica, forma și crea colaborare fructuoasă cu studenții și medicii rezidenți aflați în formare. Intenționez să mă implic în redactarea unui îndrumar de practică în semiologie medicală și gastroenterologie pentru studenții secției cu predare în limba franceză. În sfera clinică, obiectivul este actualizarea și acumularea cât or mai multe cunoștințe de specialitate, dobândirea și practicarea de competențe specifice și corelate domeniului gastroenterologiei și hepatologiei. Intenționez sa asimilez și să efectuez tehnica enteroscopiei, eco-endoscopia și ecografia tubului digestiv. Din punctul de vedere al evoluției pe plan științific, în primul rând vor fi continuate studiile în domeniul explorării prin CE a tubului digestiv. În principal, urmăresc stabilirea unui scor comprehensiv de apreciere a activității bolii Crohn – și corelarea lui cu remisiunea pe termen lung, evaluarea bolilor inflamatorii intestinale neclasificate și, deasemenea, studierea rolului capsulei de colon în screeningul cancerului colo-rectal. Ca direcții noi de cercetare intenționez implementarea inteligenței artificiale - care ar putea recunoaște și clasifica leziuni. Găsirea însă a locului medicului explorator în sfera investigațiilor paraclinice (la acest moment asaltată și, poate, în viitor, dominată de inteligența artificială), este o provocare a cărei rezolvare depinde de noi, și în care doresc să îmi aduc contribuția. Intenționez să completez explorarea tubului digestiv cu noi metode (atât non-invazive, cât și invazive): ecografia de tub digestiv, enteroscopia, eco-endoscopia și, mai nou, endomicroscopia confocală laser - extrem de ofertante fiecare în parte dar, mai ales, printr-o combinare judicioasă și adaptată, și să sistematizez rezultatele comparative și cumulative în articole. În sfera hepatologiei, perspectivele de cercetare sunt reprezentate de studiul complicațiilor cirozei hepatice - analiza factorilor de risc pentru complicații și stratificarea prognosticului pacienților cirotici în corelație cu acestea și de urmărirea pacienților post-transplant hepatic, din prisma dinamicii anticorpilor anti-hepatita B și a steatozei dezvoltate la nivelul grefei hepatice.

Secțiunea a treia cuprinde referințele bibliografice corespunzătoare elaborării tezei de abilitare.

ABSTRACT OF THE THESIS

The elaboration of this habilitation thesis represents first of all an opportunity and, also, a duty to recapitulate and review the professional, academic and scientific achievements from the postdoctoral period. At the same time, along with the synthesis of the results so far, the future directions of development will be formulated, as a continuation of the preoccupations so far, and the extension in related areas.

The structure of the thesis follows the recommendations of the National Council for Attestation of University Degrees, Diplomas and Certificates (CNATDCU), being composed of three sections:

- Section I - Academic, professional and research activity achievements during my postdoctoral period;
- Section II - Development directions and future research projects;
- Section III - Bibliographical references.

The first section summarizes the academic, medical and scientific achievements, obtained during the 14 years of activity, up to the current position, of associate professor.

I highlighted the main elements of the clinical and academic activity, as well as the scientific achievements, presented in published papers and awards.

The recognition of my scientific activity is proven by:

- Hirsch index – 11

- 444 citations and a cumulative impact factor for the articles in which I am principal author of 24.927.

The presentation of the scientific activity is structured in three chapters, dedicated to the scientific achievements in the main area of interest – the exploration of the digestive tube by the endoscopic capsule method, but also in adjacent areas and related areas of concern, completing the approach of the patients in the complex domain of hepato-gastroenterology.

A current challenge in medicine is to find the ideal balance between invasive and non-invasive or minimally invasive methods. In the field of hepato-gastroenterology, these different types of methods can be beneficially combined, but they can also impose a rigorous choice, based on their competitiveness, advantages and/or limits or risks.

During my professional and research activity, I aimed to contribute through personal elements to this theme of the perfect balance between invasive and non-invasive or minimally invasive investigations.

The first chapter illustrates my preoccupations in the field of capsule endoscopy exploration of the gastrointestinal tract. Innovative, non-invasive method of visualizing the digestive tract, it was introduced relatively recently into clinical practice. The technique, advantages and limitations of the capsule endoscopy are presented for each of the three existing versions (esophagus, small bowel and colon). A comprehensive presentation of the small bowel capsule endoscopy is made, as a method currently recognized as the first line of investigation in suspected small bowel pathology. Moreover, the personal results from the direct application of this method into the clinical practice are presented: the clinical impact of the exploration by capsule endoscopy of obscure gastrointestinal bleeding, unusual causes of obscure digestive hemorrhage, visual morphopathological correlations of small intestine tumors and factors influencing the rate of small bowel visualization in Crohn's disease. The first chapter ends with the presentation of the results regarding the use of the endoscopic capsule in the colon exploration, as an alternative to colonoscopy in the diagnosis of colon neoplasia, as well as in the exploration of unclassified colonic inflammatory diseases.

The second chapter is dedicated to the presentation of personal contributions in the field of hepatology, a related area of interest, an intrinsic part of the holistic approach to the patient with digestive ptahology. The main areas of interest are: viral hepatitis screening - in this sense I will present the results of a study of prevalence and risk factors for chronic viral hepatitis C, antiviral treatment and post-therapy monitoring of patients - with an emphasis on tracking liver "remodeling" , documented by non-invasive explorations as computed tomography examination, and elastometry with the parameter of controlled attenuation of steatosis, as well as the study of non-alcoholic fatty liver disease, which has an increasing prevalence and significance through comorbidities and its evolutionary potential.

The third chapter highlights aspects of approaching to patients with hepato-gastrointestinal pathology in particular settings. The first study presents the results regarding the risk factors for extraintestinal manifestations of patients with inflammatory bowel diseases, while the following studies, in the special recent context of the SARS-CoV-2 pandemic, present: the pandemic impact on Clostridium difficile infection, SARS-CoV-2 infection as a precipitating factor of encephalopathy hepatic and, also, an analysis of the particularities of carrying out endoscopic investigations during the pandemic. The last part of this chapter is dedicated to the presentation, in summary, of the results obtained from the study of the particularities of approaching the the elderly patient - gastrointestinal bleeding, inflammatory bowel diseases, antiviral treatment of hepatitis C-related liver cirrhosis.

The second section presents the main directions for my future development in teaching, clinical activity and scientific research. In terms of teaching, , the aim is to involve, form and create fruitful collaboration with students and resident doctors in training. I intend to get involved in writing a practice guide in medical semiology and gastroenterology for the French section student. In the clinical field, the objective is to update and accumulate as much specialized knowledge as possible, to acquire and practice specific and correlated skills in the field of gastroenterology and hepatology. I intend to assimilate and perform the technique of enteroscopy, echo-endoscopy and ultrasound of the digestive tract. From the point of view of scientific development, studies in the field of capsule endoscopy exploration will be continued in the first place. Mainly, I aim to establish a comprehensive score to assess Crohn's disease activity – and correlate it with long-term remission, to study unclassified inflammatory bowel disease and, also, to analyze the role of the colon capsule in colorectal cancer screening. As new research directions, I intend to implement artificial intelligence - which could recognize and classify lesions. However, finding the place of the human physician in the context of paraclinical investigations (at this moment assaulted and, perhaps, in the future, dominated by the artificial intelligence), is a challenge whose solution I want to contribute to. I intend to complete the exploration of the digestive tract with new methods (both non-invasive and invasive): bowel ultrasound, enteroscopy, ultrasound endoscopy and, more recently, laser confocal endomicroscopy. In the field of hepatology, the research perspectives are represented by the study of complications of liver cirrhosis - analysis of risk factors for complications and stratification of the prognosis of cirrhotic patients, and follow-up of liver transplanted patients, in terms of anti-hepatitis B antibodies and liver graft steatosis.

The third section includes the bibliographic references corresponding to the elaboration of the habilitation thesis.

SECTION I
SYNOPSIS OF PERSONAL ACADEMIC, PROFESSIONAL, AND SCIENTIFIC ACCOMPLISHMENTS

A. ACADEMIC ACHIEVEMENTS

My academic carrier started in 2008, when I was admitted by competition as university assistant in the Discipline of Medical Semiology and Gastroenterology, within the First Medical Department – director Professor Anca Trifan, the Faculty of Medicine of the "Grigore T. Popa" University of Medicine and Pharmacy of Iasi. In 2011, I graduated the postgraduate psychopedagogy course.

Professor Carol Stanciu and professor Anca Trifan were my key role models, and under their mentorship I accomplished my activities and I have progressed through the years.

After passing further competitions, in 2014 I became a lecturer, and since 2019 I am an associate professor.

I have been teaching undergraduate students of the Faculty of Medicine, from the Romanian and also French program, medical semiology (3rd year) and gastroenterology (5th year), as well as Nursing students (4th year), care in gastroenterology. I am the first and only titular of gastroenterology course for the Vth year students in the French section. For 4 years, I was responsible for the optional course for the 4th year students, entitled "The twilight of the invasivity, the era of the non-invasivity and minimal invasivity". I participated as invited lecturer in the optional course "Digestive Endoscopy – talent, training, competence" dedicated to the 5th year students.

Apart from knowledge transfer to students during classes, I always encouraged and mentored their personal development. I guided students along exam preparation processes, and I also coordinated graduation thesis, both for the Romanian and French program students.

Regarding postgraduate education and teaching, I have been involved in training gastroenterology fellows. I would like to mention my contribution in the elaboration of didactic materials. I am a co-editor and co-author of several chapters of the gastroenterology syllabus dedicated to the 5th year students, released in 2020, edited by "Gr. T. Popa" University. I also contributed as co-author of several chapters in the "Clinical Gastroenterology and Hepatology Treaty", elaborated under the guidance of the leading board of the Romanian Society of Gastroenterology and Hepatology, and edited by the Medical Publishing House in 2020, which stays at the base of preparation for specialty exam. I was one of the edithors of the book "Renal connections in liver diseases", A. Covic, A. Trifan, M. Apetrii, AM. Singeap, published by "Gr. T. Popa" University, 2017. ISBN 978-606-544-494-2.

In the same time, I have been taking part as organizer, course responsible and speaker, in many educational sessions and programs dedicated to fellows and physicians in training, organized by the Institute of Gastroenterology and Hepatology and the "Grigore T. Popa" University of Medicine and Pharmacy. Several examples of such events are: Days of the Institute of Gastroenterology and Hepatology (2012, 2013), Summer School of Gastroenterology (2014, 2016-2021), Winter Workshop of Digestive Endoscopy, Gastroenterology and Hepatology (2020, 2021).

I attended all the specialty national symposiums and congresses, and I gave many lectures as invited speaker.

I strongly believe that teachers are key role models, who can influence the attitudes, values and behaviors not only of their students, but also of every person they interact with. Therefore, educational and social actions are equally important. Examples of personal contribution into the service of good are: taking part in the Vaccination Marathon organized by our university, in the context of SAR-COV-2 pandemics, participation in C hepatitis screening

program under the auspices of the Romanian Society of Gastroenterology and Hepatology, and disseminating medical advices towards the general population via reliable newspapers.

Beginning with January 2022, I have been selected as **member of the editorial board** of **e-WGN** – World Gastroenterology News, the journal of the World Gastroenterology Organization, for a 2-year period.

B. CLINICAL ACTIVITY

I graduated the Faculty of Medicine of „Grigore T. Popa" University of Medicine and Pharmacy of Iasi in 2000. Between 2002 and 2006 I was a fellow resident in Gastroenterology in Iasi University Center, under the coordination of Professor Anca Trifan. In 2007, I obtained the title of specialist in Gastroenterology.

Both in the latest period of my residency, and immediately after becoming a specialist gastroenterologist, I worked abroad, for two years in total: in Charleroi, Belgium – Hospital „Notre Dame et Reine Fabiola", for one year, and in two Gastroenterology units making part of the APHP (Professional Association of Paris Hospitals), France – Beaujon Hospital and Claude Bernard Bichat Hospital, 6 months in each. Working in European hospitals was both a professional opportunity, and a beneficial conjuncture that allowed me multicultural experiences and helped me improve multilingual communication.

In 2008 I started my activity as specialist gastroenterologist in „St. Spiridon" Clinical Emergency County Hospital in Iasi, within the Institute of Gastroenterology and Hepatology, and I have been working here ever since. In 2012 I received the title of senior gastroenterology specialist, through the exam organized under the aegis of the Ministry of Health.

My professional activity includes daily gastroenterology and hepatology consultations in the outpatient clinic, and continuous care of patients hospitalized in the clinical department of the Institute of Gastroenterology and Hepatology, during usual work hours or on duty. My clinical activity is completed by specialized paraclinical investigations: abdominal ultrasound, upper and lower digestive endoscopies – the specific training was completed during the residency, small bowel and colon capsule endoscopies – for which I attended special training programs, as well as therapeutic endoscopic procedures – with competence certificate owned since 2020. I am also part of the team performing emergency endoscopies within the Hospital Emergency Department.

I had the opportunity to take part in performing capsule endoscopy investigations from 2003, during my first years of training as fellow in gastroenterology. Professor Carol Stanciu and Professor Anca Trifan has the merits of implementing at Iasi, in national premiere, the endoscopic capsule system. Ever since, I accumulated experience, I attended training courses and I extended my CE activities. I directly performed hundreds of CE investigations, and due to the non-invasive nature of this investigation and thanks to its proven utility, over time the addressability towards our unit has increased. As tertiary referral center, the Institute of Gastroenterology and Hepatology is managing nowadays patients all over the North-Eastern region of Romania and also from other centers all over the country.

Liver transplantation is another field of interest. Liver cirrhosis is a frequent advanced disease, and in many cases liver transplantation remains the only treatment. I attended the training program entitled „Developing competences in the transplantation" in 2015, POSDRU project no. 186/3.2/S/155295**.** In 2016, the Regional Center of Liver Transplantation was founded in Iasi, an ever since it has been the second liver transplantation center in the country. Under the supervision of the medical team leader, professor Anca Trifan, and together with the others physicians involved, I acted both in the process of pretransplant evaluation and, especially, in the care of patients after liver transplantation. I am a prescribing physician for immunosuppressive therapy, and I am the local coordinator of our center as part of the National Program for the Prevention of the B Hepatitis Relapse in Transplanted Patients.

I have been actively involved in **HepC Alert**, a national screening program for virus C hepatitis, organized by the Romanian Society of Gastroenterology and Hepatology and the Romanian Association for Liver Diseases, in 2019, and I enrolled in training programs for managing the B and C hepatitis (HEPATER, part of European Social Fund, POCU 91/4/8/107931, 2018).

Currently, I am involved in **LIVE-RO** program, an ample national screening program for B and C hepatitis, part of European Social Fund (beneficiary – Clinical Institute Fundeni, partner – "St. Spiridon" Emergency County Hospital), POCU 308/4/9/120640, during the interval 2018-2023, where I accessed and obtained by competition a grant member position. I truly hope bringing my contribution to the success of this programme, whose goal is to aliniate Romanian efforts to the principal objective of World Health Organization regarding eradication of viral hepatitis by 2030.

I am part, as member, of several professional societies: Romanian Society of Gastroenterology and Hepatology (where I detained the function of general secretary between 2017-2019), Romanian Society of Digestive Endoscopy, European Crohn's and Colitis Organization, International Society of Liver Transplantation.

C. SCIENTIFIC ACHIEVEMENTS

Scientific research is essential for medical progress. Clinical practice is based on scientific realities, and practitioners are the beneficiary of the scientific results.

I am extremely grateful to my mentors, role models who influenced my formation, and exceptional clinicians.

In 2008, I was admitted by a contest to the Doctoral School within the University of Medicine and Pharmacy "Gr. T. Popa" of Iași. Under the guidance of Professor Anca Trifan, the project of the thesis, entitled „The actual and the potential role of capsule endoscopy in the exploration of gastrointestinal tract" was submitted one year later, and in 2011 I the Ph.D. thesis was finalized, obtaining the Ph.D. degree in medicine.

I had the huge opportunity to be mentored by Professor Carol Stanciu and Professor Anca Trifan, distinguished and prominent professionals. By their merits, Iasi was the first gastroenterology center in Romania where capsule endoscopy was performed, after only two years after its mondial debut.

Capsule endoscopy was my first research direction, and one of my first published papers, "Outcomes after symptomatic capsule retention in suspected small bowel obstruction" (Singeap et al., *Eur J Gastroenterol Hepatol,* 2011) was cited in journals with huge impact factor, such as *Gastroenterology* (IF 20) and Clinical Gastroenterology and Hepatology (IF 7).

Over the years, I have continued to focus on this exploration, analyzing the diagnostic yield, diverse predictive factors for diagnostic, and its impact on the patients'outcome. The experienced accumulated in this field resulted in book chapters („Small bowel stromal tumors: approach by capsule endoscopy", Anca Trifan, Ana-Maria Singeap and Carol Stanciu, in NEW TECHNIQUES IN GASTROINTESTINAL ENDOSCOPY, Edited by Oliviu Pascu and Andrada Seicean, ISBN 978-953-307-777-2, 310 pages, Publisher: InTech, Published: October 03, 2011), communications in scientific congresses and many published papers in the national and the international flux, both review type and original articles, presenting results from the all aspects of capsule endoscopy exploration ("Clinical Impact of Small Bowel Capsule Endoscopy in Obscure Gastrointestinal Bleeding"*, Medicina (Kaunas), 2020*; „Visual criteria in small bowel tumors detected by capsule endoscopy - morphological description and correlations with histological type", *Rom J Morphol Embryol.* 2019, Singeap AM, Cojocariu C, Girleanu I, Huiban L, Sfarti C, Cuciureanu T, Chiriac S, Stanciu C, Trifan A. Singeap AM, Trifan A, Sfarti C, Chiriac Ş, Huiban L, Stanciu C, Danciu M.; „Capsule Endoscopy in Inflammatory Bowel Disease: Current Applications", *Archives of Iranian Medicine*. 2015,

Singeap AM, Stanciu C, Cojocariu C, Sfarti C, Trifan A; Outcomes after symptomatic capsule retention in suspected small bowel obstruction. *Eur J Gastroenterol Hepatol*. 2011, Singeap AM, Trifan A, Cojocariu C, Sfarti C, Stanciu C).

Another domain of interest is represented by the liver diseases. One major issue is represented by C hepatitis, public health problem. In the last years, antiviral treatment was revolutionized, and World Health Organization established the goal of C virus global eradication until 2030. Recently, I have co-authored the paper entitled "An update on direct antiviral agents for the treatment of hepatitis C", *Expert Opin Pharmacother*, 2021, by Stanciu C, Muzica CM, Girleanu I, Cojocariu C, Sfarti C, Singeap AM, Huiban L, Chiriac S, Cuciureanu T, Trifan A. Clinical experience with C hepatitis patients allowed me to participate in multicenter studies and elaborate original articles, as "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", by Trifan A, Stanciu C, Gheorghe L, Iacob S, Curescu M, Cijevschi Prelipcean C,Stefanescu G, Girleanu I, Chiriac S, Mihai C, Brisc C, Goldis A, Sporea I, Miftode E, Bataga S, Rogoveanu I, Preda C, Caruntu FA, Singeap AM, *Medicine (Baltimore),* 2017. Another field of interest is the liver cirrhosis, with its complications and particular follow-up depending on etiology and functional status. Thus, an important paper on the theme of hepatocellular carcinoma after viral C eradication was the review "Hepatocellular carcinoma after direct-acting antiviral hepatitis C virus therapy: A debate near the end", *World J Gastroenterol*, 2020, by Muzica CM, Stanciu C, Huiban L, Singeap AM, Sfarti C, Zenovia S, Cojocariu C, Trifan A. Follow-up of liver disease patients often needs interdisciplinary collaboration, and the results from CT imaging studies were published in the form of several articles, such as: „L3 Skeletal Muscle Index Dynamics in Patients with HCV-Related Compensated Cirrhosis Following Sustained Virological Response after Direct Acting Antiviral Treatment", *Medicina (Kaunas)* 2021, Mihai F, Trifan A, Stanciu C, Huiban L, Muzîca C, Lupașcu-Ursulescu C, Negru D, Savin ML, Gîrleanu I, Cuciureanu T, Sîngeap AM, and „Liver Remodeling on CT Examination in Patients with HCV Compensated Cirrhosis Who Achieved Sustained Virological Response after Direct-Acting Antivirals Treatment". *Medicina (Kaunas) 2020,* Mihai F, Trifan A, Stanciu C, Singeap AM, Cucuteanu B, Lupascu Ursulescu C, Pop C, Girleanu I, Cuciureanu T, Negru D, Cojocariu C. And last, but not least, my preoccupations have been recently directed towards non-alcoholic fatty liver disease, a modern disease, with important evolutive potential. My contribution in this field is represented by the co-authorship of a recently published paper, "Vibration-Controlled Transient Elastography and Controlled Attenuation Parameter for the Diagnosis of Liver Steatosis and Fibrosis in Patients with Nonalcoholic Fatty Liver Disease", *Diagnostics* 2021, by Zenovia et al.; another paper in the domain of liver steatosis is „Association between Nonalcoholic Fatty Liver Disease and Endocrinopathies: Clinical Implications", *Can J Gastroenterol Hepatol.* 2021, by Singeap AM, Stanciu C, Huiban L, Muzica CM, Cuciureanu T, Girleanu I, Chiriac S, Zenovia S, Nastasa R, Sfarti C, Cojocariu C, Trifan A.

Because patients in hepatogastroenterology are complex, and they present diverse and sometimes combined pathologies, I also approached along the years other spheres, as: inflammatory bowel disease, Clostridium difficile infection, pancreatic pathology, endoscopy during SARS-COV-2 pandemics. Consequently, part of my contribution as papers and book chapters reflects these themes. A worth mentioning paper is „Proton pump inhibitors and risk of Clostridium difficile infection: Systematic review and meta-analysis", by Trifan A, Stanciu C, Girleanu I, Stoica OC, Singeap AM, Maxim R, Chiriac SA, Ciobica A, Boiculese L., *World J Gastroenterol* 2017, which has been already cited 104 times.

In the same time, the elderly remain a major preoccupation, and consequently many papers are dedicated to the management of this particular patients.

I have been a grant member in **IBS NEURO,** a research project, funded by Executive Unit for the Financing of High Education, Research, Development and Innovation (UEFISCDI), gained by "Al. I. Cuza" University of Iasi, between 2018-2020.

I also am part of guidance committee of several PhD students' thesis.

The recognition of the value of scientific activity is proven by the list of published papers, including 42 ISI Thomson Reuters indexed papers (12 as main author, 30 as co-author) and over 40 IDB indexed papers. My **international visibility** is reflected by a **Hirsch Index of 11** and **444 citations** in Clarivate Analytics. My cumulative impact factor for the articles in which I am principal author is 24.927.

Over the years, many abstracts I presented in scientific manifestations were awarded by international scientific societies.

AWARDS OF INTERNATIONAL SCIENTIFIC SOCIETIES

Falk Foundation *1st Poster Award*	Stafie R, Stanciu C, Rotaru A, Zenovia S, Stratina E, Nastasa R, **Singeap AM**, Cojocariu C, Sfarti C, Girleanu I, Chiriac S, Cuciureanu T, Huiban L, Muzica C, Trifan A. Assessing the risk for non-alcoholic fatty liver disease development among patients with ulcerative colitis. Falk Foundation Symposium 228, 7-10 July 2022, Amsterdam
United European Gastroenterology *Poster of Excellence*	Mihai F, **Singeap AM**, Sfarti C, Negru D, Stanciu C, Trifan A. "The potential of right hepatic vein diameter as sensitive marker for liver remodeling in patients with virus c related cirrhosis who have achieved sustained virologic response after the administration of direct-acting antivirals treatment". United European Gastroenterology Week, October 2019, Barcelona
AASLD *Poster of distinction*	Chiriac Ş, Stanciu C, **Singeap AM**, Sfarti C, Cojocariu C, Gîrleanu I, Cuciureanu T, Stoica O, Huiban L, Muzica CM, Livadariu RM, Trifan A. Relative adrenal insufficiency in the setting of acute on chronic liver failure - an organ dysfunction that could indicate futility. Digestive Disease Week, 2-5 June 2018, Washington
AASLD/EASL *Travel Grant*	**Singeap AM**, Trifan A, Cojocariu C, Sfarti C, Girleanu I, Stanciu C. „Current needs and actual eligibility for antiviral treatment of chronically HCV infected patients in North-East region of Romania". AASLD/EASL Special Conference on Hepatitis C; 12-13 September 2014, New York, SUA
United European Gastroenterology *Oral Free Paper Prize*	Girleanu I, Trifan A, Stoica OC, Cojocariu C, **Singeap AM**, Sfarti C, Baluta C, Stanciu C. Terlipressin-induced hyponatremia in cirrhotic patients with variceal bleeding. United European Gastroenterology Week, October 25-28, 2015, Barcelona
United European Gastroenterology *Poster of excellence*	Girleanu I, Trifan A, Cojocariu C, **Singeap AM**, Rohozneanu A, Chiriac S, Stanciu C. The effect of non-selective beta-blockers on survival in decompensated liver cirrhosis patients. United Eur Gastroenterol J. 2015; 3 (5S): A337, October 25-28, 2015, Barcelona
United European Gastroenterology *Poster of Excellence*	**Singeap AM,** Cojocariu C, Sfarti C, Stanciu C, Trifan A. Clinical benefit and protective role against acute diverticulitis of non-absorbable antibiotics with cyclic administration in diverticular colonic disease. United European Gastroenterology Week, October 2013, Berlin, Germany
Italian Society for Hemostasis and Thrombosis *Young Researcher Award*	Girleanu I, Trifan A, Cojocariu C, Dimache M, **Singeap AM**, Stoica O, Stanciu C. Natural course of de novo partial portal vein thrombosis in patients with cirrhosis. 5th International Congress on Coagulopathy in Liver Disease, 27-28 September 2013, Padova, Italy

Chapter 1
NEW FRONTIERS IN THE EXPLORATION OF THE GASTROINTESTINAL TRACT: CAPSULE ENDOSCOPY – COULD IT BE THE IDEAL SOLUTION?

I.1.1. INTRODUCTION

Launched more than 20 years ago, capsule endoscopy (CE) has become the first-line examination in suspected small-bowel pathologies. Over the last years, many studies analyzed its performance and confirmed its value. Obscure gastrointestinal bleeding, unexplained iron deficiency anemia, inflammatory bowel diseases and small bowel tumors are the domains where small bowel capsule endoscopy revolutionized the diagnostic workup. Technical progresses were made, and along the years, esophageal and colon capsule appeared.

CE in clinical practice – current status

The introduction of the capsule endoscopy (CE) in clinical practice revolutionized noninvasive, directly visualization of small bowel, considered until then the "black box" of the gastrointestinal (GI) tract. Launched in 2000 (Iddan et al., 2000), approved for clinical use in United States and Europe in 2001 (Nakamura and Terano, 2008) and recognized in 2003 by the US Food and Drug Administration (FDA) as first-line examination for the small bowel (Gay et al., 2004), CE has been extensively used during the past years. Technical progress led to the introduction of some updated versions (second- and third- generations) of CE for the small bowel and the manufacturing of the CE designed for esophagus and colon.

Indications for small bowel CE have extended widely and are being continuously diversified. The most common indications for small bowel CE are obscure GI bleeding (OGIB) and unexplained iron deficiency anemia (IDA), suspected or known CD, suspected small intestine tumors, diagnosis as well as surveillance in patients with polyposis syndromes, and refractory malabsorptive syndromes (as celiac disease) (Neumann et al., 2014; Khan et al., 2013; Fisher and Hasler, 2012). CE has also been used in several clinical conditions such as irritable bowel syndrome, nonsteroidal anti-inflammatory drug (NSAID)-enteropathy, protein-loosing enteropathy, small bowel transplantation, and primitive intestinal lymphangiectasia (Liao et al., 2010).

The ***esophagus CE*** is used in patients unable or unwilling to undergo conventional upper GI endoscopy for suspected Barrett's esophagus, esophagitis, or esophageal varices (Ishiguro et al., 2012, Bhardwaj et al., 2009;), while ***colon CE*** is used for colon polyp screening, as well as an alternative for incomplete colonoscopy or patients unwilling to undergo colonoscopy (Eliakim et al., 2009).

CE contraindications include patients with dysphagia or swallowing disorder, known or suspected Zenker's diverticulum, gastrointestinal obstruction, strictures, fistulas, pregnancy, gastroparesis, prior major abdominal surgery of the gastrointestinal tract, and those with cardiac pacemakers or other implanted electromedical devices, although recent evidence suggests that CE can be safely used in such patients under appropriate monitoring (Harris et al., 2013). Generally, CE is a safe procedure, with very low complication rates. Global incidence of capsule retention is 1%-2%, but there are large variations according to clinical indication for CE investigation and the selected population (Singeap et al., 2011; Liao et al., 2010; Li et al., 2008). In healthy volunteers, retention rate was null. Capsule retention was 1.6% in patients suspected with CD, compared with 13%, in those known with this disease (Cheifetz et al., 2006). Liao et al. (2010), in a systematic review of English-language published studies regarding indications of small bowel CE have reported retention rates of 1.2%, 2.1%, and 2.6% for OGIB, tumors, and CD, respectively. In patients with high risk of retention many

authors recommend a prior imaging examination to exclude stenosis (Agile patency capsule, CT-enteroclysis).

Limitations of CE and future expectations

CE is not an ideal tool, as it has several limitations. CE remains a purely visual technique with no ability to obtain biopsy specimens or perform therapeutic maneuvers. The most obvious drawback is the operator's inability to control its locomotion through GI tract. The capsules presently on the market are unable to localize or mark the location of detected lesions. Visualization may be impaired by the presence of food materials or bubbles and, in contrast with conventional endoscopy, CE cannot perform flushing, suctioning, or air insufflation to obtain better images. All capsules for clinical use are powered by limited-life batteries which may be depleted before the examination is complete. The rate of missed lesions is still high for those located in the duodenum and proximal jejunum, where the transit is more rapid than in the distal segment of the small bowel. Reading time for interpretation is another shortcoming of CE, as it takes more than 1 h to read a full 8-h examination. Finally, the costs are still high.

Overcoming these limitations and developing a new generation of CE with diagnostic and therapeutic capabilities is the objective of many research groups worldwide. Development of the artificial intelligence is a premise of CE progresses and its implementation into the clinical CE practice aims to revolutionize reading, images interpretation and lesions classification. Based on the tremendous running progresses of modern technology, it is expected that in the near future CE will be one of the major forms of digestive endoscopy, covering the entire GI tract from mouth to anus as its inventors have dreamed**.**

I.1.2. THE DIAGNOSTIC PERFORMANCE OF CAPSULE ENDOSCOPY IN SMALL BOWEL PATHOLOGY – BETWEEN REVELATIONS AND UNANSWERED INQUIRIES

I.1.2.1. Introduction

Obscure gastrointestinal bleeding, unexplained iron deficiency anemia, inflammatory bowel diseases and small bowel tumors are the domains where small bowel capsule endoscopy revolutionized the diagnostic workup.

Obscure gastrointestinal bleeding and unexplained iron deficiency anemia

Obscure gastrointestinal bleeding (OGIB) is defined as bleeding of unknown origin that persists or recurs after an initial negative endoscopic evaluation including upper endoscopy and ileocolonoscopy and represents approximately 5% of all GI tract bleeding (Lewis, 2007). OGIB is classified as either overt (melena or hematochezia) or occult (positive fecal occult blood test or persistent iron deficiency anemia). Iron deficiency anemia (IDA) occurs in 3-5% of adult subjects, and esogastroduodenoscopy (EGD) or ileocolonoscopy identifies the cause of bleeding in about approximately 75% (Fireman and Kopelman, 2004). CE revolutionized the evaluation of OGIB and IDA (Lewis, 2007; Kopylov and Seidman, 2013), becoming, in recent years, the first-line diagnostic modality for both situations. CE diagnostic yield (ratio of the number of positive result examinations to the number of all procedures) in OGIB ranges from 32% to 91% depending on various factors such as type of bleeding investigated, timing of examination, and definition of positive findings. Current guidelines of all international gastroenterology – endoscopy societies recommend CE as the first test in patients with OGIB, after negative EGD and ileocolonoscpy results. In a systematic review including 227 studies and 22,840 CE procedures, the diagnostic yield for OGIB was 61% (Koulaouzidis et al., 2013). Compared with radiographic barium studies, CE has an increased diagnostic yield of 25%-50% for detection of OGIB sources in the small bowel (Laine et al., 2010; Koulaouzidis et al., 2012; Uchida et al., 2021). A meta-analysis found a CE diagnostic yield of 42%, compared to only

6% for small bowel barium radiography in patients with OGIB (Triester et al., 2005). Moreover, CE achieves superior results in patients with OGIB/IDA when compared with more advanced radiographic technologies such as CT-enterography, CT-angiography and MR-enterography (Saperas et al., 2007; Wang et al., 2013). He et al. (2014) found a significantly higher diagnostic yield for CE than for a 64-slice multiphase CT enterography (68.7% and 47.6%, respectively; P=0.01). In a systematic review of 24 studies comprising 1960 patients, global diagnostic yield for CE in IDA was 47%; however, when only those patients with confirmed IDA by established levels for hemoglobin and ferritin were included, the diagnostic yield of CE was 67% (Koulaouzidis et al., 2012). The diagnostic yield of CE was significantly higher than that of angiography (53.3% and 20%, respectively) (Leung et al., 2012).

CE has repeatedly proven superior in detecting small bowel sources of bleeding to the old classical gold standard for OGIB, the intraoperative enteroscopy (Hartmann et al., 2005), or to push enteroscopy (de Leusse et al., 2007).

When CE was compared to double-balloon enteroscopy (DBE), a similar diagnostic accuracy for OGIB was reported (Pasha et al., 2008). In a meta-analysis, CE had a higher yield than DBE using a single approach, but lower yield than DBE using a combined antegrade-retrograde approach (Chen et al., 2007). However, due to its excellent tolerability and safety profile, and being more likely to achieve total small bowel enteroscopy, CE should be the diagnostic procedure of first choice, while DBE should be reserved for therapeutic purpose only (Arakawa et al., 2009). CE may also help to select DBE insertion routes.

Timing of CE examination is associated to diagnostic yield, patients with on-going bleeding having the highest yield. In one study, CE diagnostic yield was 92% in patients with on-going obscure overt bleeding, compared to 44.2 % in patients with obscure occult bleeding, and 13% in those with previous obscure occult bleeding which stopped (Pennazio et al., 2004).

Several studies showed that CE had a significant clinical impact on patient management and outcomes for OGIB. For these patients, the objectives are to stop the bleeding or resolve anemia, diminish the necessity for transfusions, as well as reduce costs related to hospitalizations and supplementary diagnoses, and improve quality of life. CE leads to therapeutic endoscopic or surgical interventions and, consequently, to bleeding being stopped and improved outcomes. CE helps direct further therapeutic interventions or change medical therapy in 37-87% of patients (van Tuyl et al., 2007; Arakawa et al., 2009). CE also helped in localizing bleeding sites prior to intraoperative enteroscopy or surgical resection. One study reported that 51% of their patients which had a definite diagnosis on CE had a change in management, as well as in medication, undergoing an endoscopic procedure and surgery (van Tuyl et al., 2007). Other studies have reported a positive impact of CE on OGIB patients' outcome (Cañas-Ventura et al., 2013).

In our studies, SBCE has proved good performance parameters for OGIB. In an analysis of 341 SBCE examinations performed in a three-year period, OGIB was the main indication (224 examinations, corresponding to almost two thirds of the total number of cases). Analysing the examinations performed for OGIB, we showed that SBCE had an overall DY of 62%, higher in cases of overt OGIB (75%) and lower for unexplained IDA (37%). In the same time, we showed a positive clinical impact on the management of patients with OGIB. The main diagnosis were: SB angioectasias, and no further bleeding and/or correction of anemia were noted in 86 out of 92 cases, after individualized intervention; Crohn's disease - specific medical treatment was applied for all the 13 cases, with resolution of bleeding and anemia in 12 cases; 19 cases of SBT which were referred for surgical cure, solving the bleeding and the anemia; other causes were SB lymphoma (persistence of anemia), NSAID enteritis, radiation enteritis (Singeap et al., 2020). We showed that, regardless of diagnosis, the SBCE orientates the subsequent management and has a favorable clinical impact in terms of immediate and long-term outcome. Additional investigations may be needed for the final diagnosis, especially

in the case of infrequent lesions. Uncommon causes may be revealed by SBCE, and we published the case of a patient with jejunal ulcerated lipoma complicated by obscure bleeding and secondary IDA, diagnosed by SBCE, which had surgical cure (Cuciureanu et al., 2016).

Several studies have shown that CE repeated in the setting of one negative CE for OGIB gave positive findings ranging from 40% to 75% upon a second CE; a second such examination is indicated in patients with a previous nondiagnostic CE where bleeding presentation changed from occult to overt (Viazis et al., 2009).

Inflammatory bowel diseases

Crohn's disease

In the absence of a single diagnostic test, Crohn's disease (CD) diagnosis is still based on a combination of clinical, biological, radiological, endoscopic and histologic findings. Traditional ileocolonoscopy has been the most important method for diagnosis and surveillance of CD, with the disadvantage that it does not make the diagnosis if the disease is localized to more proximal segments of small bowel. Thanks to its capacity to directly visualize mucosa of the entire small bowel, CE has undoubtedly contributed to substantial progress in diagnosis, therapeutic decision, and outcome of CD patient. CE diagnostic advantage in patients with CD, apart of exploring the whole small bowel, is also based on the characteristic discontinuity of mucosal lesions (severely affected bowel segments are separated by "skip areas" of apparently normal bowel), as well as in visualization of incipient lesions.

Diagnostic criteria for CD on the basis of lesions described only by CE are still subject to a validation. An accepted set of diagnostic criteria for CD, proposed in 2004 (Mow et al., 2004), consists in the presence of more than three small bowel ulcerations detected by CE in the absence of NSAIDs use. An alternative suggestion came from Voderholzer et al. (2005) who claimed that a minimum of ten aphtoid lesions are suggestive for CD diagnosis. In order to objectively evaluate and quantify the severity and extent of small bowel lesions seen on CE in CD, several diagnostic scores have been proposed, as Lewis score (Gralnek et al., 2008), incorporated into the RAPID® software (Given® Imaging Ltd., Yoqneam, Israel) or a new scoring system called the Niv or CECDAI (Capsule Endoscopy Crohn's Disease Activity Index) which has been validated by a multicentric study (Niv et al., 2012).

Suspected CD. CE usefulness has been proved especially in patients suspected of CD, with negative ileocolonoscopy and/or radiological investigations. An international OMED (*Organisation Mondiale d'Endoscopie Digestive*) - ECCO (*European Crohn's and Colitis Organisation*) consensus stated that CE is able to identify lesions compatible with CD in patients where other diagnostic modalities have been non-diagnostic (Bourreille et al., 2009). Reviews of existent literature on CE diagnostic yield for both suspected and known small bowel CD show it to be superior to other diagnostic techniques such as small bowel follow-through (SBFT), enteroclysis, push-enteroscopy, ileo-colonoscopy, and CT-enterography (Dionisio et al., 2010; Leighton et al., 2014; Uchida et al., 2021). CE is superior to MR-enterography at identifying small bowel mucosal lesions, while MR-enterography is superior to CE at diagnosing mural and extra-enteric lesions (Crook et al., 2009). Jensen et al. (2011) evaluated prospectively the diagnostic accuracy of CE, CT-enterography and MR-enterography in comparation to ileocolonoscopy in 95 patients with suspected CD and found that sensitivity and specificity were 100% and 91% by CE, 81% and 86% by MR-enterography, and 76% and 85% by CT-enterography, respectively. Proximal small bowel CD was detected by CE in a significant greater number of patients compared to MR-enterography and CT-enterography (18 vs 2 and 6, respectively; $P<0.05$). A prospective, international, multicenter, blinded study reported that CE performed before ilecolonoscopy has a diagnostic yield higher that SBFT and equivalent to ileocolonoscopy in patients with suspected small bowel CD (Leighton et al., 2014).

Known CD. Mucosal healing, understood as the absence of inflammation at endoscopy, is considered to be a predictive factor for favourable long-term outcome, associated with low risk of complications and surgical interventions. Efthymiou et al. (2008) have evaluated mucosal healing with CE in patients with small bowel CD in a study without a validated scoring system for mucosal inflammation. More recently, in two prospective studies CE has proved useful for evaluating mucosal healing after immunomodulator or biologic therapy (Hall et al., 2014; Picco and Farraye, 2019).

There are conflicting results regarding the value of CE in detecting postoperative CD recurrence compared with ileocolonoscopy. Using the Rutgeerts endoscopic score, in one study CE had 62%-76% sensitivity and 90%-100% specificity in detecting postoperative recurrence compared with 90% and 100% with ileocolonoscopy (Bourreille et al., 2006), while according to another study, CE detected neo-terminal ileum recurrence in 62% of cases compared with 25% by ileocolonoscopy (Pons Beltrán et al., 2007). By consensus, in evaluating post-operative CD recurrence, CE is recommended as first choice investigation for patients with small bowel proximal resection in which surgical anastomosis is not accessible to colonoscopy and for those with ileal or ileo-cecal resection only when ileocolonoscopy is contraindicated, refused by the patient or impossible, while ileocolonoscopy should remain the procedure of choice in cases in which anastomosis is readily accessible at endoscopy (Swaminath et al., 2010).

Ulcerative colitis

Diagnosis of ulcerative colitis (UC) does not require CE. However, consensus statements recommend small bowel investigation in cases of UC refractory to medical treatment, prior to colectomy, as well as in cases of UC with unexplained anemia or abdominal pain. To date, only a handful of studies have evaluated colonic capsule for diagnosis and monitoring of UC, two of them reporting a significant correlation of findings (i.e., disease severity and extent) between colon capsule and colonoscopy (Ye et al., 2013; Hosoe et al., 2013). However, to date, colon capsule cannot replace conventional endoscopy in diagnostic and surveillance of patients with colonic IBD.

Unclassified IBD

At least 10% of colonic IBD patients remain unclassified as UC or CD based on colonoscopic and biopsy findings. CE seems to be a useful investigation in such patients, providing a more definitive diagnosis for reclassification of unclassified IBD (Kopylov et al., 2014).

Small bowel tumors

Small bowel tumors (SBTs) are rare, accounting for 1%-3% of primary GI tumors. Once CE became routine practice, they turned to be more frequent than previously estimated, amounting to 9%-12% (Rondonotti et al., 2008). Most SBTs were detected by CE when the procedure was carried out in patients with OGIB.

SBTs frequency on CE varies, according to selection criteria. In a large multicentric European study of 5129 patients undergoing CE, Rondonotti et al. (2008) found SBTs in 2.4% (one third being gastrointestinal stromal tumors) of the cohort, while in another study including 1,000 CE investigations, the frequency of SBTs was 1.6% (Pasha et al., 2008). From Korea, a multicentre study including 1332 CE examinations demonstrated tumors in 4%, of which half were dignosed by CE and missed by radiographic investigations (Cheung et al., 2010). Other studies, including less than 200 patients, reported SBTs in 5%-10% of cases (Trifan et al., 2010). CE and DBE are comparable for the diagnosis of SBTs, but DBE has the advantage of biopsy and therapeutic potential. Generally, SBT appearance on CE resembles mass lesions (polypoid in 70-80%) or, less frequently, ulcers or stenosis (20-30%), with no capacity of distinguishing between the types of tumors. Moreover, making the difference between a true mass lesion and a false positive lesion (as in extrinsic compression, mucosal bulge, and intussusception) is often difficult. Images provided by CE cannot be manipulated in terms of

angle, recording durations or revisualization of the same lesion in a different moment of peristalsis. These impediments increase difficulty in assessing the nature of a "mass" type of lesion pointed out by CE.

CE is also useful as a screening and surveillance tool for inherited gastrointestinal polyposis syndromes such as Peutz-Jeghers syndrome or familial adenomatous polyposis (FAP). Several studies have shown that CE is superior to other imaging modalities for the diagnosis of small bowel polyps. In one study CE was more accurate than MR-enterography to diagnose intestinal polyps smaller than 15 mm, while identification of large polyps (>15 mm) was similar between CE and MR-enterography (Gupta et al., 2010). CE has proven to be accurate for the detection of polyps especially in the medium and distal small bowel, but cannot determine with precision their size or localisation. CE does not have the same reliability in detecting polyps in the periampullary region, its sensitivity for this location being inferior to that of push enteroscopy or DBE. Another study (Koulaouzidis and Plevris, 2012) evaluated the detection rate of the ampulla of Vater during CE examinations and found a low detection rate regardless of SBCE systems used (10.7% with PillCam SB 1, 8.8% with PillCam SB 2; 8.6% with MiroCam). The authors concluded that if ampulla of Vater is taken as a surrogate marker of small polyp detection, SBCE cannot replace side-viewing standard endoscope in the evaluation of periampulary polyps in FAP and that it is an infallible technique in other small bowel polyposis states. However, CapsoCam SV1 with a 360° panoramic view, pointed out the papilla in 71% of cases, in comparison with 10-44% of cases investigated with conventional CE (Friedrich et al., 2013).

In a retrospective analysis of 302 SBCE examinations performed in our center, we found a SBT frequency of 5.2% (Singeap et al., 2019); the main indications for SBCE were overt OGIB (43%), unexplained IDA with occult OGIB (36%), and isolated unexplained abdominal pain. The main challenge in diagnosing SBT by SBCE is providing characteristic features to accurately classify the lesion. SBCE is a visual technique and lacks the capability of taking biopsies. However, specific features may correlate with the histological type. In the above-mentioned study, we described and analyzed the SBT regarding the type – they all were protruding lesions, either single or multiple; the size, the shape, the color, the presence of the ulcerated surface, the active bleeding or the presence of stigmata of recent bleeding. Further investigations were performed and final diagnosis was provided in 14 out of the 16 SBT seen by the SBCE. The most frequent histological type was represented by the stromal tumors, followed by adenocarcinomas and neuroendocrine tumors. To note, an additional peculiar feature of large-sized stromal tumors is the presence of a "hidden" area, where a potentially bleeding ulcer may exist.

As a result of the personal experience in the diagnosis of SB stromal tumors by CE, I would like to mention as personal contribution the chapter "Small bowel stromal tumors: approach by capsule endoscopy" (authors Anca Trifan, Ana-Maria Singeap and Carol Stanciu), published in "New techniques in gastrointestinal endoscopy", edited by Oliviu Pascu and Andrada Seicean, Publisher: InTech, 2011.

Other clinical applications are screening and surveillance tool for inherited *gastrointestinal polyposis syndromes* such as Peutz-Jeghers syndrome or familial adenomatous polyposis (FAP), *celiac disease* - particularly in patients with antibody negative-villous atrophy, and in monitoring complications of refractory celiac disease such as ulcerative jejunoileitis and small bowel tumors (Culliford et al., 2005), *NSAIDs-enteropathy,* a*cute upper gastrointestinal bleeding (UGIB)* - CE being proposed for risk stratification in acute UGIB, aiming to identify patients which do not require hospitalization and can be investigated in outpatient clinics (Chandran et al., 2013), *chronic abdominal pain* – but no clinical benefit of such examination was confirmed (Xue et al., 2015).

PERSONAL CONTRIBUTIONS RELATED TO THE ROLE OF SMALL BOWEL CAPSULE ENDOSCOPY

	ARTICLES
1.	**Singeap AM**, Cojocariu C, Girleanu I, Huiban L, Sfarti C, Cuciureanu T, Chiriac S, Stanciu C, Trifan A. Clinical Impact of Small Bowel Capsule Endoscopy in Obscure Gastrointestinal Bleeding. *Medicina (Kaunas).* 2020;56(10):548. **IF = 2.43**
2.	**Singeap AM**, Trifan A, Sfarti C, Chiriac Ş, Huiban L, Stanciu C, Danciu M. Visual criteria in small bowel tumors detected by capsule endoscopy - morphological description and correlations with histological type. *Rom J Morphol Embryol*. 2019;60(1):159-165. **IF = 1.411**
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I.1.2.2. Clinical impact of small bowel capsule endoscopy in obscure gastrointestinal bleeding

Background and aim. The advent of small bowel capsule endoscopy (SBCE) has revolutionized non-invasive direct visualization of the small bowel, considered until then the “black box” of the gastrointestinal (GI) tract. The most frequent indication for SBCE remains OGIB, either overt or occult. Overt OGIB is represented by clinically manifest hematochezia or melena with no cause identified after upper and lower endoscopy, whereas the current understanding of occult bleeding consists in either a positive fecal occult blood test or chronic iron deficiency anemia (IDA). SBCE has become the first-line investigation in the diagnosis of both OGIB and IDA (Pennazio et al., 2015), having an important role clinical decision and patient’s evolution. We aimed to evaluate the diagnostic yield (DY) of SBCE in overt and occult OGIB, as the etiological spectrum of OGIB and, mainly, the impact of SBCE results on clinical management and outcome.

Materials and methods. Our study retrospectively included all cases of OGIB investigated by SBCE in a tertiary referral center between 1st January 2016 and 31st December 2018. The entire diagnostic work-up was conducted following the current guidelines for the use of SBCE. OGIB was defined by overt or occult gastrointestinal bleeding, with negative upper endoscopy and colonoscopy. Occult gastrointestinal bleeding was either proved by a fecal test or presumptively incriminated as a cause for IDA (defined as hemoglobin <13 g/dL in men and <12 g/dL in women). Anamnesis was rigorously conducted in order to identify other potential explanations for IDA (gynecological causes for women) or risk factors for bleeding (associate pathology, risk from ongoing medications such as NSAIDs, anticoagulants and antiplatelet agents). Before SBCE, celiac disease was excluded for all patients with unexplained IDA. In some cases of overt bleeding, a second-look endoscopy was performed in order to reassure the true negative result of the first exam. A second-look colonoscopy was also performed in selected cases, the reasons for repeating the investigations being the incomplete preparation and/or the lack of evidence that the prior exam was complete. This analysis included only patients with isolated IDA, with no other symptoms or signs that could have suggested inflammatory bowel disease in the first place. SBCE exams were performed after contraindications (mainly suspicion of intestinal obstruction) were excluded and after the patient signed the informed consent, which contained all information needed for fully understanding the procedure and the potential complications, retention risk included. Second- and third-generation endoscopic capsules for small bowel examination (PillCam SB2 and PillCam SB3) manufactured by Given Imaging, Yoqneam, Israel were used. Examinations were performed after overnight fasting for 12 h. For overt OGIB, all SBCE were performed within 14 days of a bleeding episode. They were all performed in an inpatient setting, and the results were interpreted by a single trained specialist. Patients were allowed to drink clear fluid 2 h after the capsule was swallowed and a light lunch 4 h later. Evaluation of the relevance of

the lesions was made according to Saurin classification (Saurin et al., 2003). Bleeding lesions and potentially bleeding lesions, as well as fresh blood in the lumen, were considered diagnostic findings. Diagnostic yields for overt and occult bleeding were assessed and compared. Following SBCE results, individual therapeutic decisions were made, and follow-up data were recorded. The observational interval ranged between 18 months for the procedures performed by the end of the study period and 54 months for the procedures performed at the beginning of the study period.

Statistical analysis was carried out using the SPSS 17.0 software (SPSS Inc., Chicago, IL, USA). Continuous variables with normal distribution are expressed as mean ± SD, non-normal variables are expressed as median and interquartile range. Categorical variables are expressed as absolute values and percentages. Univariate analysis was performed for each recorded variable, and variables with a p-value < 0.1 were included in multivariate analysis (logistic regression) in all patients to identify the predictive factors of positive diagnosis by SBCE. Odds ratio (OR) with 95% confidence interval (CI) was calculated (for qualitative variables included in the logistic regression). A p-value of less than 0.05 was considered statistically significant.

Results

Patients, indications and prior work-up. There were 224 SBCE examinations for OGIB, representing almost two thirds (65.6%) of the total number of SBCEs performed in the specified period (341 for all indications). The median patient age was 53 years old, and there were more females (64.2%). One hundred and fifty patients (67%) were older than 50 years. One hundred and four patients (46.4%) were on oral anticoagulant treatment (vitamin K antagonists, with international normalized ratio (INR) within therapeutic range, or non-vitamin K oral anticoagulants), or antiplatelet treatment (like aspirin, clopidogrel, ticagrelor). The most frequent associated conditions requiring risk medication were cardiovascular and/or neurological pathologies; other comorbidities were respiratory, renal, liver or rheumatological disease. The mean hemoglobin level was 9.8 ± 1.8 g/dL (range 4.8–11.7) and 60 patients (26.7%) had received blood transfusion (Table 1.I). One hundred and forty-eight patients were investigated for overt OGIB, and 76 for unexplained IDA, either with proved occult bleeding by fecal test (22 patients), or with suspected occult gastrointestinal bleeding with negative or not available fecal test (54 patients). The 148 patients with overt OGIB initially presented with either melena or hematochezia. They were either admitted in emergency settings or referred after primary management by other departments or centers, mainly of secondary care. They were all investigated after hemodynamic stabilization by upper gastrointestinal endoscopy and colonoscopy, with no evidence of potentially bleeding lesions. Thirty-two of them had also a second-look upper endoscopy, justified by the suboptimal examination in emergency conditions; none of the second examinations came with a positive diagnosis. Ten patients had also a second-look colonoscopy, mainly justified by incomplete preparation during the first examination, and no lesions were found. The SBCE completion rate, defined as visualization of the cecum, was 95%.

Findings and diagnostic yield. Significant findings, summarized in Table 1.II, were found in 139 cases out of all 224 examinations for OGIB, resulting in an overall DY of 62%, higher in overt OGIB (75%) than in occult OGIB (37%). By far, the most frequent findings were small bowel angioectasias (62.2% of patients with overt OGIB, and 78.5% of patients with IDA). Other causes for overt OGIB and unexplained IDA were small bowel tumors, Crohn's disease and NSAID-induced enteritis. One of the cases with unexplained IDA, with no findings at SBCE, was later diagnosed with atransferinemia and referred to a hematological unit. One case of retention was registered in a patient with radiation enteritis, solved surgically. The retention rate was 0.44%.

On univariate analysis, age >50 years, NSAID use, anticoagulants/antiplatelet drugs and hemoglobin level <10 g/dL were associated with positive findings in patients with OGIB and IDA, whereas on multivariate logistic regression analysis, only hemoglobin level <10 g/dL and anticoagulants/antiplatelet therapy were the variables independently associated with positive findings (Table 1.III).

Table 1.I. Patient demographics, clinical and laboratory parameters

All Patients, *n*	**224**
Gender, male/female, *n*	80/144
Age (years), median (IQR)	53 (16)
NSAID consumption, *n* (%)	129 (58.6)
Oral anticoagulants (NOAC, VKA)/antiplatelet drugs, *n* (%)	104 (v, *n* = 11, VKA, *n* = 36; antiplatelet drugs, *n* = 57) (46.4)
Hemoglobin, g/dL (mean ± SD)	9.8 ± 1.8
Blood transfusion, *n* (%)	60 (26.7)

NOAC – non-vitamin K antagonist oral anticoagulant; VKA – vitamin K antagonist

Table 1.II. Significant findings at SBCE in overt OGIB and IDA

Patients with OGIB (*n* = 224)	**Findings**	**Cases, *n* (%)**
Overt OGIB (*n* = 148) Positive SBCE, *n* = 111 Negative SBCE, *n* = 37	Small bowel angioectasias	69 (62.2%)
	Small bowel tumors	18 (16.2%)
	Crohn's disease	10 (9%)
	NSAID-induced enteritis	5 (4.5%)
	Radiation enteritis	4 (3.6%)
	Fresh blood	4 (3.6%)
	Cecum angioectasia	1 (0.9%)
Unexplained IDA (*n* = 76) Positive SBCE, *n* = 28 Negative SBCE, *n* = 48	Angioectasias	22 (78.5%)
	Crohn's disease	3 (10.7%)
	Small bowel tumors	1 (3.6%)
	Parasitosis	1 (3.6%)
	Lymphoma	1 (3.6%)

Impact on patient management and outcome. Following SBCE, individual decisions were made (Table 1.IV). Most patients with angioectasias had argon plasma coagulation by enteroscopy or were surgically treated in a few cases due to multiple small bowel angioectasias; interventional radiology with selective embolization during arteriography was performed in one case. The sole patient with cecum angioectasia was treated by argon plasma coagulation during the subsequent colonoscopy. An interclinic approach was followed for all patients with concomitant hemorrhagic risk medication, for treatment revision. Knowing that there is no unique standard diagnostic test for Crohn's disease, all patients with findings consistent with small bowel Crohn's disease were complementarily explored in order to add other arguments favoring the diagnosis. Medical treatment was initiated with good clinical evolution. All cases with small bowel tumors were fully investigated afterwards. Enteroscopy was performed to fully characterize tumors and CT enterography or MR enterography were used for stadialization. The patients were referred for surgical treatment, the majority of tumors being either stromal gastro-intestinal tumors or adenocarcinomas; the sole exception was one case of

intestinal lymphoma, which was referred to the hematological unit. The patient with parasitosis received specific antiparasitary treatment, with resolution of anemia. In the case of suspected NSAID-induced enteritis, revision of treatment was made, with no further intervention needed. The cases with fresh blood seen at SBCE were referred to further work-up, and they were afterwards diagnosed with angioectasias (two cases) and small bowel tumors (two cases), with proper subsequent interventional or surgical management. Apart from the case of retention, SBCE had less impact on clinical management for patients of radiation enteritis; they were further conservatively managed.

Table 1.III. Univariate and multivariate regression analysis of risk factors associated with positive findings in patients with OGIB and IDA

Parameter	Univariate Analysis			Multivariate Analysis				
	OR	**95%CI**	***p*-Value**	**OR**	**95%CI**	***p*-Value**	**β cof**	**Wald**
Anticoagulant/antiplatelet drug use	6.73	3.78–11.27	**0.002**	2.27	1.63–5.19	**0.018**	1.328	7.998
Hemoglobin < 10 g/dL	7.28	3.11–14.27	**0.010**	1.94	1.06–4.79	**0.037**	2.551	9.12
Age > 50 years	2.26	1.15–5.27	**0.028**	1.43	0.32–5.37	**0.499**	0.413	0.456
Male gender	2.97	1.47–6.88	**0.047**	1.76	0.29–10.44	0.534	0.565	0.387
NSAID use	3.55	2.01–7.38	**0.035**	1.61	0.98–23.11	**0.067**	1.564	3.769

Discussion. SBCE has become the first line investigation for suspected small bowel pathologies, and OGIB is its most frequent indication. In terms of SBCE indication, the two notions—occult OGIB and IDA—are overlapping. In turn, reciprocally, IDA could be classified as IDA with proved occult OGIB, and IDA with suspected occult OGIB, knowing that a negative fecal test does not exclude occult bleeding. In this study, we did not include the cases with suspected "non-bleeding" anemia, such as suspected celiac disease or other malabsorption syndromes. Before considering SBCE, all alternative possible causes for IDA were looked for. Even so, a diagnosis of atransferinemia was made after SBCE work-up was completed. The rarity of these cases exculpates this, but it is undoubtedly of utmost importance to perform an exhaustive diagnostic work-up before SBCE

Defining SBCE performance parameters in terms of sensitivity and specificity is difficult due to the fact that there is no gold standard applicable. The appearance itself of SBCE is the result of the need to better explore the small bowel. Even if until the advent of SBCE, the small bowel had its own methods of being investigated, they were imperfect, and efforts were made to overcome their shortcomings. SBCE proved itself to be more useful as a diagnostic tool than almost all the other methods (Segarajasingam et al., 2015). SBCE was compared to intraoperative enteroscopy as gold standard, but this is not a method currently applicable (Hartmann et al., 2005). Two meta-analyses have shown that SBCE is superior to radiological imaging and push enteroscopy for diagnosing OGIB, while it has a comparable DY to double balloon enteroscopy (Chen et al., 2007; Brito et al., 2018). Therefore, because sensitivity and specificity are unsuitable performance parameters for SBCE, a surrogate parameter is used, namely the DY. It is defined as the detection rate for what are thought to be clinically significant findings, reported for all investigations performed. The DY takes into account all the investigations with clinically significant findings, even if they are not necessarily directly related to the indication of SBCE. In our study, all SBCE findings were

related to the indication of administration (OGIB or IDA). Therefore, we can affirm that, in this study, the calculated DY truly reflected the SBCE diagnostic performance. The global DY of 62% is similar to other studies in the literature (Brito et al, 2018) and even higher for the cases of overt OGIB (DY = 75%). The DY in IDA cases was 36%, and could be considered modest; as discussed above, an influence factor for the performance of SBCE could be the selection of cases. Some studies reported that increased age may predict a higher diagnostic yield with SBCE (Sidhu et al., 2009), a characteristic not confirmed by our study. Another reported factor which may predict the ability of SBCE to detect small bowel pathology in OGIB (including IDA) was the use of anticoagulants (Olano et al., 2018), which was confirmed by our study.

Table 1.IV. Interventions and outcome after SBCE

Diagnosis at SBCE	**No of Cases**	**Intervention Type (*n*)**	**Outcome (*n*)**
Angioectasias	92	Endoscopic therapy (52) Surgery (2) Radiological therapy (1) Medical therapy (37)	No further bleeding (70), correction of anemia (16)
Crohn's disease	13	Specific medical treatment	No further bleeding (10), correction of anemia (2)
Small bowel tumors	19	Surgery	No further bleeding, correction of anemia
Small bowel lymphoma	1	Hematological referral	Persistence of anemia
Parasitosis	1	Specific medical treatment	Correction of anemia
NSAID enteritis	5	NSAID withdrawal, iron supplementation treatment	No further bleeding
Radiation enteritis	4	Medical treatment (3) Surgery for retention (1)	No further bleeding (2)
Fresh blood	4	Further work-up Surgery (2), endoscopic treatment (2) after final diagnosis	No further bleeding (4)

The most frequent findings were angioectasias, confirming the results of other studies (Ell et al., 2002; Pennazio et al., 2004). They are recognized as potentially bleeding lesions, especially if concomitant anticoagulant or antiplatelet medication is taken. Their identification by SCBE is diagnostic, but in almost all cases there is no active bleeding seen, so their responsibility is circumstantial. Angioectasia could be in fact innocent bystander, the more so for the cases of IDA without evidence of occult bleeding. Questions and doubts regarding the real cause of IDA could be raised in these cases. On the other hand, if we do consider angioectasia as a true cause, another scenario could be valid: the false negative results in anemia, by the paleness of angioectasia corresponding to the low level of hemoglobin. We found that hemoglobin level was an independent predictive factor of positive diagnosis by SBCE.

Our study showed a good diagnostic performance of SBCE for diagnosing small bowel tumors, confirming previous reports (Cobrin et al., 2006; van Turenhout et al., 2010); but it must be emphasized that SBCE cannot offer the final diagnosis. Additional investigations are required in order to have a histological diagnosis, a precise localization and a complete stadialization. Enteroscopy could be considered a complementary exam, while other type of investigations (CT enterography, MR enterography) will provide additional information

needed before therapeutic decision. The small bowel tumors are probably the most illustrative for the dual portrait of SBCE nowadays: the great advantage of non-invasiveness is counterbalanced by the disadvantage of the incapability of taking biopsies. However, this important limit of SBCE is not far from being overcome by the next generation of SBCE with diagnostic and therapeutic capabilities.

Even if the lesion itself was not found in the four cases of active bleeding, the presence of the fresh blood in the bowel was considered diagnostic. This is a well-accepted principle, given that a positive diagnostic of small bowel bleeding is made; SBCE is able to guide further investigations.

The retention rate in our study was 0.44%, remarkably low compared to other studies performed for other indications, such as known or suspected Crohn's disease (2.6%) or small bowel tumors (2.1%) (Rondonotti et al., 2008). However, we confirmed that patients with OGIB are at the lowest risk for capsule retention.

In addition to its inability to obtain biopsies, other CE limitations consist in interpretation difficulties (transient bulges into the small bowel lumen may appear to be submucosal tumors), and missed lesions in the duodenum and proximal jejunum (potential blind points of CE because of its rapid transit through these areas).

Several studies showed that CE had a significant clinical impact on patient management and outcomes for OGIB. For these patients, the objectives are to stop the bleeding or resolve anemia, diminish the necessity for transfusions, as well as reduce costs related to hospitalizations and supplementary diagnoses, and improve quality of life. CE leads to therapeutic endoscopic or surgical interventions and, consequently, to bleeding being stopped and improved outcomes. CE helps direct further therapeutic interventions or change medical therapy in 37-87% of patients (van Tuyl et al., 2007; Arakawa et al., 2009). CE also helped in localizing bleeding sites prior to intraoperative enteroscopy or surgical resection. One study reported that 51% of their patients which had a definite diagnosis on CE had a change in management, as well as in medication, undergoing an endoscopic procedure and surgery (van Tuyl et al., 2007). Other studies have reported a positive impact of CE on OGIB patients' outcome (Cañas-Ventura et al., 2013).

SBCE had a favorable impact on the evolution of the majority of patients, showing that, even if it is not a therapeutic modality, it has its own value as a safe, non-invasive diagnostic method. The place of SBCE in current guidelines is already justly stated, as a first choice in suspected small bowel pathologies. However, SBCE must be wisely chosen during the patient work-up, with utmost attention paid to patient selection and appropriate referral to further investigation or therapeutic techniques.

Conclusions. SBCE has good performance parameters for OGIB and proved itself as a safe technique. SBCE has a high diagnostic yield and a positive impact on the management of patients with OGIB. The overall DY was 62%, higher in cases of overt OGIB (75%) and lower for unexplained IDA (37%). Subsequent individualized management assured a good outcome for all the diagnosed cases. SBCE remains a visual technique, and small bowel tumor discovery had to be followed by supplementary work-up. Small bowel angioectasias were the dominant findings. Additional tests should follow negative SBCE exams. Regardless of diagnosis, the SBCE orientated the subsequent management and had a favorable clinical impact in terms of immediate and long-term outcome. Despite its actual limits, such as the lack of taking biopsies and of therapeutic valences - which will most likely be overcome in the near future - SBCE has an indisputable diagnostic role in small bowel pathologies, having on its side the advantage of being a non-invasive and safe procedure.

I.1.2.3. Uncommon causes of obscure gastrointestinal bleeding

Background and aim. Lipomas are benign, fatty gastrointestinal (GI) tumors, more commonly located in the colon (64%) (Zirpe et al., 2016), the small intestine being the second site, and very rarely in the jejunum (< 2%) (Kordzadeh et al., 2017). Usually, small bowel lipomas are asymptomatic, uncomplicated and discovered incidentally during investigation for other abdominal diseases such as an obstructive bowel syndrome or GI bleeding. However, they may become symptomatic as the result of a number of complications such as bleeding, intussusception, and obstruction. The intussusception is defined as the telescoping of a proximal segment of bowel into the lumen of an adjacent segment of bowel (usually resulting in obstruction), and it is more common in children, where is generally idiopathic; it seldom occurs in adults (with 5% of all cases of intussusception (Uyulmaz et al., 2018) and it is often due to a malignancy (70% of the colonic and 30% of small bowel intussusceptions are attributable to malignancy) (Manouras et al., 2007). In a retrospective study including all adult patients diagnosed with intussusception at Mayo Clinic, Rochester from 1983 to 2008, among all 196 patients, only 10 presented with small bowel lipomas (Onkendi et al., 2011). We aimed to report a very rare case of an ulcerated intussuscepted jejunal lipoma in an adult, discovered after investigating an obscure GI bleeding and managed by surgical resection, in a parallel with a short review of the literature regarding the small bowel (jejunal) intussuscepted lipoma.

Case presentation. A 63-year-old man with personal history of hypertension was admitted to our department of Gastroenterology and Hepatology to investigate an obscure GI bleeding with iron deficiency anemia (IDA). He complained of intermittent abdominal pain accompanied by nausea. He had a prior history of hypertension well controlled by treatment with angiotensin-converting enzyme inhibitors. He had no abdominal surgery. He was a construct engineer and a current smoker (15 cigarettes/d for the past 20 years). He had no serious family history. Physical examination showed pale teguments, and the abdomen was soft and tender in the umbilical and right flank area, without any palpable abdominal mases. Laboratory data showed IDA (hemoglobin 9.5 g/dL, serum iron 45 µg/dL, ferritin 10 µg/L).An upper GI endoscopy and colonoscopy were performed, excluding lesions with potential for bleeding. Then, videocapsule endoscopy was performed, revealing fresh blood in the proximal jejunum, and a protruding lesion, with discolored covering mucosa (Figure 1.1). Next, a single–balloon enteroscopy was carried out, which showed a polypoid mass with ulceration, situated in the proximal segment of jejunum (Figure 1.2). Multiple biopsies were taken from the lesion, but the histological result was inconclusive, as it frequently occurs in submucosal GI benign tumors including lipomas, due to depth factor–the amount of submucosal tissue required in biopsies of the lesion. Then, contrast-enhanced abdominal computed tomography was performed which showed a 6 centimeters elongated structure inside the intestinal lumen with homogenous fat density and smooth well-defined contour, suggestive for an intestinal lipoma (Figure 1.3). Within the next week the patient complained of abdominal pain, nausea and several episodes of vomiting. A laparotomy was performed revealing jejuno-jejunal intussusception. Intra-operative macroscopic observation identified six centimeters intussuscepted yellowish mass suggestive for lipoma (Figure 1.4). The histological examination revealed in the submucosa a nodular mesenchymal tumor consisting in mature adipocytes, with no pleomorphism and no mitotic activity. These findings were compatible with a diagnosis of lipoma (Figure 1.5). The intussuscepted jejunal segment was resected *en bloc* and the inspection of this segment showed a submucosal firm mass with ulceration of the mucosa. End-to-end anastomosis was performed. The patient made an uneventful recovery and was discharged seven days later, and at six months follow-up he had no complains and his hemoglobin returned to normal value.

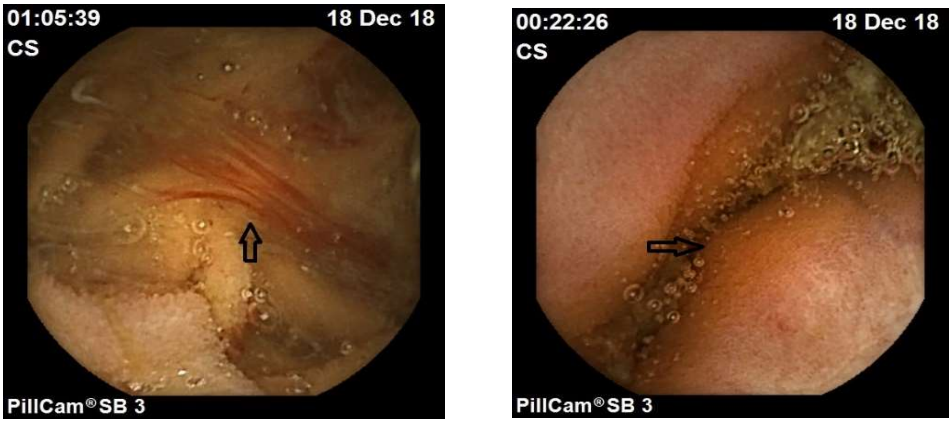

Figure 1.1. VCE findings obtained from our patient.
A. Fresh blood in the jejunum. B. Protruding jejunal lesion

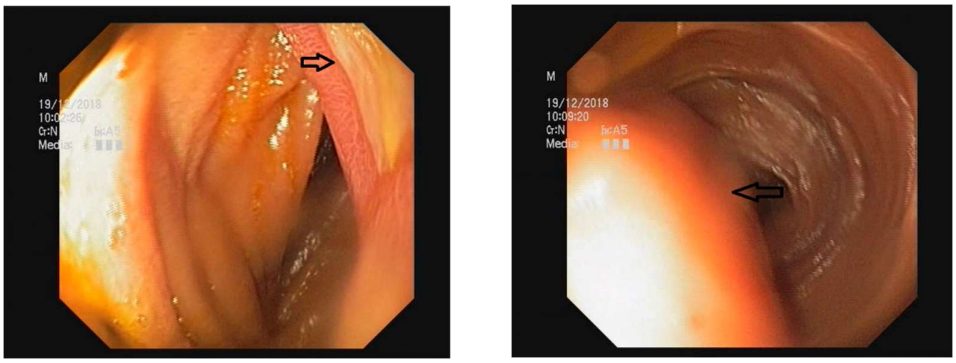

Figure 1.2. Images acquired through enteroscopy performed in our patient.
A. Ulcerated tumoral mass. B. Tumoral mass with partial bowel obstruction

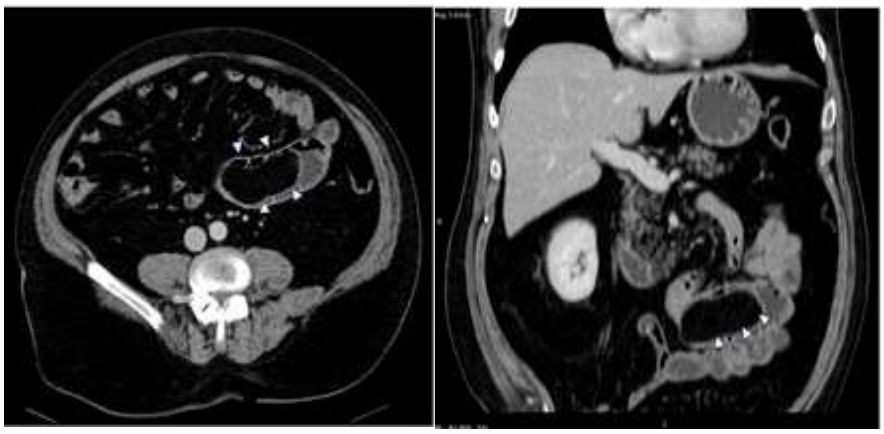

Figure 1.3. Contrast – enhanced abdominal CT scan**.** Both axial (left) and coronal (right) reformatted images show a large elongated structure inside of intestinal lumen (arrowheads) with homogeneous fat density and smooth, well-defined contour

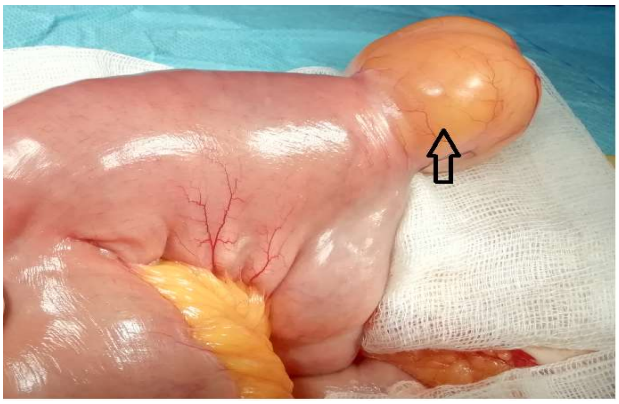

Figure 1.4. Macroscopic appearance of the jejunal lipoma

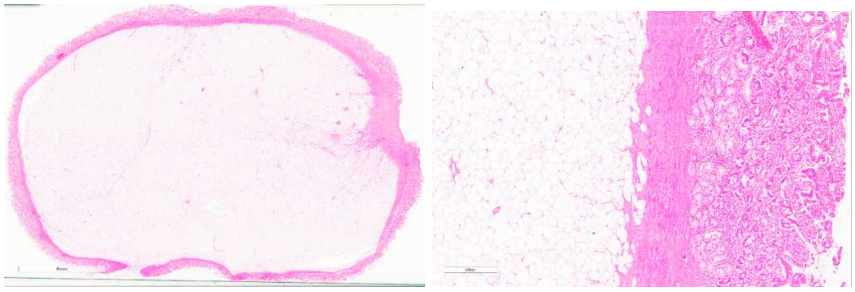

Figure 1.5. Jejunal submucosal lipoma with ulcerated area of the mucosa. A: full section (Hematoxylin-eosin staining, x40); B: detail (Hematoxylin-eosin staining, x200)

Discussion. Jejunal lipomas are rare, but they may, nonetheless, represent a diagnostic challenge when complicated by lower gastrointestinal tract hemorrhage or intussusception. Large benign tumors of the small bowel rarely include intussusception in their spectrum, which may become an important risk factor for ischemia and necrosis of the intestinal wall. The most common sites of GI lipomas reported in the literature are the colon (64%), followed by the small bowel (31%), stomach (3%) and the esophagus (12%) (Toya et al., 2014). While most of small bowel lipomas are small in size and asymptomatic, those surpassing 2 cm in size usually manifest through clinical symptoms such as abdominal pain, hemorrhage or bowel obstruction (Wilson et al., 1975). In adult patients, intussusception is more likely to present progressive misleading symptomatology with diffuse abdominal pain and rarely with classical triad-symptoms such as intense abdominal pain, vomiting and lower gastrointestinal hemorrhage, making the diagnosis complex and requiring further radiological documentation. Intussusception is documented frequently on computed tomography, a method of choice due to its accuracy of virtually staging the lesion (Heiken et al., 1982). Over 90% of intussusception cases found in adults have an organic cause (Martinez-Ubieto et al., 2015).

In order to find similar cases, we have reviewed the literature using Pub Med and found ten cases in the published accounts (Baron et al., 1996; Manouras et al., 2007; Yigitler at el., 2007; Akyouz et al., 2008; Ferrara et al., 2012; Mouaqit et al., 2012; Charalambous et al., 2012; Sarabjit et al., 2013; Pinto et al., 2018; Kida et al., 2017). The keywords used were “lipoma”, “intussusception”, “jejunum”, “bleeding”. All ten cases presented jejunal lipoma with intussusception and bleeding. Over the past decade, there has been a constant debate about the appropriate and safe treatment of small bowel benign tumors. Clinical presentation differed probably on account of the different sizes in tumoral mass. One study (Yu et al., 2007) reported

fifteen cases of gastrointestinal lipomas with different sizes that benefited from endoscopic therapy without important complications. However, it should be noted that endoscopic resection may be associated with a risk of bleeding and perforation. Thus, another study (Raju et al., 2005) reported that endoscopic removal of lipomas > 2 cm in diameter was associated with a greater risk of perforation. In our case, the patient presented a tumor over 5 cm in length with a wide base of implantation. Due to its size and vascularization, surgical resection was considered to be the optimal treatment.

Conclusions. Jejunal lipomas, very rare benign tumors of the GI tract, are mostly asymptomatic and found incidentally during investigations for other abdominal diseases. However, in some cases, they may lead to complications such as intussusception and hemorrhage. Surgical resection remains the treatment of choice for large and complicated lipomas.

I.1.2.4. Visual criteria in small bowel tumors detected by capsule endoscopy – morphological description and correlations with histological type

Background and aim. Although the small bowel represents 75% of the length of the gastrointestinal tract and 90% of its absorption surface, small bowel tumors (SBT) are rare, counting for only approximately 3-6% of all gastrointestinal neoplasms and 1-3% of all gastrointestinal malignancies (Jemal et al., 2002). There are several possible explanations for the relative rare incidence of SBT compared to other gastrointestinal tumors, especially to the colorectal neoplasms: faster transit time, shorter contact of solid carcinogenic components with intestinal mucosa, lower bacterial population, and protective role of the mucosal lymphoid tissue.

The diagnosis of SBT represents a true challenge for gastroenterologists. Besides their rarity, other conditions may render the diagnosis difficult: hardly accessible location by conventional examination tools, long asymptomatic periods and/or non-specific clinical picture. However, the advent of small bowel capsule endoscopy (SBCE) revolutionized the diagnostic of small bowel pathology. Emerged from the need to overcome the examination techniques existent so far, the SBCE proved to be a safe and valuable tool for exploring the small bowel. The main indications of SBCE are obscure gastrointestinal bleeding (OGIB), unexplained iron deficiency anemia (IDA), Crohn's disease, suspected SBT. Studies showed superior diagnostic yields of SBCE to barium studies, computed tomography (CT) enterography, magnetic resonance (MR) enterography, and push enteroscopy, for detecting the small bowel lesions (Koulaouzidis et al., 2012, Ukida et al., 2021). SBCE has become the first-line investigation for suspected small bowel pathology (Pennazio et al., 2015), and the SBCE major breakthrough consequently increased to double the diagnostic rate of SBT (Rondonotti et al, 2008).

Nevertheless, SBCE has limitations in the diagnostic work-up of SBT, among which the most important is the lack of capability of taking biopsies. It is a visual technique, which can offer a macroscopic description of the lesion, but cannot provide the definite diagnosis. Since only visual appearance is described, terminology has an important role for providing a portrait as suggestive as possible. In the same time, it has not the power to always accurately discriminate between the real tumors and pseudotumoral masses. Other inconveniences that could sometimes hamper the diagnostic yield are the low quality of visibility and the incomplete examination of the small bowel within the battery life time.

In order to achieve a final diagnosis, other investigations must follow SBCE. Both invasive and non-invasive examinations are required to provide a histologic diagnosis and staging of the tumor, if malignant, described by SBCE. Surgery will follow if appropriate, providing complete pathological evaluation of the entire surgical specimen.

SBCE stands nowadays as a valuable tool for investigating the SB, and succeeding overcoming its main limitations would assure it an even much more powerful position in the diagnostic work-up of the SBT.

Our study aimed to assess if structured visual description of SBT detected by SBCE correlates with the histological type.

Materials and Methods. We have conducted a retrospective observational study between 1st of January 2011 and 31st of December 2018, in "Sf. Spiridon" Emergency Hospital of Iasi, Romania, Institute of Gastroenterology and Hepatology, including patients with small bowel tumors, evaluated by SBCE and furthermore explored, for which a final histopathological diagnosis was made, either on biopsy samples, or on surgical specimens. Demographics, medical history, clinical examination data and paraclinical examinations results were collected from patients' medical files. SBCE exams were performed according to current guidelines, after the contraindications were excluded and after the patient signed the informed consent for the procedure. Second- and third-generation of endoscopic capsules for small bowel examination (PillCam SB2 and PillCam SB3, Given Imaging, Yoqneam, Israel) were used. The interpretation of the video recordings was made using Rapid Reader Software v.8. The SBCE findings and reports were reviewed in order to assess the main macroscopic features of the SBT, the presumed location, the transit time and any other significant finding. Concerning the appearance of the small bowel masses, the following parameters were analyzed: the number of lesions, the estimated size, the shape, the type, the color of the covering mucosa and the distribution pattern, the presence of an ulcer on its surface, the presence of bleeding or of stigmata of bleeding. The macroscopic morphological criteria which were analyzed and their respective variants of interpretation are presented in Table 1.V.

Table 1.V. Main visual parameters and features described at SBCE

Parameter	Variants
Number of lesions	Single
	Multiple
Size	Small
	Medium
	Large
Shape	Well defined
	Poorly defined
Type	Vegetant
	Submucosal
Color of the mucosa	Normal
	Discolored
Ulcer	Absent
	Present
Bleeding	Active bleeding
	Stigmata of bleeding
	Bleeding potential
	No bleeding potential

Spiral or single-balloon enteroscopy, with or without biopsy, followed SBCE in certain cases, and macroscopic features of SBT were also observed. Complete staging was performed by CT or MR scans, and the patients were managed accordingly. If surgery was performed, data regarding the final histological diagnosis were collected. All biopsy tissues and surgical specimens were routinely processed through fixation in 10% neutral buffered formalin, embedding in paraffin and sectioning. Five μm thickness sections were stained with Hematoxylin-Eosin (HE), van Gieson, and Alcian blue. Immunohistochemistry tests were performed using standard techniques with appropriate positive and negative controls. The following antibodies were used: Chromogranin A (Novocastra, 5H7, 1:400), Synaptophysin (Novocastra, 27G12, 1:150), Ki67 (Novocastra, SP6, 1:250), DOG1 (Novocastra, K9, 1:100), CD117 (Novocastra, EP10, 1:200), SMA (Novocastra, asm-1, 1:50) CD34 (Novocastra, Qbend/10, 1:100), HMB45 (Novocastra, HMB45, 1:100), S100 (Novocastra, polyclonal, 1:150), CK AE1/AE3 (Novocastra, AE1/AE3, 1:250), CK7 (Novocastra, RN7, 1:100), CK20 (Novocastra, Ks20.8, 1:50), CD5 (Novocastra, 4C7, 1:150), CD10 (Novocastra, 56C6, 1:100), CD20 (Novocastra, L26, 1:150), Bcl6 (Novocastra, LN22, 1:60), MUM1 (Novocastra, EAU32, 1:100). All morphological data were analyzed, correlating the descriptive features provided by SBCE with the definitive histological diagnosis.

Results

SBT frequency. Three hundred and two SBCE examinations were performed in the mentioned period in our center. The main indications were obscure gastrointestinal bleeding either overt or occult, and unexplained IDA, followed by suspected or known Crohn's disease, celiac disease, unexplained abdominal pain.

In 16 patients, SBCE showed findings consistent with SBT; consequently, the calculated frequency of SBT at SBCE for all indications was 5.2%. For two patients, a definitive diagnosis was not available. For the remaining 14 patients who entered the study, a histological diagnosis was provided, either by enteroscopy with biopsy or by analysis of surgical specimen if surgery was performed.

General characteristics – demographics, indications of examination, and histological diagnosis. Among the 14 patients, mean age 51+/-2 years, the majority were men (64% male, 36% female). The SBCE was indicated for overt OGIB (6 cases, 43%), unexplained IDA with occult OGIB (5 cases, 36%), or isolated abdominal pain (3 cases, 21%). All the 14 patients were previously investigated by upper and lower endoscopy, without significant lesions.

Following SBCE, six patients, with duodenal or jejunal lesions, underwent enteroscopy. In two cases, the biopsy was not conclusive, and in four cases, a histological diagnosis was made – two cases of stromal tumor (one duodenal, one jejunal), one case of SB lymphoma, and one case of metastatic melanoma, respectively. The two patients with stromal tumors, as well as the two patients with macroscopical suspicion were referred to surgery, and a complete histological diagnosis was established: one jejunal lipoma and one jejunal gastrointestinal stromal tumor (GIST) (Figure 1.6, A-D). The patient with SB lymphoma had a complete staging and referred to the onco-hematology department. The case of metastatic melanoma had an oncological management. The remaining 8 patients were directly referred to surgery immediately after SBCE; the final diagnosis confirmed the previous suspicion – GIST (Figure 1.7, A,B) in another 3 cases, adenocarcinoma in three cases (one jejunal, and two ileal – Figure 1.8, A-C), and neuroendocrine tumors (NET) in two patients (Figure 1.9, A-D).

The demographics, clinical characteristics of patients, SBCE indication, and histopathological diagnosis of patients with SBT are presented in Table 1.VI.

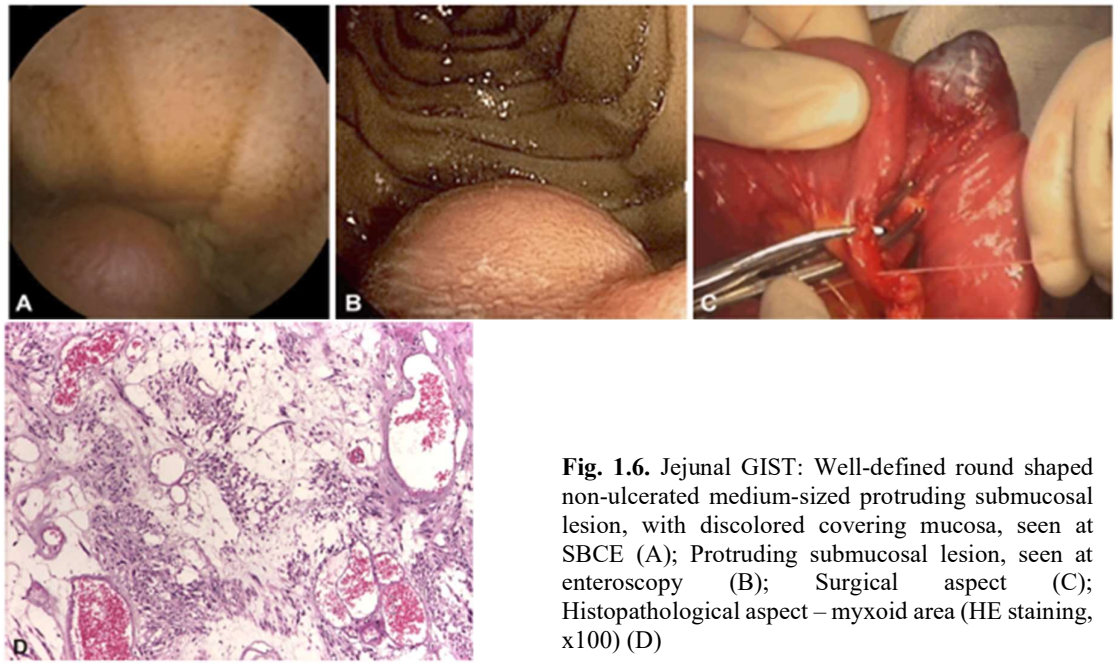

Fig. 1.6. Jejunal GIST: Well-defined round shaped non-ulcerated medium-sized protruding submucosal lesion, with discolored covering mucosa, seen at SBCE (A); Protruding submucosal lesion, seen at enteroscopy (B); Surgical aspect (C); Histopathological aspect – myxoid area (HE staining, x100) (D)

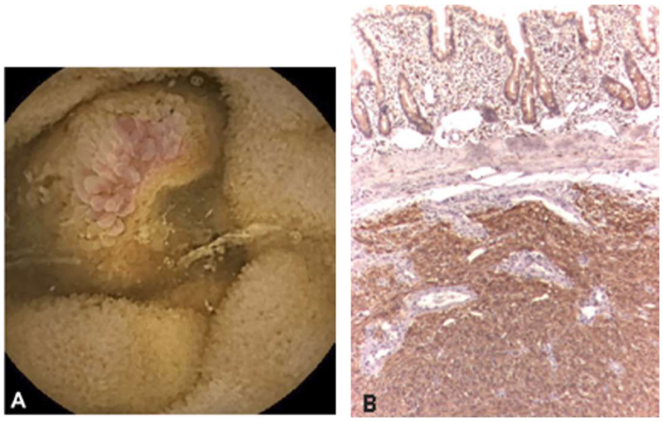

Fig. 1.7. Jejunal GIST: Well-defined shaped ulcerated protruding submucosal lesion, seen at SBCE (A); Histopathological aspect – CD117 (c-kit) diffusely positive in tumor cells (Anti-CD117 antibody immunomarking, ×100) (B). GIST: Gastrointestinal stromal tumor; SBCE: Small bowel capsule endoscopy; CD117: Cluster of differentiation 117

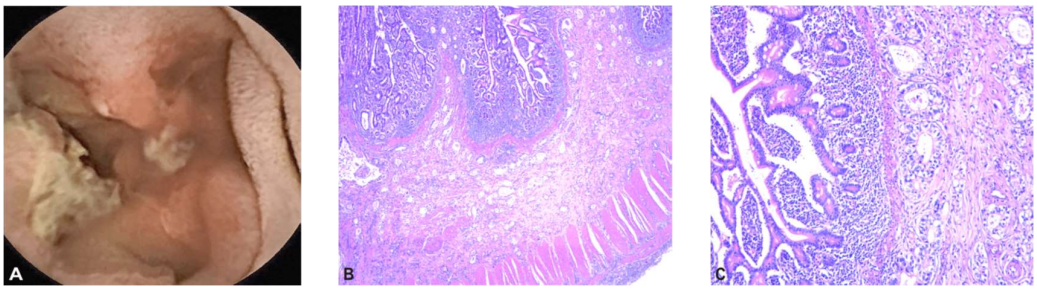

Fig. 1.8. Jejunal well differentiated adenocarcinoma:Bleeding ulcerated vegetant protruding lesion, seen at SBCE (A); Histopathological aspect on surgical specimen – atypical tubular structures infiltrating the whole thickness of the jejunal wall (HE staining, ×25) (B); Microscopic detail (HE staining, ×100) (C)

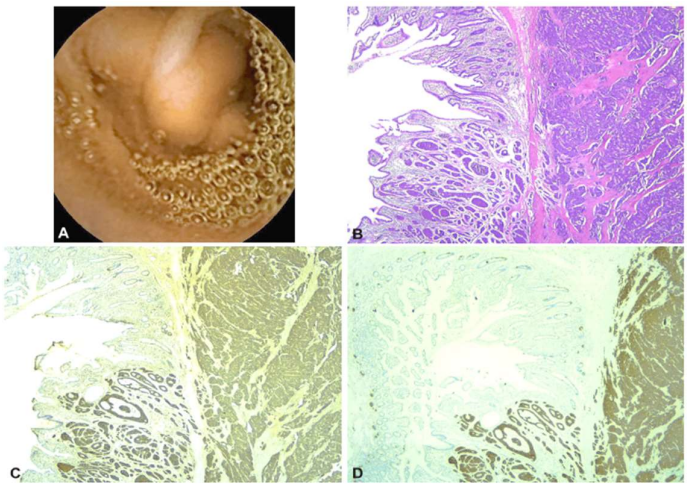

Fig. 1.9. Ileal NET G1: Poorly-defined shaped ileal protruding submucosal lesions, seen at SBCE (A); Histopathological aspect on the surgical specimen – isle and trabecular architecture, monomorphic tumor cells, low mitotic rate, infiltrative growing (HE staining, ×25) (B); Chromogranin A – intense diffuse positivity in tumor cells (Anti-chromogranin A antibody immunomarking, ×25) (C); Synaptophysin – intense diffuse positivity in tumor cells (Anti-synaptophysin antibody immunomarking, ×25) (D)

Table 1.VI. Characteristics, SBCE indication, and histological diagnosis of patients with SBTs

Patient, no.	Gender, Age (yrs)	SBCE indication	Histological diagnosis after enteroscopy with biopsy	Histological diagnosis after surgery
1. GM	M, 54	Overt OGIB	Jejunal GIST	Jejunal GIST
2. FR	M, 48	Unexplained IDA	Inconclusive Biopsy	Jejunal GIST
3. FI	F, 52	Abdominal pain	Duodenal GIST	Duodenal GIST
4. LM	F, 62	Overt OGIB	NA	Jejunal GIST
5. DV	M, 25	Overt OGIB	NA	Jejunal GIST
6. BM	F, 71	Overt OGIB	NA	GIST
7. PT	M, 65	Overt OGIB	NA	Jejunal adenocarcinoma
8. MV	M, 70	Abdominal pain	NA	Ileal adenocarcinoma
9. BD	F, 61	IDA	NA	Ileal NET G2
10. CI	F, 59	Abdominal pain	NA	Ileal NET G1
11. BA	M, 32	IDA	Lymphoma	NA
12. OI	M, 54	Overt OGIB	Metastatic melanoma	NA
13. SC	M, 64	IDA	Inconclusive Biopsy	Ulcerated lipoma
14. EC	M, 68	IDA	NA	Ileal adenocarcinoma

Macroscopic morphological criteria. For all the lesions proved as SBT, the cardinal designation term was "protruding lesion". All SBT presented as intraluminal mass lesions, small, medium or large.

The majority of lesions (11 cases) were single, while in three cases, multiple protruding lesions with similar characteristics were described: two cases of multicentric NET and one case of metastatic melanoma.

After tumor size estimation, lesions were classified into small (lymphoma, melanoma, NET), medium (GIST, adenocarcinoma, NET) and large (GIST, lipoma).

Regarding the type of the lesion, the submucosal type of the lesion was observed in all the cases confirmed latter as GIST, NET or lipoma. For the three cases of adenocarcinoma, a vegetant phenotype was described.

The shape was described as well-defined (mainly round) or poorly defined. All cases of GIST were well-defined as shape, with visible limits from the adjacent mucosa. On the contrary, adenocarcinoma and NET presented poorly defined shape, either irregular or barely identified besides surrounding mucosa.

An important feature was the color. None of the SBT presented normal colored mucosa, even in the cases of submucosal tumors. Discolored covering mucosa was described in all cases of submucosal tumors: GIST had a purplish shade surface, NET appeared as whitish-colored masses, while the jejunal lipoma had smooth slightly discolored aspect. The patient with metastatic melanoma had atypical lesions, white ulcerated protruding masses, being proved after biopsy as amelanotic melanoma. The distribution pattern of the discolored mucosa was also analyzed, and we found that for almost all cases (13 out of 14) the modified aspect was localized, while only for one case (the SB lymphoma), there was a diffusely modified aspect of the mucosa.

The presence of ulcer on the surface of the SBT was noticed by SBCE in 4 cases of the stromal tumor, in all the 3 cases of adenocarcinoma, and in the melanoma case. The other 2 cases of stromal tumor, the 2 cases of NET, and the lymphoma had no obvious ulcer on their surface. For the patient with lipoma, during enteroscopy the ulcerated aspect was observed. In the same time, it must be mentioned that the macroscopical examination of the surgical specimen performed afterwards in the 2 cases of GIST revealed the presence of ulcer on the surface of the tumor, on the side not visualized by the SBCE.

The active bleeding or the stigmata of recent bleeding were important features, as well as the estimated potential of bleeding of the lesion. For the patients who had OGIB or unexplained IDA as indications for SBCE, the detection of bleeding or potentially bleeding lesions had a positive diagnostic role. The images provided by the SBCE revealed actively bleeding in two cases of GIST and in one case of SB adenocarcinoma, stigmata of recent bleeding in the case of melanoma and bleeding potential in the other two ulcerated cases of GIST and in the other two cases of adenocarcinoma. The lesions with no active, recent or potential of bleeding corresponded to the cases investigated for other reasons than overt OGIB, namely IDA, or abdominal pain.

General description and **characteristic features** of SBT, by histological type, as seen at SBCE, are presented in Table 1.VII; beside the so-called common characteristic features, **additional peculiar features** have been described, for some of the SBT. For instance, large stromal tumors had unseen versants which were afterwards proved to be ulcerated. Adenocarcinomas presented as vegetant or ulcerated-vegetant masses, with obstructive effect, proven by the large amount of time the capsule passed around the tumor. The NET tumors were multicentric, presenting as multiple similar multilevel lesions. The case of SB lymphoma presented diffuse discolored modified mucosa, with abnormal villi all around the tumor. The metastatic melanoma appeared as multiple ulcerated lesions, with similar localization, size, and shape; it was a particular case of amelanotic melanoma, proved by enteroscopy with biopsy. The lipoma was described as a large sized submucosal tumor, with incompletely visible versants, with smooth slightly discolored covering mucosa.

Discussion. SBCE is nowadays recommended as first-line investigation for suspected small bowel pathology, with incontestable proved value in the diagnostic work-up of OGIB, unexplained IDA, celiac disease, suspicion of SBT, unexplained abdominal pain, after negative or inconclusive upper and lower endoscopy. The major indications for SBCE remain OGIB and unexplained IDA, which are in the same time the main circumstances of discovery of SBT.

Table 1.VII. General description and characteristic features of SBT, by histological type

Type of SBT (no. of cases)	**General term**	**Common characteristic features (no. of cases) at SBCE**	**Additional peculiar features**
GIST (6)	Protruding lesion	Single Well-defined shape Medium/large Submucosal mass Discolored surface Ulcerated (4)/Non-ulcerated (2) Bleeding (2)/non-bleeding (4)	Hidden side
Adenocarcinoma (3)	Protruding lesion	Single Medium Poorly defined shape Ulcerated Bleeding (1)/non-bleeding (2)	Vegetant aspect Obstructive effect
NET (2)	Protruding lesion	Multiple Small/medium Poorly defined shape Submucosal mass Discolored surface Non-bleeding	Multilevel lesions
Lymphoma (1)	Protruding lesion	Multiple Small Discolored surface Non-ulcerated Non-bleeding	Diffuse discolored modified mucosa Abnormal villi
Metastatic melanoma (1)	Protruding lesion	Multiple Small Discolored surface Ulcerated Stigmata of bleeding	Multiple similar lesions
Lipoma (1)	Protruding lesion	Single Large Well-defined shape Submucosal mass Non-ulcerated	Smooth light surface Hidden side

The frequency of SBT at SBCE varies widely in the literature; the prevalence is between 1.6% as reported by Pasha and al. in a meta-analysis including 1000 examinations (Pasha et al., 2008) and much higher, above 10% in other studies with lower number of patients, (Almeida et al., 2009; Marmo et al., 2009). The differences in prevalence probably rely mainly on the size of the series, as highlighted by a multicenter European study, where an inverse correlation between the number of the procedure performed and the frequency of detection of SBT was described (Rondonotti et al., 2008). In the same time, other circumstances may explain the differences in prevalence, such as inclusion criteria or lack of definitive diagnosis. In our study, the frequency of SBT at SBCE for all indications was 5.2%, comparable with other studies of similar size. The frequency was calculated taking into account the lesions subsequently proved as SBT, while only the patients with definitive histopathological diagnosis

entered the study. Thus, the analysis did not include those cases for which a final diagnosis was not microscopically confirmed.

In our study, indications for SBCE were OGIB (43%) and unexplained IDA (36%), followed by unexplained abdominal pain (21%). As already stated by now, OGIB and IDA remain the main revealing circumstances of SBT, due to their bleeding potential. Regarding abdominal pain as indication for SBCE, the overall diagnostic yield of SBCE is variable, but rather low in different series, facilitating diagnosis in 9-24% of cases (Fry et al., 2006; Mimidis et al., 2011; Egnatios et al., 2015). Even in the studies that reported the highest diagnostic yields, the spectrum of significant lesions included mostly inflammatory lesions and much scarcely SBT (Yang et al., 2014). However, in our study, chronic unexplained abdominal pain was the cardinal symptom which justified further investigations and permitted a diagnostic of SBT in three cases, among which one case of duodenal GIST, one case of adenocarcinoma and one case of neuroendocrine tumor.

Following SBCE, individualized work-up decisions were made. Some cases were further explored by enteroscopy with biopsy. Enteroscopy provided in some cases a more accurate description of the lesion, and in the same time, offered localization information. For SBCE, lesion localization is difficult, because of the lack of landmarks; conventionally, lesions are described as situated in one of the three tertiles of the SB, and also as projected in one of the four abdominal quadrants. However, SBCE was accurate, a correspondence of the sites of the lesions being noticed, as described by SBCE, enteroscopy and surgery. The biopsies performed during enteroscopy provided or not a histological diagnosis; the cases with positive results were: ulcerated GIST, lymphoma and metastatic melanoma, inconclusive biopsies being from protruding lesions developed in the submucosa. In some cases, patients were referred to surgery immediately after SBCE, being given the macroscopical features, localization and bleeding complications (GIST with overt bleeding, ileal NET, suspicion of adenocarcinoma with bleeding or obstructive effect). So, SBCE gains value as standalone technique mandating therapeutic decision.

The appearance of SBT at SBCE was the main parameter when describing the lesions. The macroscopical description of SBT in structured terminology and enriched with individual observations permitted framing the findings in certain supposed types of SBT. All SBT are defined as protruding lesions, and afterwards a sum of parameters is added, in order to enrich the description. Features as size, color, type, shape, presence of ulceration on the surface of the mucosa, bleeding stigmata or potential, contributed outlining a prototype. Furthermore, some peculiar characteristics were noticed, and even if they are not yet be not extrapolated, they may be highly indicative: large-sized stromal tumors have “hidden” areas at SBCE where an ulcer might exist, adenocarcinomas present with prolonged transit time of the SBCE, NET may present as multilevel lesions. The size of the lesion seemed correlating with the circumstances of diagnosis: little-sized lesions, discovered in clinical circumstances of abdominal pain or unexplained IDA were subsequently proved as aggressive type (NET, lymphoma, SB metastases), while medium- or large-sized lesions were rather discovered in more dramatic circumstances, revealed by the hemorrhagic complications (GIST, adenocarcinomas). The discoloration of the covering mucosa, as well as the appearance of the adjacent mucosa, was also indicative parameters, as presented.

Undoubtedly, empowering the SBCE predicting the most accurately possible the definitive diagnosis equally preoccupied the gastroenterologists and the researchers. There have been registered much progress in SBCE technology, in terms of image resolution, wider angle view, software innovations, such as flexible spectral imaging color enhancement or the “optical biopsy”, including the wireless spectroscopic compact photonic for detecting microscopic malignancy (Yung et al., 2017). Nevertheless, no matter how much SBCE will

progress as technique, it will remain an artificial smart one. Only the human intelligence and sense could empower SBCE as highest valuable and not only purely visual technique.

Conclusions. SBCE has become a valuable tool in the investigation of small bowel pathology. Its real advantages, consisting in safety, non-invasiveness, and patients' comfort may be counterbalanced by its limitations, mainly lack of capability for biopsy and lack of therapeutic abilities. However, the introduction of SBCE doubled the diagnostic rate of SBT, and even if, so far, SBCE has been considered a visual technique, with no discriminating power between different histological types, we may affirm that thorough examination and rigorous analysis of macroscopical features may successfully predict the final diagnosis. SBCE proved to be both a trust comrade in the diagnostic pathways of SBT, and a valuable standalone technique guiding the definitive therapeutic decision.

I.1.2.5. Completion rate of SBCE in suspected Crohn's disease

Background and aim. SBCE has excellent sensitivity, positive predictive value and negative predictive value for small bowel Crohn's disease (Triester et al., 2006), being even more sensitive for Crohn's disease diagnosis than entero-IRM due to its ability to visualize incipient lesions and to superior detection of lesions in the proximal small bowel (Jensen et al., 2011; Kopylov et al., 2017). However, the specificity of SBCE for Crohn's disease is still a matter of debate, due to the fact that it is only a visual technique but also related to the procedure itself - namely incomplete visualization and completion rate. Because examination of the whole small bowel is of utmost importance for the diagnosis of Crohn's disease, our study aimed to assess the completion rate of SBCE in suspected Crohn's disease and the influencing factors, and estimate if it could possibly limit the SBCE diagnostic performance.

Patients and methods. Our study retrospectively analysed all the patients with suspected Crohn's disease, investigated by SBCE, in the Institute of Gastroenterology and Hepatology of Iasi, Romania, tertiary referral care centre in North East Romania, in a three-year period, between 1st of January 2015 and 31st of December 2017. Patients' medical files were analysed. The foreground of the clinical suspicion consisted in the association of at least one clinical gastrointestinal symptom (chronic abdominal pain, chronic diarrhoea and/or weight loss) with either an extraintestinal manifestation or an abnormal suggestive laboratory (inflammatory syndrome, anaemia, faecal calprotectin) or imagistic exam result (computed tomography, IRM, small bowel follow through). The investigational work-up followed present guidelines; for all patients, an indication of ileocolonoscopy was made as first line examination. If ileocolonoscopy was not feasible, complete or diagnostic, SBCE was performed after contraindications were excluded. For subsequent analysis, patients were divided into two age groups: under 65 years and 65 years or older, respectively; they were also classified after the presence of diagnosed diabetes mellitus, into diabetic patients and non-diabetic patients, respectively.

Small bowel capsule endoscopy. The SBCE procedure was made according to usual recommendations. The patients were informed about the procedure and signed the informed consent form. The small bowel examination by capsule endoscopy was made after overnight fast. Third-generation of endoscopic capsule for small bowel examination (PillCam SB3, Given Imaging, Yoqneam, Israel) was used. The interpretation of the video recordings was made using Rapid Reader Software v.8. The evidence or the strong suspicion of obstruction represented absolute contraindications for the procedure.

Completion rate. The completion rate consists in the proportion of complete examinations, reported to all the investigations performed. Ideally, SBCE accurately visualizes the entire small bowel, offering images throughout all the segments of the small bowel, and reaching the caecum within battery life. Still, complete examination is not always performed, SBCE failing to pass into the caecum in due time. We assessed the number of incomplete

examinations and we correlated the completion rates according to age and to the presence of diabetes.

Diagnostic yield. As performance parameter for SBCE, the diagnostic yield is used. There is no usual gold standard for the investigation of the small bowel, and in the same time, there is no unique diagnostic test for Crohn's disease. The theoretical gold standard for the small bowel, to which the SBCE could be objectively compared, is represented by intraoperative enteroscopy, difficult to apply in clinical practice. Therefore, diagnostic yield is the surrogate parameter proposed for appreciating the performance of SBCE. The diagnostic yield is defined as detection rate of findings considered clinically significant, reported to all the investigations performed. Following Mow's diagnostic criteria (Mow et al., 2004), the diagnosis of Crohn's disease was considered if three ulcerations or more were visualized. Lewis score (Gralnek et al., 2008), incorporated in SBCE software, played a role in quantification of inflammation. Three distinct specific diagnostic yields were defined and calculated: the overall diagnostic yield, reporting the cases with significant lesions consisting in Crohn's disease to all the investigations performed, the effective diagnostic yield, reporting the cases with significant lesions consisting in Crohn's disease exclusively to complete examinations, and a hypothetical diagnostic yield, reporting the cases with significant lesions consisting in Crohn's disease together with incomplete examinations (as if they were all diagnostic) to all the examinations performed.

Results. During the analysed three-years period, ninety-six patients were investigated by SBCE for suspected Crohn's disease. Among them, 72 patients (75%) were under 65 years old, while 24 patients (25%) were 65 or older. 82 were complete investigations, while 14 capsules failed to reach the caecum within battery life, resulting in a completion rate of 85%. Following current diagnostic criteria, the SBCE examination was conclusive for Crohn's disease in 28 cases, consisting in an effective diagnostic yield of 34%, and in an overall diagnostic yield of 29%. If all the incomplete examinations were diagnostic, a hypothetical diagnostic yield was also calculated, of 44%. According to age, the completion rate was significantly higher in the younger group compared to older group (93% vs. 62.5%, respectively, $p<0.01$). According to the presence of diabetes, completion rate was significantly lower in diabetic patients compared to the group of patients without diabetes (55% vs. 92%, respectively, $p<0.01$).

Discussion. Complete accurate visualization is of extreme importance for the diagnosis of Crohn's disease. Due to its discontinuous distribution of lesions, with any possible localization of lesions along the gastrointestinal tract, the diagnosis of Crohn's disease is challenging. A complex work-up is often needed in order to objectivate the specific lesions. SBCE gained a valuable role in the algorithm of investigation of suspected Crohn's disease, as first choice examination after a non-diagnostic ileocolonoscopy (Pennazio et al., 2015). Nevertheless, SBCE achieve its maximum diagnostic potential only if the examination of small bowel is complete, otherwise diagnostic doubts persist and negative predictive value is impaired. Incomplete examinations could be caused by prolonged transit time, due to intrinsic factors or could be determined by technical limitations as shorter battery life. Our study aimed to assess the completion rate in patients investigated for Crohn's disease, both overall and by special categories defined by age or the presence of diabetes mellitus.

The completion rate of 85% was satisfactory, higher compared to other studies which reported incomplete examinations in up to 20-30% of cases (Mergener et al., 2007; Lim et al., 2015). Incomplete examinations were more frequent in older group (patients aged 65 years or older), probably related to slower transit time or maybe also to the lesser ability to walk around in order to facilitate capsule progression. Diabetes was also a risk factor for incomplete examinations, suggesting the influence of gastrointestinal autonomic neuropathy. In these

patients, additional prokinetic measures could be of benefit. Also, technical progresses regarding prolonged battery life could be useful.

In our study, the effective diagnostic yield of SBCE for suspected Crohn's disease was 34%, which could seem low at first sight, compared to other studies; for instance, a meta-analysis including 12 studies with more than 400 examinations showed higher yields for CE, when compared to small bowel follow through (52% vs 16%), CT-enterography (68% vs 21%), and ileocolonoscopy (47% vs 25%) in patients with suspected CD (Dionisio et al., 2010). The lower diagnostic yield in our study probably correlates to wider examination criteria. More strictly SBCE administration criteria maybe would have assured a higher diagnostic yield, but with the price of potential missed diagnosis. In the same time, other diagnoses than Crohn's disease were established by SBCE examination; the absence of lesions suggestive for Crohn's disease classified the SBCE investigation as non-diagnostic, but taking into account all positive diagnosis, the universal diagnostic yield was higher.

However, the overall specific diagnostic yield was even lower, 29%, due to incomplete examinations. Even if lesions could be observed along visualized segments, moreover even suggestive of Crohn's disease, the unrevealed part of the small bowel could hide anything, so an incomplete investigation couldn't be possible considered as valid.

Finally, a hypothetical diagnostic yield was calculated, if all incomplete examinations would have described lesions consistent with Crohn's disease, and the result was 44%. Because Crohn's disease has a discontinuous pattern, non-visualized segments could theoretically be responsible for the real diagnosis. Once more, it is of extreme importance that SBCE offers images from entire length of the small bowel, up to caecum. Otherwise, the performance of SBE is impaired and additional investigations, or even additional capsule endoscopy exams are required.

Conclusion

Small bowel capsule endoscopy is a valuable tool in the work-up of suspected Crohn's disease. Completion rate may influence performance of SBCE. Effectiveness of SBCE is related to diagnostic yield and to proportion of valid examinations. Risk factors for incomplete visualization are older age and diabetes; by adopting in these cases special measures, as additional preparation or prokinetics use, SBCE performance could be improved and in the same time, additional costs related to supplementary investigations could be avoided.

I.1.3. CAPSULE ENDOSCOPY IN THE COLON EVALUATION – A PROMISING TOOL, BUT WITH FINAL STATUS STILL PENDING

I.1.3.1. Introduction

Colon capsule endoscopy (CCE) has been developed with the aim of colorectal cancer (CRC) screening. Low compliance of the general population worldwide in CRC screening programs is due to fear of pain, need for sedation, concerns about possible complications, and the unease about the invasion of one's privacy. Certainly, CCE offers an alternative as a painless procedure, and no need for sedation, air insufflation, radiation, or invasion of one's privacy. However, the results for first generation CCE-1 (PillCam COLON 1) have been disappointing. Comparative trials that used colonoscopy as the gold standard reported CCE2 sensitivity between 72% and 95% for patients with polyps ≥ 6 mm and between 75% and 92% for polyps ≥ 10 mm, while specificity between 64% and 91% for patients with polyps ≥ 6 mm, and between 89% and 100% for patients with polyps ≥ 10 mm (Eliakim et al., 2009; Spada et al., 2011a). Using the second-generation CCE (CCE-2), one study reported the sensitivity and specificity for detecting polyps ≥6 mm of 89% and 76%, respectively, while for polyps ≥10 mm the corresponding figures were 88% and 89%, respectively (Eliakim et al., 2009). In a

recent prospective, blinded trial to determine the accuracy of PillCam CCE-2 and CT-colonography, Spada et al. (2015) found that both methods had comparable efficacy in completing colon evaluation after incomplete colonoscopy, although overall diagnostic yield of CCE-2 was superior to CT-colonography. Subsequently, CCE-2 is now recommended in patients with incomplete colonoscopy (FDA approved), in addition to those who are unwilling or unable to undergo colonoscopy. Negreanu et al. (2013) found CCE-2 an effective procedure in detecting significant lesions and an adequate alternative diagnostic tool in patients unwilling or unable to undergo colonoscopy. A prospective, multicenter international (10 centers in the United States and 6 in Israel) study in an average-risk screening population reported that CCE-2 identified subjects with 1 or more polyps 6 mm or larger with 88% sensitivity and 82% specificity; the authors concluded that CCE is an appropriate method for detecting such polyps for patients who cannot undergo optical colonoscopy or had incomplete colonoscopy, although colonoscopy remains the gold standard for the diagnosis of colorectal polyps (Rex et al., 2015). Adrián-de-Ganzo et al. (2015) in a prospective study of 329 asymptomatic first-degree relatives of patients with colorectal cancer found that CCE-2 was as effective as colonoscopy in detecting significant lesions, and thus, CCE could be a valid strategy for individuals unwilling undergo screening colonoscopy. However, despite a good sensitivity and specificity of CCE for detecting polyps and cancers, the rigorous data of CCE on colorectal cancer screening are lacking. In one study (Holleran et al., 2014), CCE was effective in detecting polyps and cancer in a positive fecal immunochemical test (FIT) cohort. However, in the absence of specific studies in colorectal cancer screening, CCE cannot be included in any screening programs. CCE may be useful for evaluating IBD, particularly in UC patients. Thus, Hosoe et al. (2013) reported that CCE-2 might be feasible for assessing the severity of mucosal inflammation in patients with UC. The same team performed a second CCE-2 study (Usui et al., 2014) in UC patients with a low-volume (2L) PEG and prokinetics regimen and found that 85% of the patients achieved total examination. In pediatric UC, one study compared the diagnostic accuracy of CCE-2 with colonoscopy and found a 96% sensitivity and 100% specificity for CCE-2 (Oliva et al., 2014). Sung et al. (2012) in a multicenter study involving 100 suspected or known patients with UC reported the sensitivity of CCE to detect active colonic inflammation of 89% and specificity was 75%. More recently, a multicenter pilot study assessed the safety and feasibility of the CCE-2 in evaluating the severity of CD; the study included 40 patients with active colonic CD who underwent CCE-2 and optical colonoscopy procedures (D'Haens et al., 2015). The authors found substantial agreement between CCE-2 and optical colonoscopy in the measurement of the Crohn's Disease Endoscopic Index of Severity (CDEIS). CCE-2 had 86% sensitivity and 40% specificity in detecting colonic ulcerations, it had no adverse effects, and was better tolerated than colonoscopy; the majority of patients would favor CCE-2 for a future endoscopic examination, a finding which supports previous assessments of patient preference (Oliva et al., 2014).

The clinical indications for CCE and detected findings have been published as a ESGE (European Society of Gastrointestinal Endoscopy) guidelines (Spada et al., 2012). Despite encouraging results, CCE cannot replace conventional colonoscopy as every positive CCE finding needs colonoscopy for a definitive diagnosis. According to the European Society of Gastrointestinal Endoscopy (ESGE) guidelines, CCE is indicated in patients with incomplete colonoscopy, in those patients unwilling or unable to undergo colonoscopy (Spada et al., 2012).

Similar recommendations are sustained in more recent guidelines, the use of CCE being not advised as a routine substitution instead colonoscopy (Enns et al., 2017).

Although CCE is a promising tool, there are still gaps. Standardized criteria should be developed for documenting CE findings, for training and credentialing. More data is needed concerning the optimal preparation regimen before CCE. More studies are required in relationship with the role of CCE in IBD evaluation – the extent and the severity of the disease.

PERSONAL CONTRIBUTIONS RELATED TO THE ROLE OF CAPSULE ENDOSCOPY IN THE COLON EVALUATION

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1.	**Singeap AM**, Stanciu C, Trifan A. Capsule endoscopy: The road ahead. *World Journal of Gastroenterology*. 2016;22(1):369-78. **IF = 3.365**
2.	**Singeap AM,** Stanciu C, Cojocariu C, Sfarti C, Trifan A. Capsule Endoscopy in Inflammatory Bowel Disease: Current Applications. *Archives of Iranian Medicine*. 2015;18(6):379-83. **IF = 0.931**
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4.	**Singeap AM**, Trifan A, Cojocariu C, Stanciu C. Colon capsule endoscopy compared to colonoscopy for colorectal neoplasms diagnosis: an initial experience and a brief review of the literature. *Rev Med Chir Soc Med Nat Iasi*. 2012 Jan-Mar;116(1):145-9. Review. PubMed PMID: 23077887

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1.	Videocapsula endoscopică. **Singeap AM**, Negreanu L, în GASTROENTEROLOGIE ȘI HEPATOLOGIE CLINICĂ, SRGH, Anca Trifan, C. Gheorghe, D. Dumitrașcu, M. Diculescu, Liana Gheorghe, I. Sporea, M. Tanțău, T. Ciurea. Editura Medicală, București, 2018, ISBN 978-973-39-0846-3.
2.	Cancerul de colon. Tarcoveanu E, Scripcariu V, Carasevici E, Trifan A, Ursulescu C, Vasilescu A, **Singeap AM**, Lupascu C, Makkai-Popa ST, Dragomir C. În TRATAT DE CHIRURGIE, sub redacția Irinel Popescu, Constantin Ciuce, vol. 8 (Chirurgie generală) - coordonator Irinel Popescu, Editura Academiei Române, București, 2015. ISBN 978-973-27-2530-6. Pag. 218-287.
3.	Rolul videocapsulei endoscopice în bolile inflamatorii intestinale, **Singeap AM,** Trifan A, Sfarti C, Stanciu C. BOLILE INFLAMATORII INTESTINALE, sub redacția Carol Stanciu, Anca Trifan, Ioan Sporea. Editura „Gr. T. Popa" U.M.F.Iași, 2014, ISBN 978-606544-220-7

I.1.3.2. Colon capsule endoscopy compared to colonoscopy for colorectal neoplasms diagnosis

Background and aim. Colorectal cancer (CRC) is a leading cause of morbidity and mortality worldwide. Approximately 90% of CRC develop from benign adenomatous polyps, and current guidelines recommend screening colonoscopy with subsequent polypectomy to prevent progression into cancer (Levi et al., 2008). With such screening program in the United States, a decrease in incidence and mortality of CCR was reported (Winaver et al., 1997; Thiis-Evensen et al., 1999). However, colonoscopy has several limitations including invasiveness, discomfort, embarrassment, high cost, sedation, and potential complications. Therefore, many patients are reluctant to undergo this procedure, and generally, participation in colonoscopy screening is unacceptable low (Knopnadel et al., 2003). Colon capsule endoscopy (CCE) is a new, safe non-invasive and patient-friendly procedure for visualizing the colon with no need of sedation, intubation, air insufflation or radiation; thus, new technology might become an alternative method to the invasive conventional colonoscopy in CCR screening and polyp detection. We aimed to present our personal experience with CCE.

Material and methods

Patients. We have analyzed the first results with ***PillCam Colon capsule*** 1 (PPC1) at our institution in patients with or without personal or family history of CRC or polyps and compared them with those obtained at colonoscopy. CCE was not performed in patients with contraindications - dysphagia, known or suspected bowel obstruction, surgical anastomosis, diverticulosis, cardiac pacemaker or other implanted electromedical devices, pregnant female patients, known allergy to PEG, sodium phosphate or bisacodyl.

Colon capsule endoscopy: device description. The PillCam Colon capsule 1 (PCC1) endoscope (Given Imaging Ltd., Yoqneam, Israel) measures 11 x 31 mm and has two cameras

that enables the device to acquire video images from both ends with a frame rate of 4 images per second, and a total operation duration of about 10 hours (Adler et al., 2011). After an initial few minutes of image transmission, the capsule enters a delay mode of approximately two hours, after which (after capsule is probably in terminal ileum) it "wakes up" and restarts the transmission of video images. The recorded data are downloaded into the Given Imaging RAPID workstation for review of the colon video. **The PillCam Colon 2** (PCC2) – the second-generation capsule endoscopy system – measures 11.6 x 31.5 mm, and the angle of view has been increased to 172 degrees for each camera (from 154° in PCC1), offering a panoramic view. The new Data Recorder 3 is a "thinking" recorder which controls the capsule image rate in real time (4 images per second when the capsule is stationary and 35 images per second when it is moving) as opposite to the fixed rate of 4 images per second of PCC1; this technical improvement conserves battery energy (Adler et al., 2011). The Data Recorder 3 also recognizes location of the capsule (stomach, small intestine) and how long it has been there, thus assisting medical staff and patient through the procedure (administration of a prokinetic or laxative). The new RAPID software includes a graphic interface tool for polyp size estimation in millimeters.

Colon preparation. The bowel preparation procedure is similar to that used for conventional colonoscopy. However, it is more rigourous since no suction of liquid remnants is possible during capsule endoscopy, unlike during colonoscopy. Briefly, it consists of clear liquid diet on the day before capsule ingestion and 3 liters of polyethylene glycol (PEG) ingested in the evening before procedure; another liter of PEG is ingested in the next morning, then the capsule is swallowed with a glass of water (Iobagiu et al., 2008). One or two "boosters" (sodium phosphate) are administered after 1-2 hours of the capsule has entered the small bowel, followed by a laxative (bisacodyl suppository) if the capsule has not been excreted (Sieg et al., 2011). The reason for the "vigorous" bowel preparation is to maintain the colon cleanliness and to promote the capsule progression through the gastrointestinal tract.

Results

Comparison of capsule endoscopy and conventional colonoscopy. Patients included were 17 men and 11 women, aged 26 to 74 years (mean age: 56 $\pm$ 12 years) who underwent both capsule and colonoscopy examinations at the Iasi Institute of Gastroenterology and Hepatology between May 2008 – December 2010. colon preparation with PEG was similar for capsule endoscopy and colonoscopy. All CCE recordings were read by the same physician, and all colonoscopies were performed by two experienced endoscopists. For statistical analysis, colonoscopy was considered the gold standard, and per-patient comparisons were made as in other studies. No CRC were detected by CCE and colonoscopy, but colorectal polyps were found in 9 (32.1%) patients by colonoscopy and by CCE in 7 (25%) patients, the agreement between these two examinations being significant (p =0 .19) (fig. 1.10).

The sensitivity and specificity of CCE for the detection of colorectal polyps (any size) were 73.5% and 81.2%, respectively. The positive predictive value of CCE was 69.8% and the negative predictive value was 83.1%. The tolerance of CCE procedure was excellent, and no complications were reported.

Discussion. Colon capsule endoscopy was compared to conventional colonoscopy for the detection of colorectal polyps and cancer in several studies (Sieg at al., 2011). One prospective, multi-center study found that sensitivity of CCE for detecting colonic polyps, advanced adenomas, and colorectal cancer was relatively low in comparison with colonoscopy (Van Gossum et al., 2009). A low sensitivity of CCE for detection of relevant colorectal polyps also was reported in another recent published study (Pilz et al., 2010). One prospective, multi-center trial designed to compare CCE (first generation of colon capsule) and colonoscopy in asymptomatic subjects enrolled in screening or surveillance programmes for the detection of colorectal neoplasia, have found that CCE cannot replace colonoscopy as a first-line

investigation for screening and surveillance of patients at risk of cancer (Sacher-Huvelin et al., 2010). On the other hand, several studies have reported that CCE was effective in detecting colorectal polyps. Thus, in a multi-center european study of detecting colon cancers and polyps using CCE (PillCam Colon 1) compared to conventional colonoscopy found encouraging accuracy of CCE for detecting polyps and suggested that this new method for visualizing the colon may be an alternative for patients who cannot or are unwilling to undergo colonoscopy (Eliakim et al., 2009). CCE was found to be an effective method in detecting clinically relevant colonic findings in patients with an indication of conventional colonoscopy (Gay et al., 2010). Two meta-analysis (Spada et al., 2010; Rokkas et al., 2010) reported CCE to be a reasonable method for screening asymptomatic subjects for colorectal polyps.

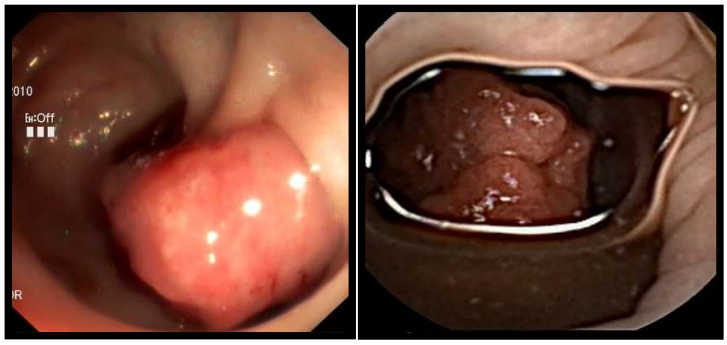

Fig. 1.10. Colonic polyp seen at colonoscopy and, respectively, at colon capsule endoscopy

The most above-mentioned studies were performed with the first generation of CCE. An improved accuracy of the second-generation CCE compared with the first-generation system (PCC1) in detecting colorectal polyps was reported (Eliakim et al., 2009). Thus, the sensitivity to diagnose patients with colorectal polyps increased from 60% in a previous study with PCC1 (Eliakim et al., 2006) to 90%, and the only reason for greater diagnostic yield was the technological improvements in the PCC2 system (Eliakim et al., 2009). Finally, it is worth to mention that, using a Markov mathematical model to simulate a comparison of CRC screening comparing CCE to colonoscopy, another study (Hassan et al., 2008) have found the cost-effectiveness of CCE potentially superior to colonoscopy.

Conclusions. Colon capsule endoscopy is a safe, minimally invasive, very well accepted by patients and which with new technological improvement could become an option for the screening of colorectal polyps and cancer.

I.1.3.3. Capsule endoscopy in unclassified inflammatory bowel disease

Background and aim. Ulcerative colitis (UC) and Crohn's disease (CD) are idiopathic inflammatory bowel diseases (IBD) with no unique, gold standard diagnostic test. UC and Crohn's colitis are in approximately 10% of cases impossible to be distinguished. The term IBD type unclassified (IBD-U) is officially proposed for the cases of chronic colitis showing overlapping endoscopic, radiological, and biopsy histological features between UC and CD, while indetermined colitis is reserved for colectomy specimen. Our aim was to evaluate the role of small-bowel capsule endoscopy (SBCE) in the diagnostic work-up of IBD-U, and impact on patients' evolution.

Material and methods. We prospectively studied all cases of IBD-U explored by SBCE in the Institute of Gastroenterology and Hepatology, tertiary referral gastroenterology care center. Patients were investigated by SBCE after contraindications were excluded. Diagnostic criteria for small bowel CD consisted in more than 3 ulcerations, irregular ulcers or

stenosis; if fulfilled, Crohn's colitis was sustained. The absence of CD features in the small bowel (SB) strengthened the assumption of UC. Follow-up data were recorded.

Results. Fifteen patients with IBD-U were explored by SBCE. Seven patients had SB lesions meeting the diagnostic criteria for CD. The remaining eight examinations showed no SB significant findings; therefore, they were classified as UC. No complications occurred. The patients were treated accordingly. Follow-up data, for a mean period of three years, recorded a case of colectomy for refractory UC.

Discussion. The term of IBD type unclassified is recommended for chronic colitis presenting with superposing and/or mixed endoscopic, radiological and/or histologic after colonic biopsies features. When a definite diagnosis cannot be established even after colectomy and post-operative histologic assessment, the term „indeterminate colitis" is proposed. At least 10% of colonic IBD patients cannot be classified as CD or UC only by colonoscopy and histological findings (Mow et al., 2004). Furthermore, studies showed that, during the course of their illness, approximately 3% of patients with UC have been reclassified as having CD, while 0.6-3% of patients initially diagnosed with CD have been reclassified as having UC (Lamb et al., 2019).

There are still questions in the literature if IBD-U represents a unique IBD phenotype or if it reflects the difficulties in diagnosis, from various reasons – atypical onset, variable clinical picture, imaging studies without definite result, or without categorical histological features.

In practice, IBD-U patients are often being reclassified as CD after identification of SB involvement, previously unknown. SBCE is a useful method in providing the presence of small-bowel lesions suggestive for CD, and, thus, to allow some of IBD unclassified patients to be recognized as CD.

Reclassification rates vary in different studies, between 16-44% (Lopes et al., 2010; Monteiro et al., 2018), according to diagnostic method and to the study population. The most frequently used diagnostic criteria are the presence of three or more SB ulcerations; due to its high ability to detect precocious ulcerative lesions, SBCE enables reclassification in a higher number of cases than non-endoscopic imaging (Di Nardo et al., 2011). In the same time, it appears that proportion of reclassification is higher when SBCE as discriminative method was administered due to a flare of underlying inflammatory disease (Cohen et al., 2008). Taking into account only the cases with definite CD after SBCE, we found a reclassification rate of 46%.

Even if negative SBCE cannot completely rule out CD, our follow-up data showed that in the lack of SB lesions, reclassifying patients as UC and treating them accordingly correlated with a favorable outcome. There was a single exception of refractory UC which finally needed colectomy, but it was related to the intrinsic UC evolution, and not to the diagnostic process; otherwise, histological postoperative analysis was consistently with UC.

Further studies are needed in order to understand better the natural history of IBD-U. Even if some cases are due to a primary misdiagnosis in early stages, or incomplete investigations, other cases are special and challenging by their atypical phenotype. Capsule endoscopy studies may contribute by a better description in terms of localization, extension and severity. Nevertheless, cautions are required due to contraindications of CE – extensive Crohn's disease, suspected stenosis, or previous complex surgery. In this regard, patency capsule is an adjunctive tool which could assure a safe CE examination.

Conclusions. SBCE is a useful safe tool in the management of IBD unclassified. Due to its diagnostic valences, SBCE plays a particular role in the process of proper clinical decision-making. Refining diagnosis and treatment regimens are the benefits of supplementary data brought by CE studies.

Chapter 2
ADVANCES IN THE APPROACH OF CHRONIC LIVER DISEASES - FROM SCREENING TOWARDS ERADICATION AND BEYOND

I.2.1. GLOBAL ELIMINATION OF HEPATITIS C VIRUS – EVERY JOURNEY BEGINS WITH A FIRST STEP

I.2.1.1. Introduction

Chronic hepatitis C virus (HCV) infection represents nowadays a public health problem, but also a socio-economic burden. The global estimated viremic HCV prevalence is around 1%, corresponding to a number of 71 million positive persons worldwide. The mean prevalence in Europe is 0.65%, and Romania occupies the first place with 2.5% HCV prevalence, corresponding to 550,000 patients with positive viral loads (Han et al., 2019). Due to the asymptomatic character of the infection, most people are unaware of HCV positivity, are not tested, diagnosed and treated on time, in this way spreading the virus and discovering their condition when in advanced stages of liver disease. An estimation of the number of people infected with HCV in the population is very important for the health policy of a given country. This allows planning of preventive and therapeutic interventions, and also determines the need for treatment of infected persons.

The prevalence of anti-HCV antibodies in populations from Central and Eastern Europe varies between 0.27 and 3.5%, the number of people infected with HCV in the general population being approximately 1.16 million (Madalinski et al., 2015). Launched in 2016 by the World Health Organization (WHO) and worldwide known, the Global Sectorial Strategy of Health for elimination of Viral Hepatitis as a threat for the health status of the population by 2030 has ambitious objectives: decrease of the incidence of the viral hepatitis by 90%, diagnosis of 90% of the infected people, access to therapy to 80% of the diagnosed and eligible persons, reduction with 65% of the liver mortality through integrated actions of awareness, testing and access to treatment.

The ambitious goal of eliminating viral hepatitis as a public health problem by 2030 will require major efforts to increase the screening rates and consequently the diagnosing rates, as well as to link the HCV positive patients to care in Romania, similar to other countries. With direct-acting antiviral agents (DAAs) therapy and its ≥95% cure rate, HCV elimination is clearly achievable. Increased treatment coverage and excellent sustained virological response (SVR) even in the later stages of liver disease have the greatest short-term impact on reducing morbidity and mortality, but treatment of any fibrosis stage (including F0-F1 fibrosis stage) is necessary to achieve reductions in total viremic infections and prevent ongoing transmission. Similar to other European countries, removing HCV treatment reimbursement restrictions in 2020 in Romania achieved great progress towards HCV elimination (Gheorghe et al., 2020).

Micro-elimination, by targeting smaller and clearly delineated HCV risk groups, allows faster and better delivery of interventions. Development of possible micro-elimination scenarios breaking down national elimination goals into individual population segments enables policy makers to understand current disease landscapes on a hospital-based or regional level (Lazarus et al., 2018). Thus, smaller scale policy initiatives targeting specific populations or localities are a tangible step towards achieving global elimination of HCV.

In this direction, I would like to mention as personal contributions the participation in two major screening projects, with recently published results regarding the prevalence and the risk factors for HCV. The first one is a national, multicentric hospital-based population

screening program, and the results following the prospective data gathering were systematized under the name of HepC ALERT study (Gheorghe et al., 2020). In total, 25.141 subjects signed the informed consent and were consequently enrolled into the study. The prevalence of anti-HCV antibodies, according to a rapid diagnostic test, was 1.39%. There was a positive association with female gender, rural area of residence, and advanced age ($p<0.001$ for each factor), as well as negative association with the education level ($p<0.001$). The second project is a micro-elimination project carried out in a rural area in North-Eastern Romania; following the screening, we found a 2.64% HCV prevalence (Huiban et al., 2021), higher than previously reported in the hospitalized population by Gheorghe et al., in 2020, explained probably by the aging population in rural communities, deficitary hygiene conditions, lifestyle and difficult access to a specialized hepato-gastroenterology center. The linkage-to-care analysis in our study showed high rates of treatment completion (98%) and sustained virological response (99%).

Regarding the linkage-to-care concept, it was previously debated in one of our articles from a socio-economic perspective, showing that the lack of treatment carries an important social burden and implies finally additional resources (Cuciureanu et al., 2018). In this study, carried out in a period when the access to treatment was granted only for advanced stages of fibrosis, we aimed to highlight the perception of untreated patients with HCV on the healthcare system and professionals involved in care or therapy as well as the social impact on this particular category. We included 140 patients with chronic HCV infection which were investigated using a designed satisfaction survey that consisted of 13 questions. Our study highlights that patients with chronic HCV infection have a significant degree of impairment in quality of life. The majority of the study lot consider that the delay in receiving viral treatment is also an economic burden on the healthcare system due to frequent visits to the physician and hospitalizations needed for the periodical evaluation of their liver disease progression.

The standard-of-care for the aaproaach of HCV has been continuosly evolved. Until recently, the treatment relied on various combinations of therapeutic agents, which were promising in terms of gain of response, but which were coming with their respective adverse events and limitations. Every step in the progress of the HCV eradication represented a major preoccupation, reflected in papers or book chapters. I co-authored two chapters dedicated to the treatment with the first direct antivirals (such as boceprevir) in naive genotype 1b patients, showing the benefits and the limitations (in « Actualities in the diagnostic and the treatment of viral chronic hepatitis » edited by Mircea Grigorescu, Carol Stanciu, Editura Medicala Universitara Iuliu Hatieganu Cluj-Napoca, two volumes, 2011 and 2012).

Fortunately, nowadays, the tremendous progresses in the antiviral medication are the premises of a brighter future. The development of direct-acting antiviral agents has completely transformed the management of this disease. Aiming to synthetize the current status, we underlined in a review recent developments, current limitations and future challenges, showing that current treatment regimens, with their high effectiveness and safety profiles, have changed patient perception of HCV infection from a disease that requires complex evaluation and long-term monitoring to a disease that can be cured (Stanciu et al., 2021).

However, there are special groups of patients which require additional expertise and multidisciplinary care. In this regard, I would like to mention the co-editing of a thematic book entitled ***"Renal connections in liver diseases"***, published by "Gr. T. Popa" University of Medicine and Pharmacy in 2017, covering various aspects on the topic of the associations of renal and liver pathologies, and answering to the most important questions regarding the interdisciplinary management of these complex patients.

PERSONAL CONTRIBUTIONS IN THE FIELD OF SCREENING AND THERAPEUTIC APPROACH OF HEPATITIS C

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I.2.1.2 Hepatitis C virus prevalence and risk factors in a village in North-Eastern Romania: a population-based screening -the first step to viral micro-elimination

Background and aim. Micro-elimination allows faster and targeted delivery of interventions. A necessary condition to apply treatment strategies is o identify persons who need them, fact that could represent a real challenge if they are asymptomatic. We aimed to assess the prevalence of HCV in a rural population from Moldavia, the Romanian region with the highest prevalence of HCV (Gheorghe et al, 2010) and to link the population to antiviral treatment. In this integrated project of testing-diagnosis-treatment, we established as objectives the following: micro-elimination of HCV, prevention of advanced HCV liver disease, prevention of HCV transmission among the healthy population and updating the epidemiological data regarding HCV in this region.

Materials and methods. We conducted a single-center prospective study based on the strategy of a project designed to educate, test and treat HCV with the aim of eliminating HCV infection in all adults in a village in the Moldavia region in north-eastern Romania - a region

considered to have a poor socio-economic level and with limited access to the healthcare system. Our project was carried out from 1 March 2019 to 28 February 2020. To achieve these objectives, it was necessary to include all stakeholders - the local government authorities, associations of healthcare providers and patients. A mobile team of gastroenterologists, residents and nurses from the Institute of Gastroenterology and Hepatology, "St Spiridon" Hospital Iasi was created, undertaking community mobilization with the aid of the local leadership (mayor, village council, general practitioners, teachers and priest). The entire population of the village over the age of 18 was invited to be tested by direct door-to-door communication. In some particular situations, the testing was organized at a household level, in order to perform screening at the level of the whole village.

The screening was carried out using rapid diagnostic orientation tests for HCV diagnosis and was performed for all subjects. All patients with anti-HCV antibodies were referred to a tertiary gastroenterology and hepatology department to confirm the active infection, staging and treatment prescription (linkage-to care). All demographic data were assessed through a questionnaire (Table 2.I).

The study was approved by the National Ethics Committee, and written informed consent was obtained from each patient in accordance with the principles of the 1975 Declaration of Helsinki.

Statistical Analysis. The collected data were statistically analyzed using the SPSS 20.0 (Chicago, IL, USA) software. The prevalence of anti-HCV antibodies subjects was calculated with a 95% confidence interval (CI). Groups were compared using the Chi-square test or Fisher's exact test for categorical variables and by the independent t Student test or Mann–Whitney U test for continuous variables (depending on data distribution). Most of the investigated variables were calculated using logistic regression and odds ratio (OR) together with the corresponding 95% CI. All statistical tests were two-tailed, with a p-value ≤ 0.05 considered statistically significant.

Results.

The study population consisted of 1973 females and 972 males. The mean age of subjects was 56.1 $\pm$ 14.5 years. Detailed baseline demographic characteristics of the study cohort are presented in Table 2.II.

Prevalence of HCV infection. All the village inhabitants - in total, 3507 subjects - were invited to be screened by rapid diagnostic orientation tests. Of these, 2945 (84%) subjects signed the informed consent and were consequently tested and enrolled in the study. The prevalence of positive HCV antibodies in the rural population that presented for testing was 2.64% (Figure 2.1).

Overall, the distribution of subjects according to gender was similar for both negative and positive HCV antibodies statuses. Regarding age groups, the prevalence of HCV antibodies was found to be higher in those between 80–97 years (30.8% versus 14.1%). Furthermore, the prevalence of HCV was higher in widowed subjects (85.9% versus 12.3%) and lower in those married or living together as a couple (14.1% versus 87.7%). According to social and educational status, the prevalence of positive HCV Ab was lower in employees (7.7% versus 27.2%) and those with university studies (6.4% versus 12.5%).

Cascade of care. All subjects with positive HCV antibodies (78, respectively 2,64%) were scheduled for further complete evaluation in a tertiary gastroenterology and hepatology center nearby in order to be linked to care. In total, 66 (85%) subjects were presented for evaluation, and 55 (83%) subjects had detectable HCV RNA. Of these, 54 (98%) patients completed antiviral treatment and 53 (99%) obtained SVR. The cascade of care is shown in Figure 2.2.

Table 2.I. Addressed questionnaire in screened subjects

Questions
Part 1 – Demographic data
1. Age: o 18-29 years, o 30-39 years, o 40-49 years, o 50-59 years, o 60-69 years, o 70-79 years, o 80-97 years
2. Sex
- male
- female
3. Residence
- rural
- urban
4. Etnicity
- romanians
- roma
- other
5. Educational status
- subjects without university studies
- subjects with university studies
6. Marital status
- widowed or divorced
- married or living together as a couple
7. Social status
- retired
- housewive
- employee
Part 2 – Risk factors for HCV infection (answer from the questionnaires)
1. Previous known HBV / HDV Yes/No
2. Known HCV (+) family members Yes/No
3. Professional exposure to blood products Yes/No
4. Blood transfusions before 1992 Yes/No
5. Abortion before 1990 Yes/No
6. Multiple surgeries Yes/No
7. Multiple dental interventions Yes/No
8. Hemodialysis Yes/No
9. Sexual contacts with multiple / unknown partners Yes/No
10. IV (intravenous) drugs Yes/No
11. Tatooing / piercing Yes/No
12. Sharing personal hygiene objects Yes/No

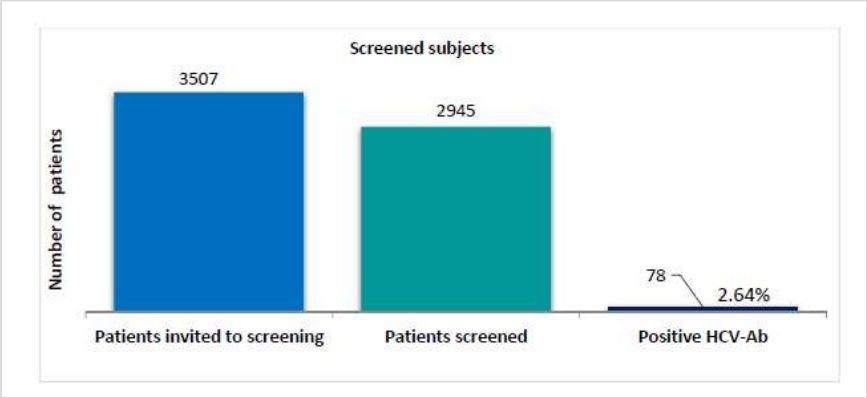

Fig. 2.1. Results of screened participants and prevalence of positive HCV antibodies in the rural population

Table 2.II. Baseline demographic characteristics

	Variable	Patients with Negative HCV Ab (n = 2867)	Patients with Positive HCV Ab (n = 78)	p-Value
Sex, n (%)				
	Male	947 (33)	25 (32.1)	0.815
	Female	1920 (67)	53 (67.9)	
Age, years, n (%)				
	18-29	284 (9.9)	1 (1.3)	0.163
	30-39	211 (7.4)	4 (5.1)	
	40-49	358 (12.5)	6 (7.7)	
	50-59	552 (19.2)	11 (14.1)	
	60-69	614 (21.4)	14 (17.9)	
	70-79	443 (15.5)	18 (23.1)	
	80-97	405 (14.1)	24 (30.8)	
Marital status, n (%)				
	Widowed or divorced	353 (12.3)	67 (85.9)	0.037
	Married or living together as a couple	2514 (87.7)	11 (14.1)	
Social status, n (%)				
	Retired persons	1277 (44.5)	44 (56.4)	0.26
	Housewives	810 (28.3)	28 (35.9)	
	Employees	780 (27.2)	6 (7.7)	
Educational status, n (%)				
	Subjects without university studies	2510 (87.5)	73 (93.6)	0.188
	Subjects with university studies	357 (12.5)	5 (6.4)	

Association between risk factors and chronic HCV infection. According to the addressed questionnaire, three (3.84%) patients were identified with a history of HBV/HDV, five (6.41%) had a history of HCV infection, eight (10.25%) individuals had undergone abortions before 1990, six (7.69%) had experienced multiple surgeries, four (5.12%) had blood transfusions before 1992, eleven (14.10%) patients had multiple dental interventions, two (2.56%) declared sexual contacts with multiple partners, one (1.28%) was using intravenous drugs, three (3.84%) patients had undergone tattooing/piercing procedures, eight (10.25%) shared personal hygiene objects, and no patients had professional exposure to blood products or hemodialysis (Table 2.III). The main risk factors associated with chronic HCV infection were a family history of HCV (OR = 2.23, 95%CI = 1.37–3.5, $p < 0.0001$), blood transfusions performed before 1992 (OR = 3.21, 95%CI = 2.25–4.52, $p < 0.0001$), abortions conducted before 1990 (OR = 1.35, 95%CI = 1.02–1.9, $p = 0.023$), multiple surgical interventions (OR =

1.32, 95%CI = 1.05–1.72, p = 0.038) and sharing personal hygiene objects (OR = 1.45, 95%CI = 1.12–1.73, p = 0.002).

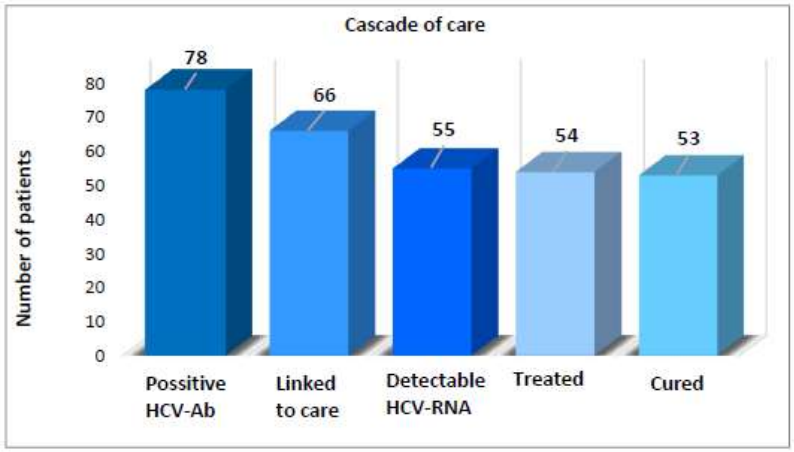

Fig. 2.2. Cascade of care step by step. Positive HCV Ab: Number of people estimated to have viremic HCV infection. Linked to care: Number of patients evaluated for treatment. Detectable HCV-RNA: Number of patients who received a diagnosis of viremic HCV infection. This number excludes patients who were cured of their infection or who had experienced the spontaneous clearance of their infection before 2019. Treated: Number of patients who initiated HCV treatment (all types of treatment, interferon-based regimens). Cured: Number of patients who obtained SVR.

Table 2.III. Risk factors associated with chronic HCV infection

Risk Factors	HCV Negative (N = 2867) n (%)	HCV Positive (N = 78) n (%)	OR	95% CI	p-Value
Known HBV/HDV	46 (1.60)	3 (3.84)	0.62	0.30–1.31	0.302
Known HCV (+) family members	47 (1.63)	5 (6.41)	2.23	1.37–3.50	0.0001
Professional exposure to blood products	78 (2.72)	0 (0.00)	0.25	0.11–0.53	0.0001
Abortion before 1990	141 (4.91)	8 (10.25)	1.35	1.02–1.90	0.023
Multiple surgeries	83 (2.89)	6 (7.69)	1.32	1.05–1.72	0.038
Blood transfusions before 1992	45 (1.56)	4 (5.12)	3.21	2.25–4.52	0.0001
Multiple dental interventions	67 (2.33)	11 (14.10)	1.12	0.67–1.45	0.303
Hemodialysis	33 (1.15)	0 (0.00)	0.34	0.06–1.03	0.062
Sexual contacts with multiple partners	133 (4.63)	2 (2.56)	0.88	0.52–1.38	0.615
Intravenous drugs	78 (2.72)	1 (1.28)	0.71	0.40–1.44	0.302
Tattooing/piercing	81 (2.82)	3 (3.84)	1.25	0.72–1.84	0.251
Sharing personal hygiene objects	103 (3.59)	8 (10.25)	1.45	1.12–1.73	0.002

OR, odds ratio; CI, confidence interval; HBV, hepatitis B virus; HDV, hepatitis D virus

In our subjects, we observed that the main risk factors associated with chronic HCV infection were a family history of HCV (OR = 2.23, 95%CI = 1.37–3.5, p < 0.0001), blood transfusions performed before 1992 (OR = 3.21, 95%CI = 2.25–4.52, p < 0.0001), abortions conducted before 1990 (OR = 1.35, 95%CI = 1.02–1.9, p = 0.023), multiple surgical interventions (OR = 1.32, 95%CI = 1.05–1.72, p = 0.038) and sharing personal hygiene objects (OR = 1.45, 95%CI = 1.12–1.73, p = 0.002).

Discussion. The global elimination of HCV has become the ultimate endeavor and final objective since the introduction of DAAs. However, the simple availability of these drugs, which can reduce the burden of HCV infection, is not enough to achieve a real impact on morbidity and mortality, much less to target viral eradication (Maticic et al, 2020).

A more pragmatic approach is the concept of "micro-elimination", which involves the

elimination of hepatitis C in defined segments of the risk population, as well as in geographical areas (regions, cities, villages) as a strategy to incrementally achieve national elimination (Gheorghe et al, 2020).

Thus, we consider this work as a small step forward in our public health achievements for the elimination of viral hepatitis by 2030 in Romania. The success of this ambitious goal, at least in countries such as Romania, is possible only by dividing it into different micro-elimination campaigns such as the project we have carried out at a sub-regional level in a population with difficult access to the healthcare system. In this screening micro-elimination program conducted in a sub-region of Romania, the prevalence of HCV was lower (2.64%) than previously reported (Gheorghe et al, 2020).

As far as we know, there have been no similar projects in Europe carried out in rural areas. Worldwide there are several HCV micro-elimination projects conducted at the community levels an Egyptian project in which HCV micro-elimination was also initiated in rural areas had successful outcomes; 99.9% of patients completed the antiviral treatment and 97% achieved an SVR (Shiha et al, 2020). In our study, 98% of positive screened HCV patients received antiviral therapy, 100% completed the treatment and 99% achieved SVR.

The main risk factors identified in our subject for the increased prevalence of HCV are family members known to be positive for HCV and sharing personal hygiene items. Also, subjects with abortions or blood transfusions conducted before 1990, multiple surgical interventions and who shared personal hygiene items show the effects of the inappropriate use of medical or surgical practices on the population.

The data from this study show that a real challenge for people with positive HCV antibodies in this rural area was access to a tertiary gastroenterology/hepatology center located at a distance for further evaluations.

The elimination of hepatitis C worldwide is becoming a possibility, with higher chances of success if micro-elimination strategies based on mass screening are implemented.

A sustained effort on the part of all stakeholders is required, including governmental authorities at the national and local levels, associations of healthcare providers, patients, and representatives of at-risk populations. Before attempting nationwide elimination, breaking down national elimination goals into smaller, achievable goals for individual population segments may be more realistic (Safreed-Harmon et al, 2019).

Conclusions. This micro-elimination project carried out in a rural area is the first in Romania and among a small number of such projects in the world. Micro-elimination involves fewer resources than large-scale country-level initiatives to eliminate HCV and can represent a boost for small victories that inspire large and ambitious efforts. The development of screening programs is crucial for the accessibility of treatment and the achievement of WHO objectives.

Our micro-elimination project showed a 2.64% HCV prevalence in rural areas, higher than reported by Gheorghe L, et al in the hospitalized population (Gheorghe et al, 2020). According to our findings, the HCV prevalence in Moldavia is 1.41%, much lower than that reported in another study from 2010, where the seroprevalence of HCV was higher compared to that in the Southern Region 4.06% vs. 3.26% ($p = 0.065$); the logistic regression analysis showed that the risk of having HCV is 1.26 times higher for a resident from the North-East of Romania compared to the one from the South of the country (Gheorghe et al, 2010).

The higher prevalence of HCV infection in patients from rural area in Modovia Romania could be explained by the aging population in rural communities, deficitary hygiene conditions, lifestyle and difficult access to a tertiary gastroenterology center.

I.2.2. LIVER RESHAPING AFTER VIRAL ERADICATION – EVIDENCES FROM NON-INVASIVE EVALUATION

I.2.2.1. Introduction

Traditionally, advanced liver disease, and especially cirrhosis, were considered as irreversible end stages, mostly due to lack of treatment possibilities. Nowadays, this irreversibility is no longer considered a "dogma" as liver fibrosis is potentially reversible on condition that the trigger is removed (Zoubek et al., 2017). For two decades, the most used treatment of HCV was represented by interferon (IFN)-based regimens with a modest efficacy for achieving sustained virological response (SVR), poor tolerability, and many adverse events. Instead, the oral therapy with direct acting antivirals (DAAs) is an innovation for the treatment of HCV infection, with a successful viral eradication in over 95% of patients across different genotypes, offering a good safety profile with minimal side effects (Stanciu et al., 2015).

Not only ***liver fibrosis***, but also ***steatosis*** are prognostic factors for liver disease in HCV-infected patients. Hence, SVR obtained by using DAAs therapy has been linked with liver fibrosis regression and consequently with a reduction in decompensation, other liver-related complications and mortality (Kogiso et al., 2018). In the same time, data from literature are limited and contradictory regarding steatosis (Ito et al., 2003; Zhang et al., 2009), and currently there are still unanswered questions about fatty liver dynamics in patients with C hepatitis.

Liver biopsy is the histological gold standard for inflammation grading and fibrosis stage assessment, while the definition of steatosis relies on pure histological changes. Nevertheless, because of its limits and disadvantages (requires expertise, has morbidity and mortality risks), **non-invasive methods** have been developed and are used nowadays successfully.

In this context, I will firstly present two relevant studies with the results of steatosis evaluation by ***controlled attenuation parameter,*** as well as of fibrosis evaluation by liver stiffnes measurement by ***transient elastography***, in patients with chronic hepatitis C infection treated with direct-acting antivirals therapy who achieved sustained virological response. We found that, after viral eradication, even if most patients presented normalization of aminotranspherases levels, trygliceride level increased significantly from baseline to SVR, and there also was an increase of the hepatic steatosis (Trifan et al., 2022). Thus, a long-term follow-up is mandatory to identify HCV-infected patients with hepatic steatosis post-SVR and the risk factors for more severe outcomes. We also demonstrated that achieving SVR was associated with significant improvement of liver stiffness measured by transient elastography, but the regression of fibrosis must be constantly evaluated in dynamic (Trifan et al., 2021).

The last two presented studies are dedicated to the non-invasive evaluation by **computed tomography** of patients with compensated hepatitis C - related cirrhosis following SVR after direct-acting antivirals, in terms of liver remodelling and muscle mass dynamics. Analysing CT examinations performed on 56 patients before and after antiviral treatment, we found that SVR in patients with HCV-related compensated cirrhosis treated with DAAs is associated with some improvements of hepatic morphology detectable by CT, the most constant being the increase of right hepatic vein diameter, which is significantly wider after treatment (parameters median: 8.12 mm; IQR: 4.20) than before treatment (median: 6.36 mm; IQR: 3.94, $p < 0.001$) (Mihai et al., 2020). Improvement in muscle mass was demonstrated in cirrhotic patients presenting with baseline sarcopenia following SVR, using as measurement tool the L3 skeletal muscle index (Mihai et al., 2021). Reduction of splenic volume was also demonstrated after SVR (Mihai et al., 2020).

As additional subdomain of interest, I would like to mention the major controverse regarding the risk of hepatocellular carcinoma (HCC) after viral eradication. In a study analysing 483 patients with a median observation period of 540 days, we found an increased, but not significant probability of HCC development in the elderly patients (5.3%) compared

to the young patients (1.8%), significantly correlated with the alpha fetoprotein value and the edge of decompensation (Trifan et al., 2021). However, in another single center observational study we found no evidence of a more aggressive pattern of HCC after viral eradication (Muzica et al., 2020).

PERSONAL CONTRIBUTIONS IN THE AREA OF NON-INVASIVE LIVER EXPLORATION FOLLOWING VIRAL ERADICATION

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BOOK CHAPTER
Utilizarea tomografiei computerizate in evaluarea pacienților cu ciroza hepatica. Mihai F, Trifan A, **Singeap AM**, Cojocariu C, Negru D. NOI CONCEPTE ÎN GASTROENTEROLOGIE SI HEPATOLOGIE. Editura „Gr. T. Popa" U.M.F. Iași, 2016, ISBN 978-606-544-430-0.

I.2.2.2. Changes in liver steatosis using *Controlled Attenuation Parameter* among patients with chronic hepatitis C infection treated with direct-acting antivirals therapy who achievedsustained virological response

Background and aim. The prevalence of liver steatosis among HCV-infected patients is about 55%, ranging from 40 to 86 % in different genotypes (Del Campo and Romero-Gomez, 2009). Moreover, approximately 10% of the patients with chronic HCV-infection, have characteristics of non-alcoholic steatohepatitis. The type of hepatocyte steatosis in HCV patients depend on their HCV genotype (GNT).

In patients with HCV-infection, GNT 3 is considered to be a steatogenic factor leading to a "viral steatosis" caused by the cytopathic effect induced by the suppression of viral proteins (Adinolfi et al., 2001).

In the case of HCV non-3 genotypes the host factors such as age, gender, higher body mass index (BMI) or insulin resistance add on to the development of the "metabolic steatosis" (Rubbia-Brandt et al., 2000). Therefore, both viral and host metabolic-related factors are involved in steatosis development, and the routine evaluation of the severity of hepatic fibrosis and steatosis must be a part of the management of HCV-infected patients after completion of DAAs therapy (Hezode, 2018).

Recent epidemiological studies have suggested that the complex interactions between HCV and glucose metabolism lead to a higher prevalence of type 2 diabetes mellitus (T2DM) (Hammerstad et al., 2015). Furthermore, in patients with a higher BMI (BMI $\geq$25 kg/m2), the presence of chronic HCV infection increases the risk to develop T2DM by 11-fold. The main mechanism by which HCV infection induces T2DM is insulin resistance (IR). HCV infection alters the hepatocyte insulin-signaling pathways and enhances the release of proinflammatory cytokines and the serine phosphorylation of the insulin receptors, which contribute to IR (Bose et al., 2012).

Liver biopsy (LB) still remains the gold standard method for assessing the hepatic fibrosis and steatosis; however, LB is not a routinely used technique because of several limitations such as intra- and interobserver variability, sampling errors, poorly tolerability by the patient and high cost (Regev et al., 2002; Trifan et al., 2012). Also, LB is associated with risk of rare, but potentially life-threatening complications (Szymczak et al., 2012).

Vibration-controlled transient elastography (VCTE) with controlled attenuation parameter (CAP) represents a non-invasive technique for the assessment of liver fibrosis and steatosis (Sasso et al., 2010). The advantages of this technique are represented by quickness, painfulness, and easiness to perform, with great repeatability and reproducibility, and has been successful implemented in clinical practice. CAP is determined simultaneously with liver stiffness measurements (LSM) using the same ultrasonic signal probe and measures the coefficient of attenuation of the liver at 3.5 MHz frequency (Vuppalanchi et al., 2018).

Several recent studies found a significant regression of liver fibrosis after DAA treatment, but on the other hand, data from literature are limited and contradictory regarding steatosis (Kobayashi et al., 2018; Sadeghi et al., 2021). Hence, this study evaluated changes in liver steatosis using CAP in patients with HCV infection who achieved SVR, after 12 weeks of therapy completion.

Materials and methods

Patients. This prospective study enrolled 280 patients with HCV infection, who were treated with DAAs between November 2019 and November 2021 in a tertiary referral hospital in North-East Romania. Eligibility criteria were represented by: (1) age > 18 years; (2) detectable HCV RNA before treatment; (3) the achievement of a sustained virological response at 12 weeks (SVR12) after end-of treatment; (4) patients without any history of liver cancer at the baseline or during the follow-up period; (5) assessment of liver fibrosis and steatosis using VCTE at the baseline and at SVR12.

The study was performed in accordance with the principles of the Declaration of Helsinki and was approved by the Ethics Committee of our Institute. Each individual signed an informed written consent.

Clinical and laboratory assessment. Demographic and clinical details were collected including sex, age, alcohol consumption, body mass index (BMI), the presence of diabetes (taking anti-diabetic drugs or fasting glucose ≥ 126 mg/dL), and arterial hypertension (antihypertensive drugs use, systolic blood pressure ≥ 140 mmHg, or diastolic blood pressure ≥ 90 mmHg). Fasting blood tests were collected at inclusion and 12 weeks after treatment, including hemoglobin, platelet count, international normalized ratio (INR), fibrinogen, alanine aminotransaminase (ALT), aspartate aminotransferase (AST), γ-glutamyl transpeptidase (GGT), alkaline phosphatase (ALP), direct and total bilirubin (DB, TB), albumin, total proteins, urea, serum creatinine, total cholesterol, triglycerides, low-density lipoprotein (LDL-c), high-density lipoprotein (HDL-c), ferritin, c-reactive protein (CRP), α-fetoprotein, and serum HCV-RNA level.

BMI was calculated using height and weight. Weight class was defined using standard cut-offs (normal 18.5 to <25 kg/m2; overweight 25 to <30 kg/m2; obesity ≥ 30 kg/m2), as endorsed by WHO classification. Weight change was defined as the difference between weight at the baseline and at SVR12. The weight gain was considered as weight change ≥ 1 kg from the baseline to SVR12, and this was chosen to be clinically significant (Schlevogt et al., 2021).

VCTE examination. The patients included in our study were examined for liver fibrosis and steatosis using the FibroScan® 502 touch model (Echosens, Paris, France) equipped with the M-(standard) or XL-(obese) probe by a single operator with more than 300 determinations in VCTE practice. After at least four hours of fasting, patients were placed in the supine position with the right arm in maximum abduction, leading to a greater intercostal window to the right lobe liver scanning. The examination was first performed using the M-probe with a transducer frequency of 3.5 MHz, while the XL-probe (2.5 MHz) was automatically used at machine indication if the distance between skin-to-liver capsule was higher than 25 mm. The criteria for a reliable measurement were considered to be if 10 acquisitions were made with an interquartile range divided by the median (IQR/M), which does not exceed 30%. The LSM and CAP measurement were performed at the baseline and at SVR12 (Castera et al., 2005).

In accordance with CAP measurement, which is a quantitative method and is expressed in decibel-milliwatts (dB/m) ranging from 100 to 400 dB/m, the cut of values for mild (S1), moderate (S2), and severe steatosis (S3) were as follows: ≥248 dB/m, ≥268 dB/m, and ≥280 dB/m, respectively (Karlas et al., 2017). Regarding LSM, we categorized the cut-off for staging liver fibrosis according to the Metavir scoring system: F0 (no fibrosis) ≤ 5.5 kPa; F1 (mild fibrosis) ≤ 6.9 kPa; F2 (significant fibrosis) ≥7.0 kPa; F3 (advanced fibrosis) ≥ 9.5 kPa; F4 (cirrhosis) ≥ 12.5 kPa. LSM results were expressed in kilopascals (kPa) ranging from 1.5 to 75 kPa (Wong et al., 2010).

Statistical analysis. All data were analyzed using SPSS software (IBM SPSS Inc, Chicago, IL, USA, version 22.0). Continuous variables are expressed as median and interquartile/median range (IQR) or as mean ± standard deviation (SD). The Mann–Whitney test was performed to assess the difference in CAP values according to the weight gain at SVR. For comparing group's variables, Student t-tests were used for continuous variables, or the Chi-squared test was used for comparing qualitative data as appropriate. Statistical significance was taken as $p < 0.05$ (two-tailed).

Results

Patients' characteristics. A total of 280 HCV-infected patients who fulfilled the inclusion criteria were evaluated between November 2019 to November 2021. At the baseline, the mean age was 59.91 ± 12.185 years, mostly females (67.1%), and BMI was 26.96 ± 4.15 kg/m2. Before the eradication of HCV infection, 173 patients (61.78%) had different stages of liver

steatosis. All patients were treated with DAAs and obtained SVR. No patients had previous DAA treatment. Regarding the oral therapy used for the treatment of HCV infection, ombitasvir/paritaprevir/ritonavir + dasabuvir were used in 66.4% of patients and sofosbuvir/ledipasvir in 33.6% of patients.

Changes in CAP and LSM. The median (IQR) LSM and CAP values before treatment were 9.9 ± 3.7 kPa and 225 ± 48.28 dB/m, respectively. According to the baseline LSM values, the grades of liver fibrosis were as follows: F0 in 21.8% of patients, F1 in 15% of patients, F2 in 20.7% of patients, F3 in 13.9% of patients, and F4 in 28.6% of patients. Regarding the steatosis degree at the baseline, the results were as follows: S0 in 38.2% of patients, S1 in 16.5% of patients, S2 in 20.7% of patients, and S3 in 24.6% of patients (Table 2.IV). Moreover, in the overall cohort, the LSM decreased from baseline value 9.9 ± 5.89 (5.2–15.4) kPa to 8.79 ± 6.63 (3.8–12.1) kPa at SVR12, a decline that is considered significant. The LSM reduction was observed in all drug regimens (Figure 2.3). The median value of CAP increased after HCV eradication. One hundred eighty-six patients (66.4%) had steatosis, with a mean CAP score of 257 ± 65.49 compared to a mean CAP score of 225 ± 48.25 dB/m at the baseline ($p < 0.0001$) (Figure 2.4).

Changes in clinical and biological parameters. The factors that were associated with an increase in CAP values from the baseline to SVR are represented by impaired fasting glucose (fasting plasma glucose at baseline 106.37 ±18.86 mg/dL vs. 114.96 ± 47.19 mg/dl at SVR, $p < 0.024$), triglycerides (124.03 ± 113.49 mg/dL vs. 153.78 ± 94.53 mg/dL, $p < 0.004$), cholesterol (191.61 ± 67.19 mg/dL vs. 216.52 ± 50.85, $p = 0.031$), and higher body mass index (26.96 ± 4.15 kg/m2 vs. 27.87 ± 4.23 kg/m2). After treatment, ALT and AST decreased to normal levels at SVR12 compared to the baseline (28.72 ± 24.71 U/L vs. 40.72 ± 27.34 U/L for ALT, $p < 0.013$ and 27.21 ± 11.15 U/L vs. 33.35 ± 23.37 U/L for AST, $p < 0.029$). The BMI increased at SVR12 compared to the baseline (27.87 ± 4.23 kg/m2 vs. 26.96 ± 4.15 kg/m2, $p = 0.042$) (Table 2.IV). In addition, 156 patients (56%) had impaired fasting glucose after the completion of treatment with DAAs, and 34 (22%) patients had a BMI ≥ 25 kg/m2. Regarding the patients with impaired fasting glucose, 125 (80%) of them had CAP values ≥ 248 dB/m.

Clinical and biological parameters in patients with liver steatosis at SVR12 and in those with no hepatic steatosis at SVR12. The patients with steatosis had higher LSMs (7.1 ± 1.5 kPa; SVR12- 8.3 ± 3.8 kPa, $p = 0.038$), while patients without steatosis did not have higher LSMs (7.5 ± 1.4; SVR12 5.5 ± 1.2 kPa, $p < 0.0001$) (Table 2.V).

Thus, 38.2% of the patients with steatosis had advanced fibrosis (F3), while patients without steatosis did not have advanced fibrosis (Figure 2.5).

At SVR12, both weight and BMI increased in patients with steatosis (73.1 ± 11.21 kg vs. 85.05 ± 10.4 kg, $p = 0.006$, respectively, 25.19 ± 5.17 kg/m2 vs. 29.23 ± 4.51 kg/m2, $p = 0.003$).

Evaluation at SVR12 also showed that the levels of transaminases decreased in patients with or without steatosis, both ALT levels (45.6 ± 50.8 U/L to 20.3 ± 4.5 U/L in patients with steatosis, $p < 0.0001$, and 57.78 ± 42.1 U/L to 19.4 ± 12.5 U/L in patients without steatosis, $p < 0.0001$, respectively), and AST levels (36.3 ± 35.6 U/L to 18.3 ± 4.4 U/L, $p < 0.0001$, and 71.3 ± 58.7 U/L to 21.8 ± 10.8 U/L, $p < 0.0001$, respectively); and alkaline phosphatase (81.4 ± 42.1 U/L to 60.8 ± 24.5 U/L, $p = 0.01$, and 79.5 ± 22.8 U/L to 61.2 ± 18.4 U/L, $p = 0.05$).

In a univariate regression model, fasting plasma glucose, increased cholesterol and triglycerides levels, and a higher BMI were significantly associated with an increase in CAP values between the baseline and 12 weeks after completion of DAAs therapy.

Multivariate linear regression analysis showed that BMI ($\beta = 0.328$, $p < 0.001$) and triglycerides ($\beta = 0.148$, $p = 0.017$) were independent risk factors associated with the CAP score in all patients.

Table 2.IV. Patients characteristics

	Overall Cohort *n* (%)	**Characteristics Baseline**	**Characteristics SVR 12**	
Variable	Overall (*n* = 280)			***p*-value**
Gender (female), *n* (%)	188 (67.1)			
Age, yr.	59.91 ± 12.185			
BMI (kg/m^2)	27.13 ± 3.62	26.96 ± 4.15	27.87 ± 4.23	0.042
HGB (g/dl)	13.23 ± 1.67	13.04 ± 1.48	13.32 ± 1.56	0.651
Platelet count (G/L)	192.18 ± 66.18	188.42 ± 71.39	193.53 ± 68.23	0.798
ALT (IU/L)	30.24 ± 26.76	40.72 ± 27.34	28.72 ± 24.71	0.013
AST (IU/L)	31.77 ± 22.61	33.35 ± 23.37	27.21 ± 11.15	0.029
GGT (IU/L)	41.89 ± 48.91	40.64 ± 31.07	43.33 ± 38.03	0.237
ALP (IU/L)	80.70 ± 36.92	76.67 ± 30.65	79.37 ± 35.01	0.709
Total bilirubin (mg/dL)	0.72 ± 0.68	0.75 ± 0.39	0.69 ± 0.38	0.465
Albumin (g/dL)	4.56 ± 0.38	4.53 ± 0.44	4.57 ± 0.36	0.559
Creatinine (mg/dL)	0.73 ± 0.13	0.71 ± 0.13	0.73 ± 0.13	0.505
Urea (mg/dL)	36.56 ± 10.81	34.40 ± 37.08	37.08 ± 11.22	0.188
Fasting glucose (mg/dL)	111.37 ± 43.77	106.37 ± 18.86	114.96 ± 47.19	0.024
Total cholesterol (mg/dL)	211.68 ± 55.04	191.61 ± 67.19	216.52 ± 50.85	0.031
Triglycerides (mg/dL)	148 ± 98.78	124.03 ± 113.49	153.78 ± 94.53	0.004
CAP dB/m	293 (245.5–339)	225 ± 48.28	257 ± 65.49	<0.001
CAP ≥ 248 dB/m *n* (%)		173 (61.8%)	186 (66.4%)	
Steatosis degree				
S0 *n*(%)		107(38.2%)	94 (33.6%)	
S1 *n*(%)		46 (16.5%)	56 (20%)	
S2 *n* (%)		58 (20.7%)	62 (22.1%)	
S3 *n* (%)		69 (24.6%)	68 (24.3%)	
Fibrosis score (mean ± SD)		9.98 ± 5.89	8.79 ± 6.63	0.019
Fibrosis stages				
F0 *n* (%)		61 (21.8%)	72 (25.7%)	
F1 *n* (%)		42 (15%)	54 (19.3%)	
F2 *n* (%)		58 (20.7%)	47 (16.8%)	
F3 *n* (%)		39 (13.9%)	44 (15.7%)	
F4 *n* (%)		80 (28.6%)	63 (22.5%)	

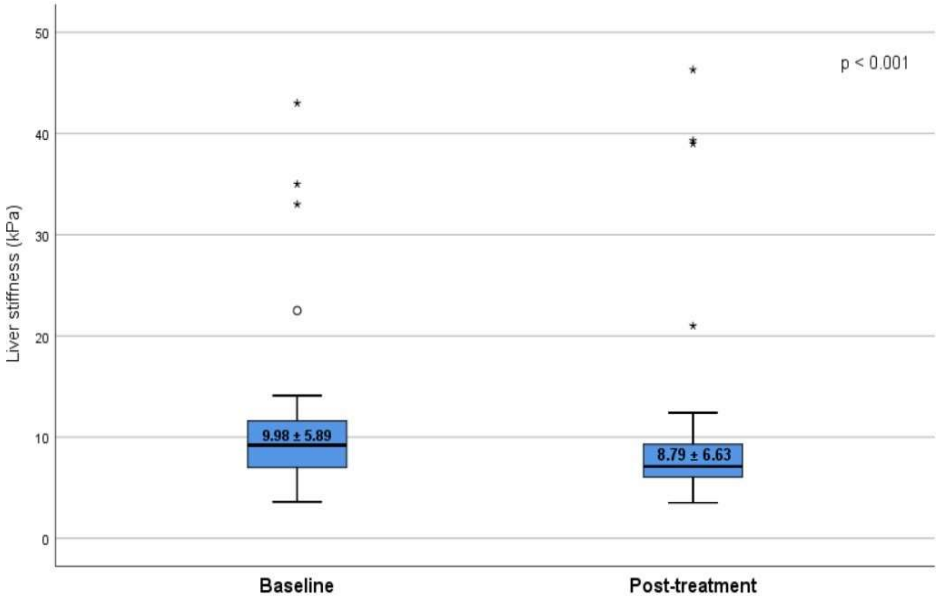


Figure 2.3. Changes in the LSM values after DAAs therapy. The bottom and the top of each box represent the 25th and 75th percentiles, while the lines through the box indicate mean value. The errors bars indicate the 10th and 90th percentiles, excluding asterisk (*). LSM, liver stiffness measurement

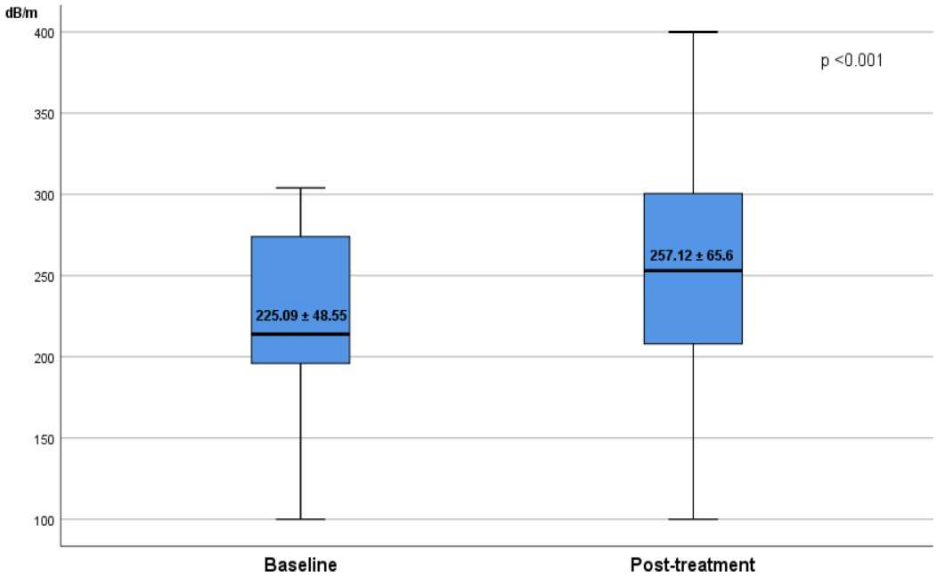


Figure 2.4. Alteration of the CAP level before and after HCV eradication

Factors associated with increased CAP. We performed a univariate linear regression analysis to identify the risk factors associated with a CAP increase at SVR12, after which only those with a significant *p* value were included in the multivariate regression analysis (Table 2.VI).

Furthermore, we noticed that patients with significant weight gain at SVR12 had higher CAP values than patients without weight gain (CAP = 263.78 ± 55.86 dB/m, $p = 0.008$ vs. CAP = 236.76 ± 6.33) (Figure 2.6).

Table 2.V. Comparison of patients with and without steatosis at baseline vs. SVR12

	Patients without steatosis (n=94)			**Patients with steatosis (n=186)**		
	Baseline	SVR12	p-value	Baseline	SVR12	p-value
Body mass index (kg/m^2)	25.3 ± 5.0	26.1 ± 5.9	NS	25.19 ± 5.17	28.15 ± 4.51	0.003
Weight (Kg)	72.4 ± 4.53	73.4 ± 3.27	NS	73.1 ± 11.21	85.05 ± 10.4	0.006
Laboratory panel (mean ± SD)						
HCV viral load log10 IU/mL	7.1 ± 1.4	0.0 ± 0.0	0.0001	7.1 ± 1.4	0.0 ± 0.0	0.0001
AST (U/L)	71.3 ± 58.7	21.8 ± 10.8	0.0001	36.3 ± 35.6	18.3 ± 4.4	0.0001
ALT (U/L)	57.78 ± 42.1	19.4 ± 12.5	0.0001	45.6 ± 50.8	20.3 ± 4.5	0.0001
Alkaline phosphatase (U/L)	79.5 ± 22.8	61.2 ± 18.4	0.05	81.4 ± 42.1	60.8 ± 24.5	0.01
Fibrosis score (kPa)	7.5 ± 1.4	5.5 ± 1.2	0.0001	7.5 ± 1.5	8.3 ± 3.8	0.038

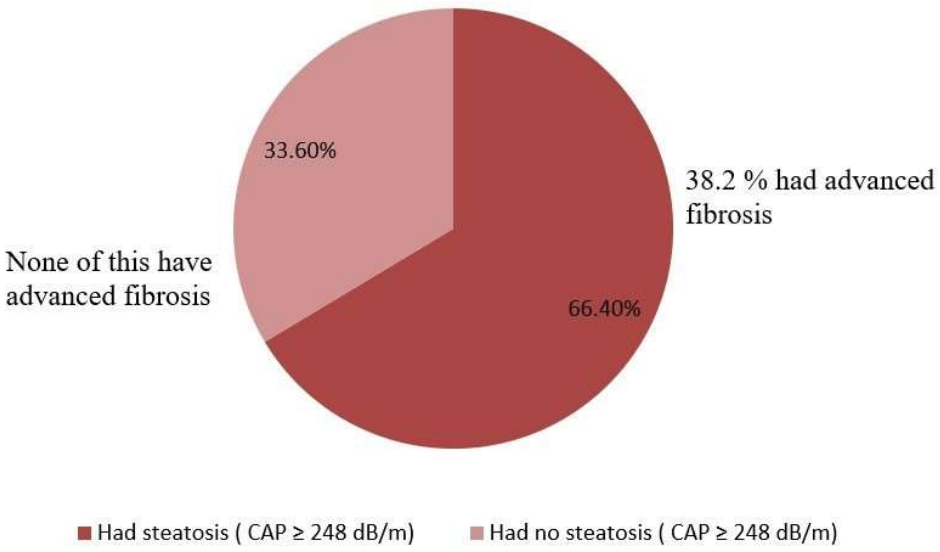


Figure 2.5. The prevalence of liver steatosis in patients with HCV infection at SVR12

Table 2.VI. Univariate and multivariate analyses of factors associated with increased CAP values

Variable	Univariate		Multivariate	
	β	*p*	β	*p*
Age	0.126	0.287		
Gender	0.07	0.912		
BMI (kg/m^2)	0.561	<0.001	0.328	<0.001
HGB (g/dL)	0.143	0.728		
Platelet count (G/L)	−0.072	0.896		
ALT (IU/L)	−0.15	0.872		
AST (IU/L)	−0.218	0.726		
GGT (IU/L)	0.182	0.787		
ALP (IU/L)	−0.139	0.639		
Fasting glucose (mg/dL)	0.299	0.041	0.187	0.056
Creatinine (mg/dL)	0.331	0.648		
Urea (mg/dL)	−0.013	0.627		
Total cholesterol (mg/dL)	0.310	0.008	0.108	0.052
Triglicerides (mg/dL)	0.426	<0.001	0.148	0.017
Albumin (g/dL)	−8.44	0.711		
Total bilirubin (mg/dL)	−0.23	0.580		
Baseline CAP	−0.594	<0.001	−0.596	<0.001

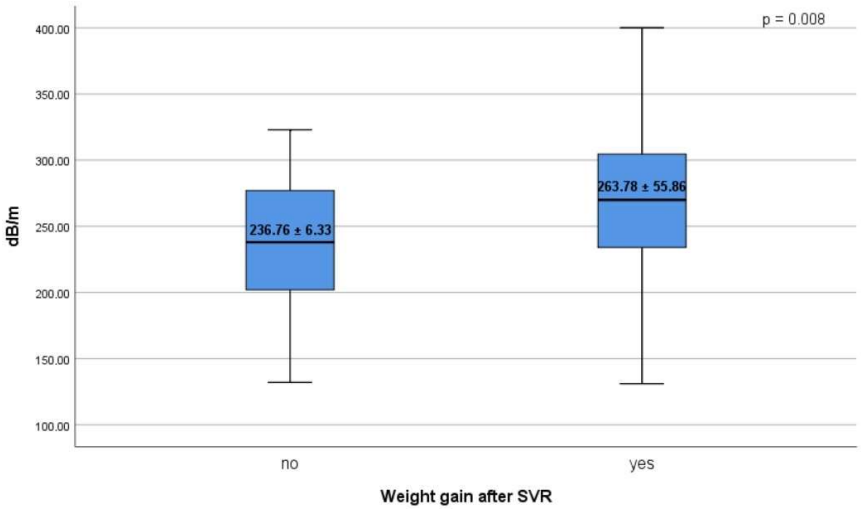


Figure 2.6. Weight gain after SVR, stratified by CAP values

Discussion. HCV infection and liver steatosis are closely associated. Furthermore, the HCV-infected patients with superimposed conditions such as metabolic syndrome and obesity should be evaluated for liver steatosis after SVR (Lonardo et al., 2014). In several studies, the

hepatic steatosis in HCV patients before SVR was assessed by liver biopsy, and 40–80% of cases had liver steatosis (Saldarriaga et al., 2021; Siphepho et al., 2021).

In our study, the prevalence of liver steatosis in patients who achieved SVR with DAAs was 66,42%, independent of the type of regimen used. In contrast, patients had normal levels of liver enzymes in the presence of steatosis, and this should be of concern to clinicians.

The American Association of the Study of Liver Diseases (AASLD) recommends the assessment for modifiable risk factors of liver injury, such as liver steatosis and diabetes mellitus, and to continue disease-specific management to optimize weight loss and glycemic control in those who achieved SVR (Jacobson et al., 2017). Furthermore, an abnormal BMI and insulin resistance (IR) at the baseline are important risk factors for liver steatosis and weight gain after DAAs treatment. In patients who obtained SVR, the bidirectional relationship between weight gain and the development of diabetes causes the progression of hepatic steatosis and fibrosis (Lonardo et al., 2009).

In our study, clearance of HCV was not associated with an important improvement in glycemic control, with 156 patients (56%) having impaired fasting glucose after the completion of treatment with DAAs. Thirty-four (22%) of them also had a significant weight gain, suggesting that high glycemic values were due to the improvement of the quality of life and the elevation of the levels of hunger-inducing hormones in those who achieved SVR. Moreover, a CAP score $\geq$ 248 dB/m was found in 80% of patients with impaired fasting glucose. In addition, upon multivariate analysis, we found that BMI ($\beta = 0.328$, $p < 0.001$) and triglycerides ($\beta = 0.148$, $p = 0.017$) were independent risk factors associated with an increased CAP score. Similar results were observed by Azad et al., who noted a higher mean weight gain in diabetic patients compared to non-diabetics treated with sofosbuvir + ledipasvir (Azad et al., 2016). Moreover, Strauhs-Nitsch et al. also demonstrated in a recent cohort study that achieving SVR after DAAs did not improve IR after one-year by using homeostatic model assessment for insulin resistance (HOMA-IR) (Strauhs-Nitsch et al., 2020). In contrast, Ciancio et al. conducted a prospective case-control study of 122 patients and showed that viral eradication using DAAs is associated with reduced fasting plasma glucose and the need for antidiabetic therapy (Ciancio et al., 2018).

We observed a significant increase in BMI at SVR12 (26.96 ± 4.15 kg/m2 vs. 27.87 ± 4.23 kg/m), while we could also suggest that excessive calorie intake may be responsible for the liver steatosis. Do et al. had similar findings in a large cohort of 11.,469 patients and reported weight gain in 2.293 (20%) patients after DAAs treatment who achieved SVR (Do et al., 2020). Another study by Schlevogt et al. showed that 44% of treated subjects had weight gain at their long-term follow-up after SVR (Schleyogt et al., 2017).

HCV infection promotes chronic systemic inflammation, reduces quality of life, and is frequently associated with depression and fatigue, with all of this leading to weight loss (Sugimoto et al., 2018). Other factors that may cause weight loss are represented by protein-calorie malnutrition and sarcopenia. After SVR, the chronic inflammation cycle is disrupted and there is a major improvement in liver anabolism, inducing an increased nutritional intake. These changes will finally determine IR and higher fasting glucose levels (Negro et al., 2014).

In HCV-infected patients, viral- and host-mediated factors contribute to the progress of liver steatosis. The prevalence of liver steatosis also has an important dependence on genotype. Thus, infection with GNT 3 is frequently associated with the development of HCC, liver fibrosis, and lower SVR rates with direct-acting antivirals. However, in patients with GNT 1b of HCV infection, poorer clinical outcomes and metabolic steatosis are expected (Kumar et al., 2002). In Romania, the prevalence of GNT 1b is 99.6% in HCV patients (Manuc et al., 2017).

A prospective study shows that magnetic resonance (MR)-based techniques are superior to VCTE for detecting hepatic steatosis and fibrosis in patients with HCV infection. MR-based techniques have a high sensibility for hepatic steatosis detection but are not suitable as point-

of-care methods due to high costs (Han et al., 2017). VCTE has multiple advantages, including better patient acceptability, easiness to perform, and a more affordable price than MR (Lonardo et aal., 2014). On the other hand, abdominal ultrasound (US) is used as a first-line assessment for the screening of fatty liver because of low cost and easy accessibility, but it is imprecise in measuring the extent of steatosis in those with morbid obesity, or in those with less than 20% of liver fat accumulation and is dependent by operator (Fedchuk et al., 2014). In comparison with US, CAP is quantitative tool for assessing liver steatosis with higher sensitivity and specificity. CAP numerical values measurements also correlate with the histological degree of steatosis, being a quick and easy method to perform (Eddowes et al., 2019; Semmler et al., 2020). Nourredin et al. found, in a prospective study consisting of 101 patients with HCV infection who have obtained SVR with DAAs therapy, that liver steatosis is a common finding post-SVR surveillance. A total of 48 patients had liver steatosis at 47 weeks after the end of treatment with a mean CAP score of 296.31 ± 37.4 dB/m (Nourredi et al., 2018). In line with these results, our study showed that 186 patients (66.4%) had liver steatosis at SVR12, with a mean CAP score of 257 ± 65.49 dB/m, and these results show an increase of 4.6% from the baseline. Additionally, the patients with liver steatosis had a higher fibrosis score than the patients without liver steatosis at SVR12 (8.3 vs. 5.5 kPa). Similar results were found by Ogasawara et al. who conducted a study in Japan; they analyzed a similar group, consisting of 214 patients with HCV infection treated with DAAs, and they concluded that liver steatosis increased significantly after SVR (Ogasawara et al., 2018). In another prospective study, Rout et al. had similar findings, with an increase in the CAP score in a cohort of 408 HCV-infected patients who achieved SVR after therapy with DAAs (Rout et al., 2019).

In contrast, in a retrospective study conducted by Shimizu et al., which included 70 patients with chronic HCV infection who achieved SVR at 12 weeks using DAAs therapy, they reported a decrease in CAP values at SVR12. Moreover, the decrease was higher among patients with S3 compared with those with S1 and S2 degrees of liver steatosis (Shimizu et al., 2018).

Recent studies among predominantly genotype 1 HCV-infected patients suggest an effect of DAAs therapy on cholesterol and triglyceride levels (Endo et al., 2017; Tada et al., 2018). Thus, DAAs therapy induce elevated triglycerides and cholesterol levels (Kawagishi et al., 2018; Villani et al., 2021). In line with these reports, we observed a significant increase in triglyceride levels (124.03 ± 113.49 mg/dL vs. 153.78 ± 94.53 mg/dL) and in cholesterol levels (191.61 ± 67.19 mg/dL vs. 216.52 ± 50.85), respectively. These results can explain the higher values of CAP measurements and need to be evaluated furthermore. Moreover, we documented a significant increase in the body mass index at SVR12 (26.96 ± 4.15 kg/m2 vs. 27.87 ± 4.23 kg/m2), and that fact could suggest that an excessive calorie intake may be responsible for the liver steatosis.

One strength of our study consists of assessing a detailed metabolic profile that is recognized as a cause for lipid deposition in the liver, especially in patients with HCV infection. Indeed, our study also had a few limitations. The first limitation is that the median time interval for the follow-up in our study was 12 weeks after treatment completion. Another limitation is represented by the measurements of LSM and CAP at SVR12, with no further follow-up. For assessing the progression of liver steatosis and fibrosis in HCV-infected patients, lengthier studies are needed. The absence of histological assessment should be mentioned as a limitation. Finally, we did not accumulate data about the patient's lifestyles, regarding dietary regime, or the frequency of physical activity.

Conclusions. In light of the latest evidence, it is clear that in the HCV-infected patients treated with DAAs therapy who achieved SVR, the liver steatosis increased, and fibrosis score decreased. In our study, 66.4% of HCV patients had hepatic steatosis at SVR12. Moreover, 38.2% of them had at least advanced fibrosis (≥F3), despite normal levels of liver enzymes. In patients who obtained SVR, the AASLD recommends counseling on lifestyle changes to

prevent weight gain and to improve glycemic control (Jacobson et al., 2017). Our results suggest that SVR is an opportune time to assess the importance of weight gain in the long-term as a metabolic risk factor for liver outcomes. At SVR12, the assessment of steatosis and fibrosis in those with a BMI ≥ 25 kg/m2 or other risk factors related with HCV-infection is also definitely warranted. Future studies are needed to clarify the importance of a long-term assessment of liver steatosis and the outcomes associated with weight gain post-SVR, as well as the role of clinical strategies to prevent weight gain after DAAs therapy.

I.2.2.3. Short-term changes of liver fibrosis in patients with HCV genotype 1b - related compensated cirrhosis after sustained virologic response

Background & Aim. TE is a highly accurate ultrasound - based technique, most widely used and accepted for the assessment of LF, with good sensitivity and specificity (Geng et al., 2016). The fibrosis index based on 4 factors (FIB-4) is one of the many noninvasive serological tests developed to detect LF in patients with chronic HCV infection. The FIB-4 index is a publicly available formula consisting of age, aspartate aminotransferase alanine aminotransferase and platelets, which has higher accuracy in predicting advanced fibrosis or cirrhosis when compared to LB (Turner et al., 2017).

This study aimed to assess the changes of fibrosis occurring after successful DAA treatment with noninvasive methods by analyzing LF measurements obtained with TE and FIB-4 index in patients with HCV genotype 1 b compensated cirrhosis, and to identify the risk factors associated with persistently elevated biomarker score and Fibroscan® related values after obtaining SVR.

Materials and methods. This study was a single-center, prospective observational cohort study which included 98 consecutive patients with HCV genotype 1b compensated cirrhosis who achieved SVR after DAA therapy with paritaprevir/ritonavir, ombitasvir and dasabuvir (PrOD) for 12 weeks in the Institute of Gastroenterology and Hepatology Iasi, from December 2016 to July 2017. For each patient, baseline demographics, clinical and laboratory data were recorded, and further data was collected during patient's visits at baseline, SVR and 12 months after the end of treatment. The diagnosis of cirrhosis was based on clinical, biochemical, imagistic features of liver cirrhosis, a liver stiffness ≥ 12.5 kPa measured with Fibroscan® and a FIB-4 index ≥ 3.25. Exclusion criteria were co-infection with HIV or HVB, the presence of decompensated liver cirrhosis. Depending on the FIB-4 index value LF was categorized as nonsignificant, significant (≥F2 to ≤ F4) and cirrhosis (F4) as follows: <1.45, >1.45 and <3.25 and ≥ 3.25 respectively. A significant improvement of the serological test represents a decrease by ≥1-degree METAVIR score class.

LSM was performed by using TE (Fibroscan®, EchoSens, Paris, France) at baseline, SVR and 12 months after end of treatment (EOT). For a valid interpretation, at least 10 valid measurements were required with an interquartile range median (IQR/M) <30% and a successful rate > 60% (24). The median of LSM was ex-pressed in kilopascals (kPa) and cut-off values for fibrosis were as follows: (1) less than 7.1kPa for < F2, (2) from at least 7.1kPa to less than 9.5 kPa for ≥ F2, and (3) at least 9.5 kPa for ≥ F3, and at least 12.5 kPa for F4. More than 30% regression in LF, at SVR and 12 months after EOT, was considered a significant improvement.

The study was conducted in compliance with the Helsinki Declaration and was approved by the institutional review board. Written informed consent was obtained from all patients.

Statistical analysis. Data were expressed as mean ± standard deviation, if normally distributed, and as median and interquartile-range (IQR), if not normally distributed. Friedman's test and Wilcoxon signed-rank test were carried out to detect longitudinal differences between noninvasive fibrosis assessment and clinical characteristics during follow-up-value value less than 0.005 was judged to be statistically significant. All the analyses were

performed by using IBM SPSS 19.0 statistical software package (IBM corp., Armonk, NY, USA).

Results. We included 98 patients with HCV-related cirrhosis with DAAs-associated SVR. The baseline characteristics of all patients are summarized in Table 2.VII. The mean age of the studied patients was 60.64±9.56 years with a female predominance (65.3%) and a BMI of 28.16±4.28. Thirty-four (34.5%) patients were IFN-based regimens experimented, 18 (18.36%) had T2DM and 55 (59.12%) had hypertension. Liver stiffness at baseline was 21.30 (16.9-27.1) kPa.

Laboratory data at SVR and 12 months after end of treatment. Albumin, total cholesterol and low-density lipoprotein cholesterol levels significantly increased overall, while high-density lipoprotein cholesterol levels increased at SVR and recorded a significant reduction at 12 months after end of treatment. Significant differences were recorded between baseline vs. SVR, SVR vs. 12 months after end of treatment, and baseline vs. 12 months after end of treatment (Table 2.VIII). Triglycerides and INR levels showed no significant improvement.

Fibrosis evaluation assessed by TE and FIB-4 score

Overall baseline median (IQR) of LSM was 21.3 kPa (16.97-27.1) using Fibroscan®. At SVR, the median TE measurement was 16.45 kPa (13.8-23.7) and 15.65 kPa (11.3-22.9) at 12 months after end of treatment, with an overall median (IQR) regression of 4.5 (1.8-7.8) from baseline to SVR and 5.1 (2.5-8.65) from baseline to 12 months after EOT ($p < 0.001$). Moreover, the LSM improvement is maintained from SVR to 12-month post-treatment ($p = 0.021$). LSM regression was found in 83 (84,6%) patients at 12 months after end of treatment evaluation. Subsequently, the primary outcome was obtained in 26 patients (26,53%) ($p < 0.001$), which had more than 30% LSM decrease. The distribution of LSM over time is shown in Figure 2.7.

The FIB-4 score significantly decreased at SVR and 12 months after end of treatment, similar to LSM values. At baseline, median (IQR) FIB-4 was 3.69 (2.19-6.02), while at SVR, the median (IQR) value reached 2.27 (1.55-3.26) with a decrease of 1.52 (0.37-2.75) ($p < 0.001$). At 12 months after end of treatment, the median (IQR) was 2.21 (1.51-3.28), with a decrease of 1.52 (0.45-2.72) from baseline. The improvement of liver function expressed in FIB-4 score correlates with Fibroscan® METAVIR staging fibrosis. At 12 months after end of treatment, 73 (74.79%) patients had reached a down-grade of fibrosis stage from F4 (98) to F3 - F2 (50- significant fibrosis), < F2 (23)- nonsignificant fibrosis, while 25 remained at the same stage (F4).

In 13 patients there was a significant increase value of LSM median from 16.3 to 22.8 kPa while at 2 patients a stationary TE was reported. In this subgroup of patients, the median FIB-4 score was 2.43 from 4.3 initially, mean age of 65 years.

Discussion. Several previous studies conducted in the IFN era have shown a long-term improvement of LF in patients with SVR (Martinez et al., 2012; Kim et al., 2015). Given that DAAs have recently been introduced into the management of patients with chronic HCV infection, conclusive data on the course of long-term LF are still scarce. This study seeks to enrich the current literature by prospectively evaluating long-term LF changes after obtaining SVR with DAA-based therapies. The key finding of our study consists of the long-term improvement in liver function demonstrated by a significant improvement of LSM and FIB-4 score. In our study, at one year of follow-up, 83 (84,6%) patients achieved a significant LSM decrease from baseline while 26 (26,53%) had a significant improvement of LSM (more than 30% regression in LF). A similar rate of improving LSM and FIB-4 score after SVR was reported by Mohammed et al, with a 46% reduction of LSM assessed by Fibroscan® and a >60% decrease of FIB-4 from baseline to one year (Mohammed et al., 2019). Another study which included patients treated with sofosbuvir-based DAA therapy, found a 31% reduction of liver stiffness after one year since EOT compared to baseline.

Table 2.VII. Patient characteristics

Variable	Baseline	SVR	12-month post-treatment
Age in years	60.64 ± 9.56	-	-
Female, *n* (%)	64 (65.3)	-	-
BMI (kg/m2)	28.16 ± 4.28	-	-
HCV treatment - experienced, *n* (%)	64 (65.3)	-	-
Diabetes, *n* (%)	18 (18.4)	-	-
Hypertension, *n* (%)	55 (56.1)	-	-
Esophageal varices, *n* (%)	27 (27.6)	-	-
BMI (kg/m2)	28.16 ± 4.28	28.00 ± 3.90	28.77 ± 3.85
Platelet (1000/μL)	155.74 ± 69.01	154.92 ± 62.47	171.96 ± 76.35
ALT (IU/L)	100.6 ± 75.96	39.62 ± 9.44	26.39 ± 16.31
AST (IU/L)	91.83 ± 61.88	34.14 ± 6.96	29.85 ± 14.35
GGT (IU/L)	87.46 ± 58.24	53.42 ± 9.33	43. 45 ± 49.77
Fasting sugar (mg/dL)	112.43 ± 31.73	99.60 ± 11.18	112.08 ± 29.01
Urea (mg/dL)	32.50 ± 9.94	39.20 ± 8.35	35. 81 ± 10.77
Direct bilirubin (mg/dL)	0.50 ± 0.30	0.35 ± 0.17	0.33 ± 0.17
INR	1.09 ± 0.10	1.08 ± 0.09	1.06 ± 0.07
Fibrinogen (mg/dL)	303.56 ± 66.36	311.09 ± 52.68	355.46 ± 42.37
Total cholesterol (mg/dL)	157.84 ± 36.84	174.09 ± 18.1	190.82 ± 38.55
LDL cholesterol (mg/dL)	97.09 ± 17.95	129.98 ± 25.88	125.40 ± 30.01
HDL cholesterol (mg/dL)	36.82 ± 5.68	41.38 ± 7.99	29.01 ± 6.67
Triglycerides (mg/dL)	106.79 ± 37.23	96.97 ± 22.63	121.13 ± 53
AFP (ng/mL)	15.82 ± 21.20	6.20 ± 5.89	5.46 ± 4.11
Albumin (g/dL)	4.12 ± 0.52	4.46 ± 0.39	4.50 ± 0.41
FIB-4	3.69 (2.19-6.02)	2.27 (1.55-3.26)	2.21 (1.51-3.28)
LSM (kPa)	21.30 (16.9-27.1)	16.45 (13.8-23.7)	15.65 (11.3-22.9)

Data are shown as mean ± SD, but LSM are expressed as median (interquartile range); BMI, body mass index; HCV, hepatitis C virus; ALT, alanine aminotransaminase; AST, aspartate aminotransaminase; GGT, gamma-glutamyl transferase; INR, international normalized ratio; LDL, low-density lipoprotein; HDL, high-density lipoprotein; AFP, α-fetoprotein; LSM, liver stiffness measurement

Even if LB remains the gold standard for estimating LF, in the last decade, noninvasive methods for evaluating the degree of LF are used in clinical practice. Their repeatability and cost-effectiveness, besides patient convenience, represent sustainable arguments for abandoning LB in the long-term evaluation of HCV cirrhotic patients. Accordingly, a cross-sectional study by Hendenstierna et al. in which patients with SVR obtained with IFN-based regimens was followed-up through LSM for a period ranging from 5 to 10 years, concluded that the improvement of LF after viral clearance is significant in patients with pre-treatment advanced fibrosis or cirrhosis, but concerning long-term dynamics of fibrosis, regression becomes a slow process which at one point is stabilized (Hedenstierna et al., 2018). Therefore, the patients with more than 9.5kPa cut-off baseline values had an improvement in LF. Only 21% of patients remained with more than 9.5 kPa at 10 years of follow-up. Also, the authors reported a progression of fibrosis in 5% of the patients, correlated with an age ≥ 55 years and a BMI ≥ 25 kg/m^2. In our study, we found an increase of TE score in 13 patients, while 2 patients had a stationary value. Of these subgroups, the mean values of age, BMI and FIB-4 index after DAA treatment were 65 years, 30.9 kg/m^2 and 2.43, respectively. We concluded that age and BMI values after treatment are risk factors for fibrosis progression.

Our paper has some limitations: the small number of patients enrolled, the lack of an LSM at EOT by FIB-4 score with a varied range of TE (±3 months), the short follow-up period which is a limitation because, for a true diminishing of LS, a longer follow-up period is required

with a well-defined reevaluation-time, and factors not HCV-related that might influence LSM such as alcohol consumption, steatogenic medication and lifestyle were not recorded.

Conclusion. TE was validated for assessing liver stiffness in patients with HCV-related liver cirrhosis, but long-term follow-up study based only on DAA based regimes treated patients are still limited. A rapid regression of fibrosis suggested by the improvement of LSM is secondary to the elimination of the hepatic inflammation after EOT. However, a dynamic assessment of LSM shows a tendency to settle on a constant value. In our patients, achieving SVR with DAA-based regimens was associated with significant improvement of liver stiffness measured by TE, and liver function monitored by FIB-4 index. A true regression of fibrosis must be constantly evaluated in dynamic, reason for which we intend to continue the TE follow-up of our cohort. Further studies are warranted to confirm these findings in other populations.

Table 2.VIII. Patient p-values for continuous clinical data

Variable	p-Value		
	Baseline *vs.* SVR	**SVR *vs.* 12-month post-treatment**	**Baseline *vs.* 12-month post-treatment**
BMI (kg/m2)	0.400	< 0.001	< 0.001
Platelet (1000/μL)	0.918	0.020	< 0.001
ALT (IU/L)	< 0.001	< 0.001	< 0.001
AST (IU/L)	< 0.001	< 0.001	< 0.001
GGT (IU/L)	< 0.001	< 0.001	< 0.001
Fasting sugar (mg/dL)	0.003	0.001	0.608
Total bilirubin (mg/dL)	0.002	< 0.001	< 0.001
Direct bilirubin (mg/dL)	< 0.001	0.050	< 0.001
INR	0.008	0.084	0.001
Fibrinogen (mg/dL)	0.523	< 0.001	< 0.001
Total cholesterol (mg/dL)	< 0.001	0.001	< 0.001
LDL cholesterol (mg/dL)	< 0.001	0.265	< 0.001
HDL cholesterol (mg/dL)	< 0.001	< 0.001	< 0.001
Triglycerides (mg/dL)	0.077	0.001	0.351
AFP (ng/mL)	< 0.001	0.013	< 0.001
Albumin (g/dL)	< 0.001	0.104	< 0.001
FIB-4	< 0.001	< 0.001	< 0.001
LSM (kPa)	< 0.001	0.021	< 0.001

BMI, body mass index; HCV, hepatitis C virus; ALT, alanine aminotransaminase; AST, aspartate aminotransaminase; GGT, gamma-glutamyl transferase; INR, international normalized ratio; LDL, low-density lipoprotein; HDL, high-density lipoprotein; AFP, α-fetoprotein; LSM, liver stiffness measurement;1 Wilcoxon signed-rank test was applied 2 Friedman's test was applied

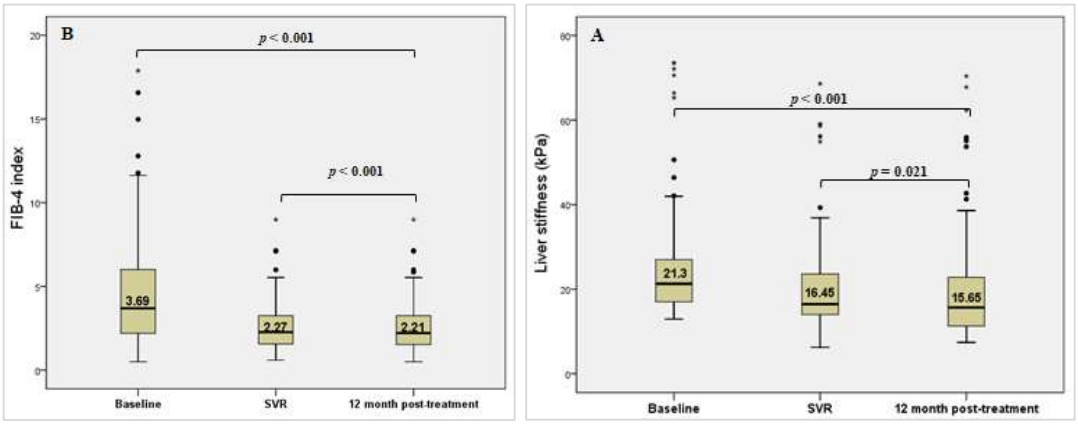


Fig. 2.7. Distribution of LSM (A) and FIB-4 index (B) over time. The lines through the boxes represent the median score. The upper and lower bottom of each box represents the 25th and 75th percentiles, representing the interquartile range (IQR). The rest of the outliner observations are plotted as dots beyond the whiskers. Each bottom error bar ends at 1.5*(IQR) < Q1, while each top error bar ends at 1.5*(IQR) > Q3

I.2.2.4. Liver remodeling on CT examination in patients with VCH compensated cirrhosis who achieved sustained virological response after direct-acting antivirals treatment

Background and aim. Traditionally, cirrhosis was considered an irreversible end stage of liver disease, mostly due to lack of treatment possibilities. Nowadays, this irreversibility is no longer considered a "dogma" as liver fibrosis is potentially reversible on condition that the trigger is removed (Zoubek et al., 2017).

There is evidence that patients who achieve SVR are less likely to develop liver-related complications, due to the regression of fibrosis after HCV eradication (Trivedi et al., 2017).

Several non-invasive methods for assessing liver fibrosis confirmed fibrosis regression in patients with SVR (Bachofner et al., 2017; Mauro et al., 2018). Liver cirrhosis is characterized by significant parenchymal and vascular architecture change with formation of septae and regenerative nodule. HCV-related cirrhosis is a consequence of an ongoing liver injury by the hepatitis C virus. Among the most important factors involved in regression of hepatic fibrosis is the elimination of the primary cause of the chronic hepatic injury. Thus, elimination of HCV achieved by the DAA treatment is the cardinal condition for regression of liver cirrhosis.

Sectional imaging techniques, such as computed tomography (CT), magnetic resonance imaging (MRI) and ultrasonography (US) have been used in cirrhosis diagnosis and staging with variable rates of success. These techniques have different independent predictive signs for the diagnosis of liver cirrhosis. US is the most accessible and most used imaging method for the evaluation of patients with chronic liver disease; liver cirrhosis diagnosis using US has an accuracy, sensitivity and specificity between 64-79%, 52-69% and 74-89%, respectively (Kudo et al., 2008; Sangster et al., 2013). MRI sensitivity and specificity in liver cirrhosis are 87% and 54% respectively, similar to those of CT (Kudo et al., 2008).

Abdominal CT scans are frequently performed in clinical studies involving cirrhotic patients due to its ability to diagnose and rapidly stage HCC following contrast administration. For this reason, CT is usually used in patients with HCV-related cirrhosis prior being involved in the therapy with DAAs in order to exclude HCC (suspected at US) or other malignancy which is contraindication to this treatment. Additionally, CT can diagnose liver fibrosis in early, pre-cirrhotic stage. It is also useful to evaluate the extrahepatic complications of cirrhosis, such as portal hypertension and its effect on the abdominal organs.

The classical imaging features of cirrhosis identifiable by CT scans include hypertrophy of the caudate lobe, as the most characteristic morphologic sign of the disease and, in more advanced stages, hypertrophy of the lateral segments of the left lobe (II and III) with atrophy of the medial segment (IV) and of the posterior segments (VI and VII) of right lobe (Sangster et al., 2013). Volume changes in the left lobe medial segment and right lobe segments lead to the widening of gallbladder fossa and the enlargement of central periportal space (defined as the distance between the anterior wall of the right portal vein and the posterior edge of the medial segment of the left hepatic lobe, easy to asses with a cut-off value of 10 mm) (Tan et al., 2008). Alteration of the caudate and right lobe morphology results in the presence of the right hepatic posterior notch sign, representing the functional lateral boundary of the hypertrophied caudate lobe and can be used as a simple and specific sign of cirrhosis (Ito et al., 2003).

However, CT overall diagnostic accuracy for liver cirrhosis is relatively low, with a sensitivity and specificity of 77-84% and 53-68%, respectively, as shown in a multicenter study (Kudo et al., 2008).

The repair process that causes the formation of regenerative nodules also determines the compression of the central hepatic veins and decrease of hepatic veins diameters, as well as changing in Doppler flow. A decreased right liver vein diameter below 7 mm should raise the suspicion of cirrhosis (Zhang et al., 2009).

The caudate lobe hypertrophy is the foundation for development of imaging-based cirrhosis scores: first cirrhosis scoring based on axial imaging measurements was developed by Harbin et al. using the caudate-right lobe ratio (C/RL) by dividing the width of the transverse caudate lobe to the width of the transverse right lobe at bifurcation of portal vein (Harbin et al., 1980). A ratio ≥0.65 is a positive diagnostic indicator for cirrhosis, with 100% specificity, good sensitivity (84%), and accuracy (94%) (Harbin et al. 1980). A modified caudate-right lobe ratio (C/RL-m) was proposed, using the right portal vein bifurcation instead of main portal as lateral boundary with more accuracy for diagnosing cirrhosis and evaluating its clinical severity (Awaya et al., 2002).

The latest imaging score for cirrhosis proposed by Huber et al uses the combination of both morphological and vascular changes, dividing the sum of liver veins diameters by the C/RL-m (Huber et al., 2014).

The objective of this study was to identify changes in hepatic morphology that evoke reversibility of fibrosis using CT scans, in patients with HCV-related compensated cirrhosis who have achieved SVR following treatment with DAAs.

Methods

Study design. This is a prospective study on patients with genotype 1 HCV-related compensated cirrhosis who have achieved SVR after treatment with DAAs (Ombitasvir/Paritaprevir/ritonavir+ Dasabuvir). Eligibility of enrolled patients was assessed following the criteria established by our National Health Insurance Agency and recommended by international guidelines: adult, treatment experienced or naïve patients with Child-Pugh class A cirrhosis assessed by Fibromax® Biopredictive (cut-off of 0.71 for F4) or liver biopsy (F4 by METAVIR). Exclusion criteria were decompensated liver cirrhosis or evidence of hepatocellular carcinoma.

Every patient had a CT examination before treatment to exclude liver malignancy and to evaluate the liver morphology. After the treatment the imaging follow-up protocol included US at every 6-months. A second CT examination was performed after the treatment, within 6 and 18 months following SVR achievement, to characterize nodular lesions or other parenchymal abnormalities detected by US.

All CT scans were anonymized and independently reviewed by three senior radiologists with experience in hepatobiliary radiology, blinded to all patient information. A fourth reader provided consensus in cases with disagreement in measurement. In order to avoid errors related to the different section level in two different examinations, measurements were made after synchronization, as close to the same section level starting from anatomical landmarks.

The local Ethical Committee approved this study. Written informed consent was obtained from each patient and the study was conducted according to the Declaration of Helsinki.

Scanning protocols. We used a Siemens Sensation® 16 slice configuration CT scanner (Siemens AG Medical Solution). Our scanning protocol was optimized for detection of potential malignant liver lesion, according with CT/MRI diagnostic Liver Imaging Reporting and Data System (LI-RADS®) recommendations for CT scanning protocol. This protocol includes anon-enhanced scan followed by i.v. administration of iodine-based contrast medium with tri-phase liver scan (arterial, portal and equilibrium phase). The contrast medium was administered in bolus with an injection rate of 3-5 ml/s. The arterial phase was acquired at 30-35 seconds (late arterial phase) and the venous phase at 75 second after contrast injection. The equilibrium phase was acquired at about 4 minutes after contrast injection. Patients were examined in the supine position, in post inspiratory apnea.

Data analytic strategy for imaging interpretation. The imaging data were evaluated for the following liver morphological changes: liver volume estimation using the following formula: volume = maximum dimension in cranio-caudal x latero-lateral x antero-posterior x 0.31 in cm^3 (Muggli et al., 2009); values of C/RL and C/RL-m, which describe the width of

the caudate lobe in proportion to the width of the right hepatic lobe; measurement of the hepatic vein diameters; measurement of the central periportal space widening; combination of hepatic vein diameters sums and caudate-right lobe ratio; assessment of right posterior hepatic notch variation; manifestations of portal hypertension: dilatation of portal system, including portal trunk, splenic vein and superior mesenteric vein diameter and splenomegaly using the index value (product of cranio-caudal dimension, maximum size in axial plane and maximum thickness in axial plane) and the splenic volume (index value x 0.58 + 30) in cm^3(Liu et al., 2009).

Statistical analysis. The statistical analysis started with the inspection of the continuous variables for assessing the normality of the distributions, including the normality of the distributions of the differences between the two repeated measurements. Since most of the differences did not meet the normality requirement, comparisons between the two sets of measurements, to see whether there were any changes in parameter levels, were performed with the Wilcoxon signed-rank test. A value of $p < 0.05$ was considered statistically significant.

Results

Patients. There were 56 patients (24 men and 32 women), mean age 57.78 ± 9.048 years (range 42–79). All the results are summarized in Table 2.IX and the graphical representation for the main variables is shown in Figure 2.8.

Hepatic vein diameters. Only the right hepatic vein diameter showed a statistically significant widening after treatment (median: 8.12 mm, IQR: 4.20), compared to the diameter recorded before treatment (median: 6.36 mm, IQR: 3.94), $z = -3.894$, $p < 0.001$. Both the middle and the left hepatic vein did not show significant changes in diameters after treatment.

The overall scores for the vein diameters were statistically significantly higher after treatment (median: 21.44 mm, IQR: 9.06) compared to the measures taken prior to treatment (median: 19.29 mm, IQR: 9.52), $z = -2.194$, $p = 0.028$, a difference mainly accountable to the right hepatic vein differences. Illustrative changes are shown in Figure 2.9.

Table 2.IX. Liver morphological changes: pre- and post-treatment

Variable	Pre-Treatment		Post-Treatment		*p*
	Median	IQR	Median	IQR	
Liver volume (cm^3)	1786.77	879.23	1716.44	840.50	0.049
Caudate-right lobe ratio	0.65	0.19	0.65	0.17	ns
Modified caudate-right lobe ratio	0.98	0.28	0.99	0.31	ns
Right hepatic vein diameter (mm)	6.36	3.94	8.12	4.20	<0.001
Middle hepatic vein diameter (mm)	5.70	2.48	5.91	2.51	ns
Left hepatic vein diameter (mm)	6.69	3.17	6.65	3.19	ns
Sum of hepatic vein diameters (mm)	19.29	9.52	21.44	9.06	0.028
Huber's score	19.16	11.50	20.58	9.73	0.035
Central periportal space widening (mm)	11.85	5.75	11.7	6.47	ns
Right posterior hepatic notch (degrees)	126.3	26.05	136.9	17.30	ns
Splenic volume (cm^3)	564.79	342.54	474.45	330.00	0.012
Portal trunk diameter (mm)	14	2.70	14.2	2.90	ns
Superior mesenteric vein diameter (mm)	12.7	2.90	12.2	2.70	ns
Splenic vein diameter (mm)	9.85	3.31	10.2	3.73	ns

ns: nonsignificant; IQR: interquartile range.

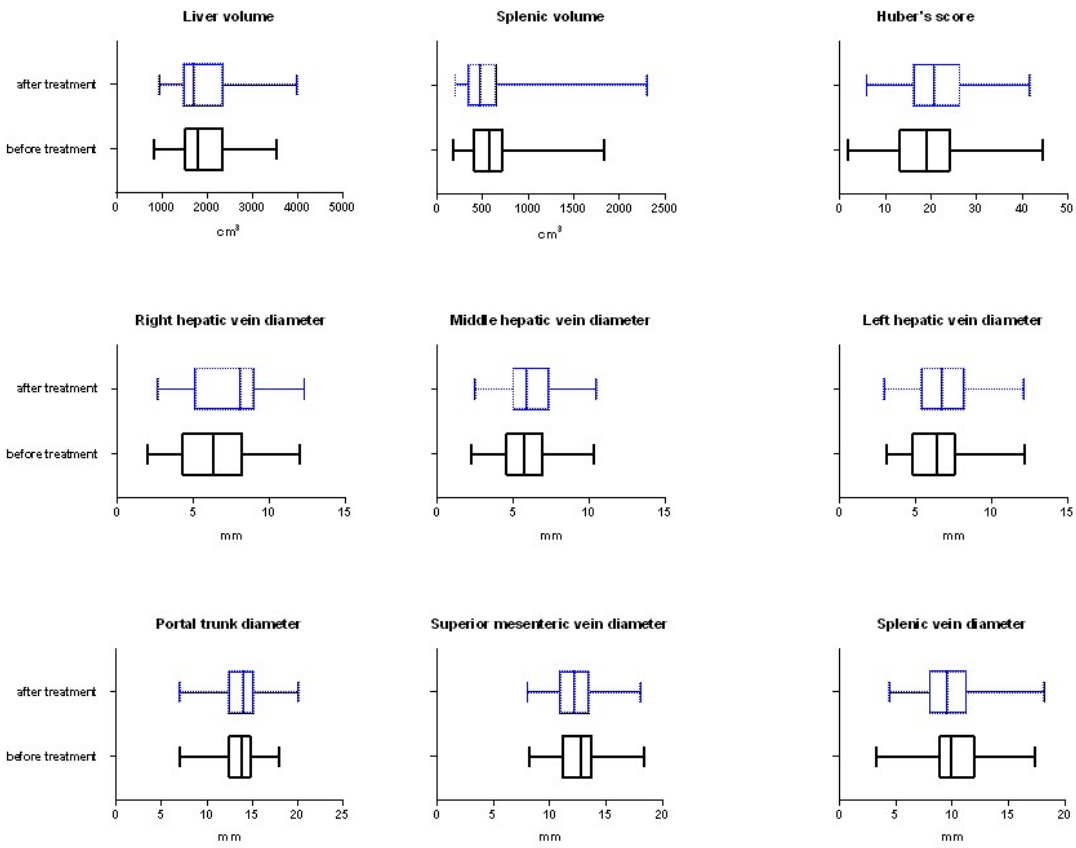
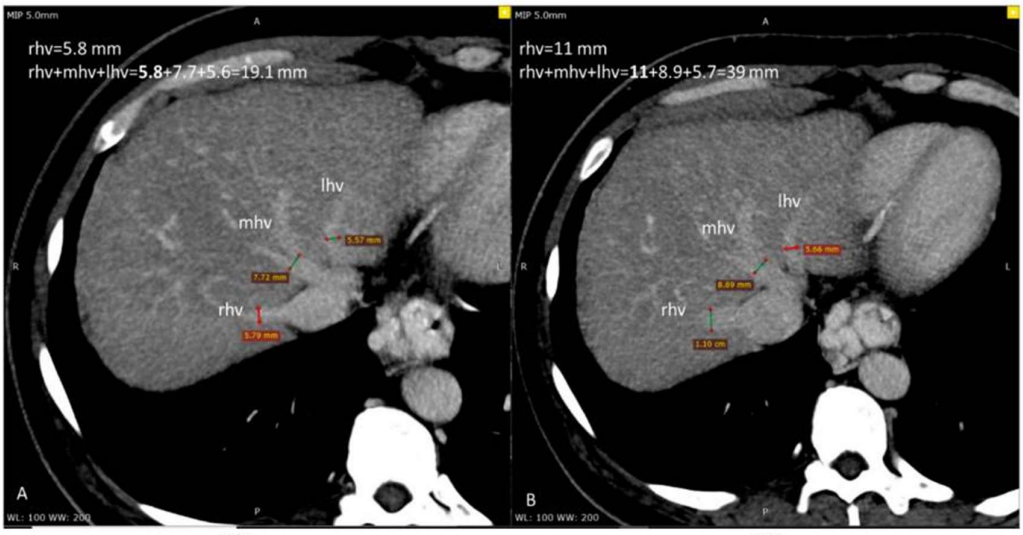


Figure 2.8. Boxplots of the main variables before and after the treatment comparison



(A) (B)

Figure 2.9. Computed tomography (CT) axial section at the level of hepatic veins before (**A**) and after (**B**) treatment. Only the right hepatic vein (rhv) showed significant widening after treatment (11 mm) compared to before (5.8 mm), while the middle hepatic vein (mhv) and left hepatic vein (lhv) showed no significant changes

The caudate-right lobe ratio and modified caudate-right lobe ratio. There were no statistically significant differences between the C/RL before (median: 0.65, IQR: 0.19) and after treatment (median: 0.65, IQR: 0.17), z = −1.283, p = 0.2, as well as between C/RL-m before (median: 0.98, IQR: 0.28) and after treatment (median: 0.99, IQR: 0.31), z = −0.597, p = 0.551.

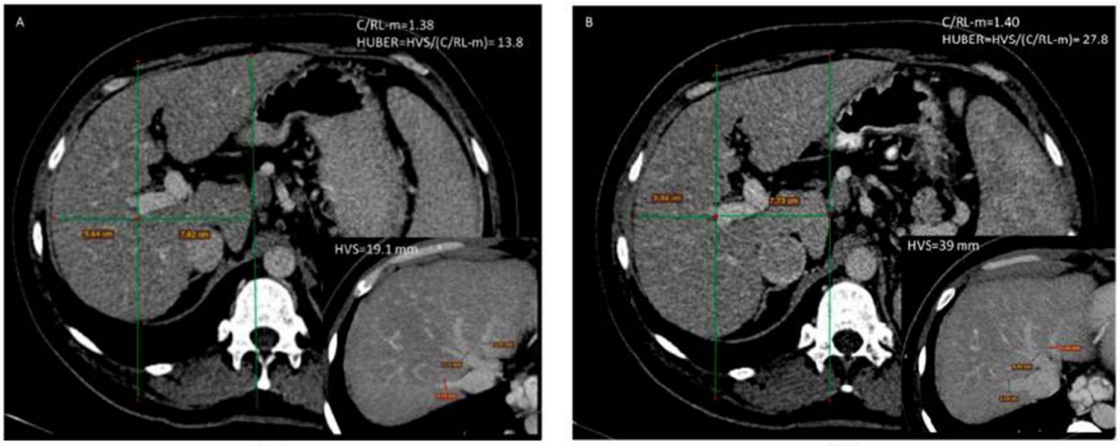
Huber's score. Huber's score before treatment (median: 19.16, IQR: 11.50) was significantly lower than after treatment (median: 20.58, IQR: 9.73), z = −2.106, p = 0.035, probably due to changes in hepatic vein diameters, because C/RL-m did not show meaningful changes (Figure 2.10).

Liver volume. Liver volume of the patients prior to the treatment was significantly higher (median: 1786.77 cm^3, IQR: 879.23) than after treatment (median: 1716.44 cm^3, IQR: 840.50), z = −1.970, p = 0.049.

Central periportal space widening. Results indicate there were no differences between central periportal space widening measured before (median: 11.85 mm, IQR: 5.75) and after treatment (median: 11.7 mm, IQR: 6.47), z = −0.368, p = 0.713.

Right posterior hepatic Notch variation. There were no significant differences between right posterior hepatic notch angle before (median: 126.3, IQR: 26.05) and after treatment (median: 136.9, IQR: 17.30), z = −1.783, p = 0.075.

Indicators of portal hypertension. Assessment of the signs of portal hypertension, such as enlargement of portal trunk, splenic vein, and superior mesenteric vein, and splenomegaly showed significant differences only for splenic volume before compared to after treatment (median: 564.79 cm^3, IQR: 342.54 vs. median: 474.45 cm^3, IQR: 330, z = −2.500, p = 0.012). There were no differences between the portal trunk, splenic vein, and superior mesenteric vein diameter before and after treatment.



(**A**) (**B**)

Figure 2.10. Huber's score—before (**A**) and after (**B**) treatment in one patient
HVS: hepatic veins sum; C/RL m: caudate right lobe ratio modified

Discussion. During these past decades, cirrhosis has evolved from an irreversible liver disease into a potentially reversible one. As it has been convincingly demonstrated by recent studies, the reversibility of cirrhosis is no longer a myth, while results show significant improvement of architecture of the liver (Grgurevic et al., 2017; Mauro et al., 2018). The main question is what happens after SVR with the liver: will the morphological changes remain as such, or will there be further morphological hepatic improvement?

Our study showed that there were some improved morphological aspects such as decrease of the liver and spleen volume, widening of the right hepatic vein diameter, increase of the sum

of hepatic vein diameters and of Huber's score. The first morphological improvement after treatment was the decrease in liver volume. Although the decrease in volume was statistically significant, it is small in value and therefore we consider that it is more likely secondary to the reduction of inflammation and may not represent a real loss of liver volume. The most dynamic changing parameter was the hepatic veins diameter, especially the right hepatic vein, significantly increased from a median of 6.36 mm at baseline to 8.12 mm ($p < 0.001$) after treatment. This variation has been also reported by other studies evaluating the fibrotic changes in pre-cirrhotic or cirrhotic patients (Huber et al., 2014). We estimate that this parameter is the most sensitive one and can be used as an early marker of liver recovery. It is unclear whether this improvement is secondary to inflammation or it is a true indicator of fibrosis reduction. The Huber's score also improved after treatment but only due to the change in the vessel diameter.

The widening of the periportal space consequently to atrophy in segment IV, as well as the caudate-right lobe ratio and its modified version, show no significant variation. A similar situation is the presence of the hepatic notch—the boundary between the hypertrophied caudate lobe and the right hepatic lobe—which also shows no improvement. We consider that the amplitude of the reversibility in fibrotic changes does not include these segment-volume variations. It is premature to set the boundaries of the reversibility in short-term follow up, as it could take longer to see a change in this parameter.

In the evaluation of the portal system vessel diameter variations, we observed no statistically significant changes. This lack of modifications may reflect a balance between two opposite tendencies: one towards a reduction in diameter as a consequence of flow reduction and the other towards an enlargement due to portal hypertension. The same situation was noticed in other studies on cirrhotic patients (Zhang et al., 2009). The improvement of portal hypertension status was revealed by a decrease in splenic volume, suggesting that this could be a sensitive marker. The decreased splenic volume may be the result of a combination of factors: decreased portal hypertension and general inflammatory status, but further research is required.

Our study has a few strengths and several limitations. Thus, as far as we know, this is the first prospective study evaluating the morphological changes by CT in HCV-related cirrhosis patients achieving SVR after DAAs treatment. One of the limitations is the low number of patients included in the study. In addition, the one-dimensional measurements may not accurately reflect all intrahepatic morphological changes.

Conclusion. SVR in patients with HCV-related compensated cirrhosis treated with DAAs is associated with some improvements of hepatic morphology detectable by CT, the most constant being the increase of right hepatic vein diameter. These results are promising but should be validated by further studies based on a larger number of patients and a longer follow-up.

I.2.2.5. L3 skeletal muscle index dynamics in patients with HCV-related compensated cirrhosis following sustained virological response after direct acting antiviral treatment

Background and aim. Sarcopenia is a syndrome characterized by progressive and generalized loss of skeletal muscle mass and impairment of muscular strength, which is commonly associated with various chronic pathologies, including chronic liver diseases. In cirrhotic patients, sarcopenia is currently defined as the loss of muscle mass, and is a remarkably frequent abnormality ranging from 40–70% of that population (Kim et al., 2015; Ponziani et al., 2018), depending on the definition criteria, the type of study population, and the assessment methods. Sarcopenia is actually seen as a prevalent complication, which has been proved as a predictor for morbidity and mortality of cirrhotic patients (Mauro et al., 2020).

The pathogenesis of sarcopenia basically results from an imbalance between protein synthesis and breakdown, but in cirrhosis, there are more complexes mechanisms, involving a

particular skeletal muscle response and the anabolic resistance (Dasarathy et al., 2016). However, sarcopenia is considered a possibly modifiable condition, and proper interventions could have a favorable impact on patients' prognosis. For these reasons routine assessment of sarcopenia is highly recommended, involving measurement of muscle mass, in addition to muscle strength and function.

Since the introduction of direct-acting antivirals (DAAs), the treatment of patients with HCV-related cirrhosis has been revolutionized. The main goal of treatment is to eliminate the virus by achieving a sustained virological response (SVR), lowering the risks of progression of the disease to liver failure and of hepatocellular carcinoma occurrence (Majumdar et al., 2016). Alongside the aforementioned proven benefits of the viral clearance, there are hopes for the improvement of cirrhosis-associated comorbidities, such as sarcopenia.

Moreover, for cirrhotic patients treated with antiviral therapies, maintaining muscle mass is crucial and identifying pretreatment factors linked to the improvement in skeletal muscle mass is important in nutritional strategy.

Computed tomography (CT) is currently the most widely used method for evaluating sarcopenia (Paternostro et al., 2019). It has the advantage of being widespread and therefore easy to access, it does not require special preparations of the patient before scanning, allowing the evaluation of muscle mass even on non-enhanced examinations. Multiple studies have considered different muscle groups, both thoracic and abdominal, to quantify muscle mass loss but the most widely used method uses total muscle area (*TMA*) at a section level passing through the third lumbar vertebra (L3) and allows the calculation of the skeletal muscle index (SMI) (Paternostro et al., 2019). The L3-SMI is easy to assess and there are well-established cutoff values depending on gender (Boutin et al., 2015). L3-SMI is the preferred CT method of muscle mass assessment in the cirrhotic patient compared to the evaluation of other muscle surface areas such as the psoas that had a lower sensitivity in these patients (Ebadi et al., 2018).

The purpose of this study was to identify the changes in skeletal muscle mass, as quantitative marker of sarcopenia, in patients with HCV-related cirrhosis who achieved SVR after DAA treatment and to assess pretreatment predictive factors for the evolution of L3-SMI.

Materials and methods

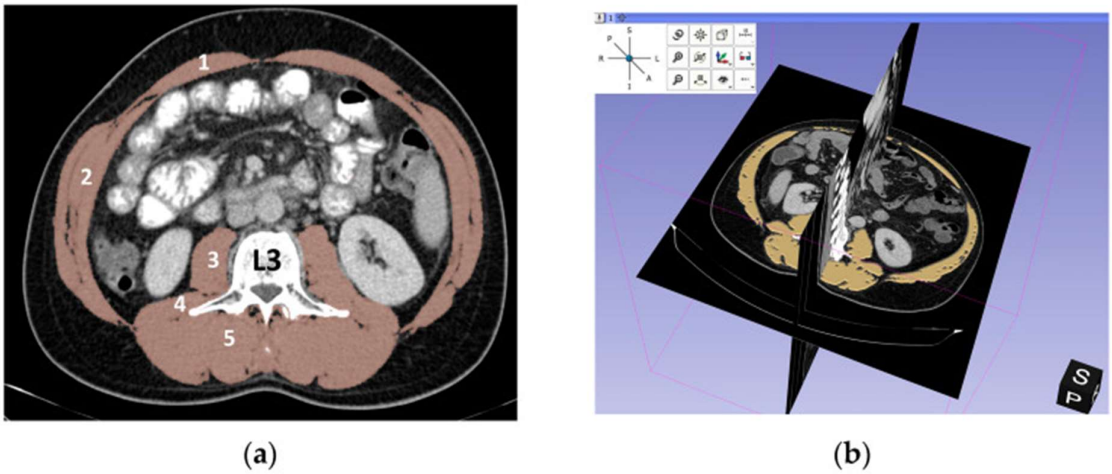
Study design. This is a single center retrospective study in patients with genotype 1 HCV-related compensated cirrhosis who obtained SVR after treatment with DAAs. Eligibility criteria for antiviral therapy were those established at the time by the National Health Insurance Agency and recommended by the international guidelines: adult, experienced or treatment-naive patients with compensated Child-Pugh A cirrhosis. The main exclusion criteria were decompensated liver cirrhosis or evidence of hepatocellular carcinoma. Demographic and biological parameters were assessed for all patients at baseline.

In order to evaluate the dynamics of muscle mass, we included in the study only patients who had at least two CT evaluations: a CT examination before starting treatment with DAAs, and a CT examination after obtaining SVR. The interval between these two CT examinations was in a range from 5 to 24 months.

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the local Ethics Committee.

Scanning protocols. We used a Siemens Sensation® 16 slice configuration CT scanner (Siemens AG Medical Solution, Erlangen, Germany) available in our institution. Patients were examined in the supine position, in post-inspiratory apnea. The scanning protocol includes a non-enhanced scan followed by intravenous administration of iodine-based contrast medium. The contrast medium was administered in bolus with an injection rate of 3–5 mL/s. The arterial phase was acquired at 30–35 s (late arterial phase) and the venous phase at 70–75 s after contrast injection.

Assessment of L3-SMI (Skeletal Muscle Index). All imaging data were acquired from venous phase images using a single slice axial CT scan of the abdomen. The third lumbar vertebral body, with both transverse processes visible was chosen as a reference for the axial section utilized for processing. The designated axial section, with 3 mm thickness, was evaluated in the soft tissue window (WL 60 WW 360) and the truncal muscles were delimited. The included muscles are the psoas muscle, erector spinae, quadratus lumborum, transversus abdominis, external and internal obliques and rectus abdominis (Figure 2.11).



(a) (b)

Figure 2.11. Axial cross-section at third lumbar vertebra (L3): (**a**) Total muscle area included: (1) rectus abdominis, (2) transversus abdominis, internal and external oblique, (3) psoas, (4) quadratus lumborum, (5) erector spine; (**b**) 3D representation of the axial section of interest at L3 level

A semi-automated demarcation of the muscle tissue was based on Hounsfield unit (HU) thresholds from −29 to +150. We used for demarcation a 3D Slicer® image computing platform, a free, open source and multi-platform software package used for medical and related imaging research. If needed, manual corrections were applied by the reader. The calculated total muscle area (*TMA*) was expressed in cm^2. Skeletal muscle index (L3-SMI) was calculated dividing *TMA* to square height (H^2) of patient expressed in m^2 as follows: SMI=TMA (cm2)H2(m2). Low muscle mass was defined according to established *SMI* cut-offs for each sex: males: <52.4 cm^2/m^2, females: <38.5 cm^2/m^2 (Sinclair et al., 2016; van Vuygt et al., 2016).

Statistical Analysis. The Shapiro-Wilk test was used to assess the normality of the distribution of the continuous variables. The differences between two independent groups for the continuous variable were assessed with the non-parametric Mann–Whitney U test, given the small sample size. For predicting changes in L3-SMI, candidate variables were identified by creating a linear regression model. Throughout the analysis a 95% confidence level was considered satisfying, setting the significance at $p < 0.05$.

Results

Baseline Data. Baseline characteristics of the patients included in our study are presented in Table 2.X. The study group included 52 patients (20 men and 32 women) with a median age of 59 years (range 42–79). L3-SMI in males at baseline ranged from 29.96 to 73.39 cm^2/m^2 (median 50.73 cm^2/m^2), whereas for women the baseline ranged from 21.74 to 60.52 cm^2/m^2 (median 37 cm^2/m^2). Considering the cut-off values specific for each sex, the overall percentage of patients with low L3-SMI value at baseline was 63.46% (33/52), with 80% (16/20) in the male group and 53.12% (17/32) in the female group.

Table 2.X. Baseline characteristics of the patients

Variables	Count or Median (Range)
Age (years)	59 (42–79)
Gender, male/female	20/32
Body mass index (kg/m^2)	26.94 (19.27–38.67)
Skeletal muscle index (cm^2/m^2), male	50.37 (29.96–73.30)
Skeletal muscle index (cm^2/m^2), female	37.00 (21.74 -60.52)
Total bilirubin (mg/dL)	1.09 (0.43–2.47)
Serum albumin (g/dL)	3.91 (2.28–5.25)
Platelets ×10^4/mm^3	10.9 (3.7–29.3)
Total cholesterol (mg/dL)	151.5 (80–221)
INR	1.14 (0.95–2.27)
Alpha-fetoprotein (ng/mL)	12.92 (2.14–131)
Serum creatinine (mg/dL)	0.72 (0.4–1.06)
AST (IU/L)	79 (28–224)
ALT (IU/L)	72 (15–264)

Comparison of Baseline Characteristics between Patients with Low L3-SMI and Normal L3-SMI (Table 2.XI). Analysis of data began with testing for significant differences in baseline records between the group of patients who had low L3-SMI pretreatment and those who had normal L3-SMI values according with gender-specific cut-off values. The following parameters were evaluated: age, body mass index (BMI) and laboratory values for total bilirubin, serum albumin, platelets, total cholesterol, INR, alpha-fetoprotein, serum creatinine, AST and ALT.

Table 2.XI. Comparison between patients with low L3-SMI and normal L3-SMI

	Normal L3-SMI (n = 19)		Low L3-SMI (n = 33)		Mann-Whitney	
	Median (Range)	Mean Rank	Median (Range)	Mean Rank	U	p
Age (years)	55.6 (42–73)	22.58	60 (42–79)	28.76	239.000	0.156
BMI	28.32 (20.2–34.42)	30.16	26.82 (19.27–38.67)	24.39	244.000	0.187
Total bilirubin	0.99 (0.6 -2.3)	24.29	1.16 (0.43–2.47)	27.77	271.500	0.425
Serum albumin	3.9 (2.49–5.25)	26.79	3.94 (2.28–4.75)	26.33	308.000	0.917
Platelets	10.6 (5.6–29.3)	26.76	10.9 (3.7–26.10)	26.35	308.500	0.924
Total cholesterol	148 (98–221)	23.61	158 (80–212)	28.17	258.500	0.296
INR	1.2 (0.95–1.55)	29.71	1.12 (0.97–2.27)	24.65	252.500	0.246
Alpha-fetoprotein	10.15 (3.82–131)	25.71	13.06 (2.14–110)	26.95	298.500	0.776
Serum creatinine	0.73 (0.53–1.06)	29.94	0.68 (0.4–0.92)	20.53	200.000	0.031*
AST	84 (28–197)	25.34	78 (35–224)	27.17	291.500	0.676
ALT	68 (33–264)	27.74	75 (15–229)	25.79	290.000	0.655

The statistical analysis showed that there was a significant difference for the laboratory values of serum creatinine between the two groups. Patients with normal L3-SMI values had higher serum creatinine (median 0.73) compared to patients with low baseline L3-SMI (median 0.68, $p = 0.031$). The other parameters investigated did not reveal significant differences between the two groups of patients at baseline.

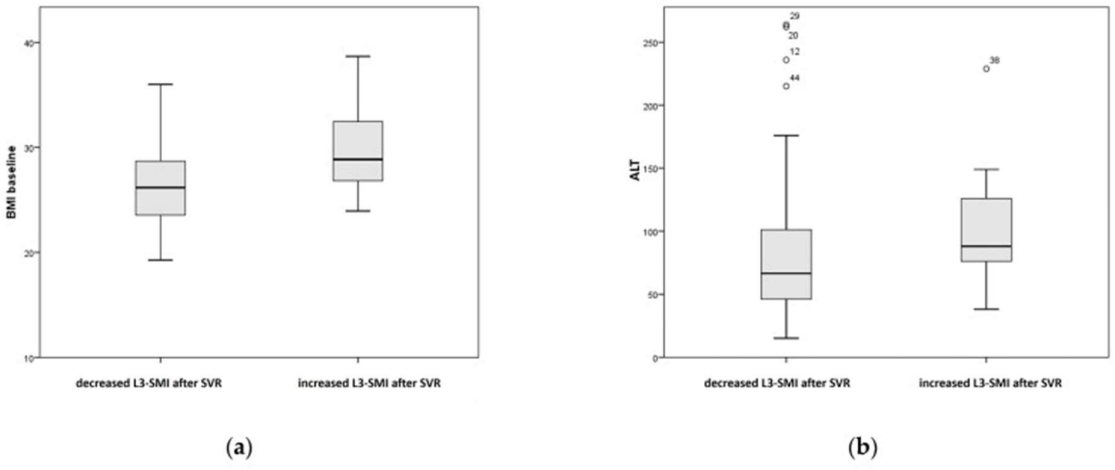
Comparison of Baseline Characteristics between Patients with Decreased L3-SMI and with Increased L3-SMI Assessed after Sustained Virological Response (SVR). To assess the dynamics of L3-SMI after treatment, the difference between L3-SMI values at baseline and those obtained after SVR was calculated. The differences were reported as a percentage of the initial value. The results were grouped in increased and decreased groups depending on the positive or negative values. In the studied group, the loss of muscle mass had

a median of −7.5% (−1% to −28%) and in those with growth the median was 7.2% (0.9% to 34%). After obtaining SVR, 14 patients showed an increase of L3-SMI, and 38 patients had a decrease in L3-SMI values. To assess whether there are significant differences between patients with decreasing muscle mass (according to L3-SMI values) and those with increasing muscle mass with respect to the initial values of the collected parameters, we performed the Mann–Whitney U test. The results are summarized in Table 2.X.

Table 2.X. Comparison between patients with decreased L3-SMI and increased L3-SMI

	Decreased (n=38)		**Increased (n=14)**		**Mann-Whitney**	
	Median (range)	**Mean Rank**	**Median (range)**	**Mean Rank**	**U**	***p***
Age (years)	59.5 (42-79)	27.04	57.07 (45-69)	25.04	245.500	0.672
BMI	26.17 (19.27-36)	23.55	28.84 (23.94-38.67)	34.50	154.000	0.021*
Total bilirubin	1.1 (0.48-2.47)	26.71	1.0 (0.43-2.4)	25.93	258.000	0.869
Serum albumin	4.04 (2.28-5.25)	28.79	3.69 (2.44-4.5)	20.29	179.000	0.073
Platelets	11.6 (5.6-29.3)	27.20	10.25 (3.7-17.5)	24.61	239.500	0.584
Total cholesterol	150.5 (80-221)	26.51	157.5 (102-198)	25.93	265.500	0.992
INR	1.14 (0.95-1.98)	26.13	1.14 (0.97-2.27)	27.50	252.000	0.772
Alpha-fetoprotein	11.2 (2.14-131)	24.26	26.15 (3.07-110)	32.57	181.000	0.080
Serum creatinine	0.71 (0.4-0.95)	26.39	0.7 (0.52-1.06)	26.79	262.000	0.934
AST	69 (28-224)	24.05	107.5 (38-146)	33.14	173.000	0.055
ALT	66.5 (15-264)	23.95	88 (38-229)	33.43	169.000	0.045*

The statistical analysis showed that BMI in the decreased L3-SMI group was significantly lower (median 26.17) than those without decreased L3-SMI (median 28.84, p = 0.021). Similarly, ALT values in the decreased L3-SMI group (median 66.5) were significantly lower than those without a decrease in L3-SMI (median 88, p = 0.045). Figure shows the boxplots for the two variables analyzed.



(a) (b)

Figure 2.12. Boxplot for BMI (**a**) and ALT (**b**) values for patients with decreased L3-SMI and patients with increased L3-SMI after SV

Linear Regression Model. All parameters available at baseline were assessed as predictive factors in multiple linear regression and multiple logistic regression models. The only viable model was linear regression with a single predictor represented by BMI. Linear regression (Figure 2.13) targeting the percentage change in L3-SMI values after SVR showed significant prediction ability of the independent variable assessing the BMI at baseline (ANOVA F (1.50) = 14.83, sig = 0.00). The variance of the single significant predictor obtained through the model, BMI at baseline, is able to predict at least 23% of the variance in the target variable L3-SMI (R2 = 0.229).

Comparison of Muscle Mass Dynamics after SVR between Groups with Low L3-SMI and Normal L3-SMI at Baseline. After treatment, 14 (28%) patients had an increase in L3-SMI values and 38 (72%) had a decrease in L3-SMI values. Comparing the evolution of L3-SMI values after treatment according to baseline groups, normal L3-SMI and low L3-SMI, we found a statistically significant difference between patients with low L3-SMI values (median −1.3) and patients with normal L3-SMI (median −3.98, $p = 0.02$) (Table 2.XI).

A higher number of patients in the sarcopenic group at baseline showed a post-SVR increase in L3-SMI values compared to the number of patients with normal baseline values. In patients with baseline low L3-SMI, 11 (33%) presented increased values after SVR while in patients with normal L3-SMI only 3 (16%) had an increase (Figure 2.14).

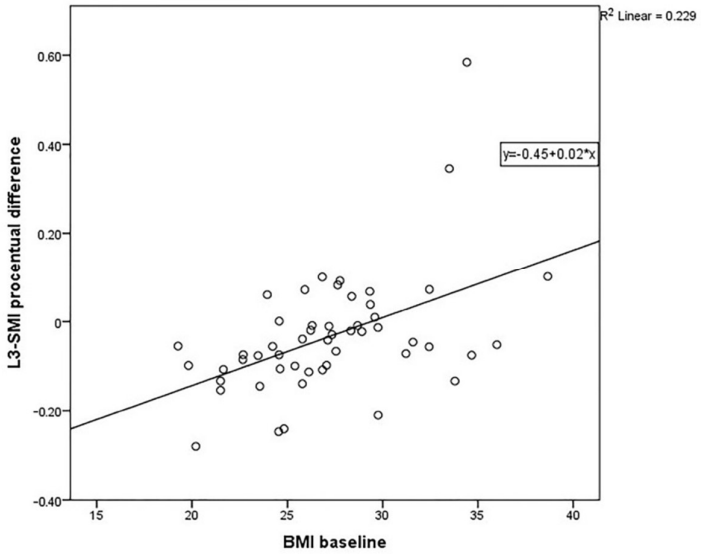


Figure 2.13. Linear regression for L3-SMI percentage change and BMI values at baseline

Table 2.XI. Comparison of muscle mass dynamics after SVR between groups with low L3-SMI and normal L3-SMI at baseline

	Normal L3-SMI (n=19)		**Low L3-SMI (n=33)**		**Mann-Whitney**	
	Median (range)	**Mean rank**	**Median (range)**	**Mean rank**	**U**	***p***
L3-SMI difference (numeric)	-3.98 (-16.94 to 23.81)	20.16	-1.3 (-6.94 to 17.34)	30.15	193.000	0.022*

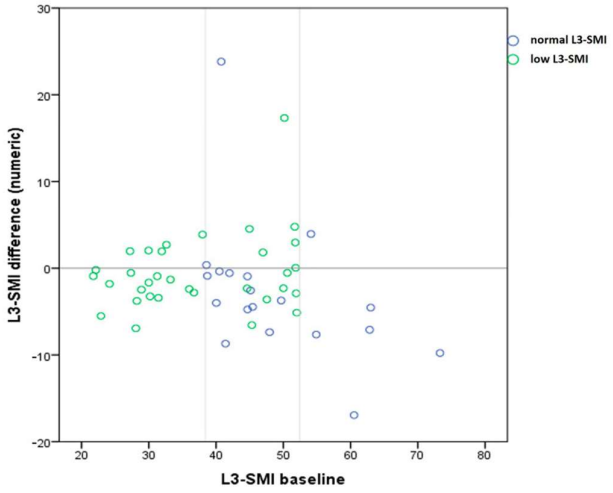


Figure 2.14. Scatter plot for L3-SMI difference after SVR in the baseline groups with low L3-SMI and normal L3-SMI. The difference in evolution is illustrated by the small number of patients in the group with initially normal values who showed an increase in L3-SMI values after SVR (above the zero line) compared to patients with low baseline values

Discussion. Our study included patients with HCV-related compensated cirrhosis, treated with direct-acting antivirals. A comprehensive pre-treatment characterization of demographic and biological parameters was compiled, and CT evaluations were performed both at baseline and during follow-up after sustained virological response, aiming to identify changes in skeletal muscle mass status, eventually related to the improvement of liver function.

Analysis of baseline data showed the global predominance of patients with low L3-SMI (63.5% of all patients were underneath definition values, and median L3-SMI was inferior to cut-off values for both male and female patients' groups), and proportion of patients with low L3-SMI values was higher in male patients compared to female patients (80% and 53.1%, respectively). Sarcopenia has been already identified as a frequent and important comorbidity related to liver cirrhosis and its high prevalence in cirrhotic patients is not unexpected. So far, data showed that between 30% and 70% of cirrhotic patients are sarcopenic (Kim et al., 2017). Nonetheless, because of its association with poor clinical outcome, such a high frequency of sarcopenia in a selected group of compensated cirrhosis must warn us about the importance of early nutritional care.

A special consideration seems to be necessary in male patients, which appear to be more predisposed to sarcopenia, as previous data have already shown, both in the general population and in patients with liver cirrhosis (Du et al., 2019). Furthermore, analysis of baseline data showed a significant difference regarding serum creatinine levels according to L3-SMI values. Patients with low L3-SMI had decreased serum creatinine levels compared to patients with normal L3-SMI. Because serum creatinine is a classical biomarker reflecting the body's muscle tissue mass, the results in our study are consistent with the current association between low creatinine levels and sarcopenia. No possible conditions that could have theoretically interfered with serum creatinine level dosage leading to falsely low values, such as advanced liver disease, fluid overload, and augmented renal clearance (Stevens et al., 2006) were identified in our study, consequently low serum creatinine levels found in the low L3-SMI group of patients are interpreted as a direct image of their poor muscle mass status.

To assess the dynamics of muscle mass status following antiviral treatment, we compared L3-SMI results before and after SVR. Our analysis showed that, globally, most patients (72%) presented a decrease of L3-SMI values, while only a few more than a quarter (28%) obtained an increase of L3-SMI values. According to baseline muscle mass status, more patients with initial low L3-SMI presented an increased index after SVR, compared to patients with normal muscular status at baseline (33% vs 16%, respectively). Therefore, even if a benefit in terms of muscle mass was noted only in a minority of all patients, improvements were more frequent in patients who were most in need of muscle mass status correction. It should be noted that although most patients in the baseline group with normal L3-SMI values showed a decrease after treatment, only three patients fell below the threshold values, the rest remaining within the normal range.

Undoubtedly, muscle mass dynamics has multiple underlying mechanisms, and sarcopenia in compensated cirrhosis is not completely decoded yet. Even if recent evidence suggests an increase or no more loss of skeletal muscle mass after antiviral treatment (Sakamori et al., 2021) the muscular improvement cannot be guaranteed solely by the viral clearance. As our results show, sarcopenia appears not to be solved only by the viral clearance, and supplementary nutritional interventions are necessary.

In our analysis, low BMI and low ALT were correlated to the decrease of L3-SMI after SVR. Classically, BMI is an indicator of nutritional status, and a higher BMI should consequently implicate a lower risk of sarcopenia. Even if sarcopenia is not excluded in overweight or even obese subjects, current thresholds showed lower sarcopenia prevalence with increases BMI (Linge et al., 2020). Therefore, all efforts to counteract underweight and malnutrition must be made. ALT (alanine aminotransferase) is found in plasma and in various body tissue, being the most common in liver. Increased ALT is used as a biomarker for hepatocellular injury, while there is limited evidence about the clinical implications associated with low ALT levels. Even if ALT is traditionally part of liver function tests, its levels do not reflect liver disease severity, being dually linked to liver function status - usually increased in most liver injury, with possible normal results in advanced liver disease (Ahmed et al., 2018). Several studies so far have demonstrated low serum ALT levels as predictive for long-term all-cause mortality among adults (Ramaty et al., 2014), while a recent study on patients with non-small lung cancer suggested a potential relation between low ALT serum activity and low muscle mass with increased frailty (Portal et al., 2019).

Thus, ALT has gained recognition as surrogate marker of frailty and shortened survival. In our study, lower ALT levels were predictive for the lack of benefit regarding muscle mass dynamics after SVR. In our relatively homogenous study group based on stage of liver disease, where all our patients had compensated cirrhosis, lower ALT levels appear to have predictive power concerning sarcopenia.

Our study has a few strengths and several limitations. As far as we know, this study is the first to discuss the CT evaluation of L3-SMI dynamics in cirrhotic patients who obtained SVR after DAAs treatment. As limitations, we could mention the relatively small number of patients included and the variable CT scan follow-up interval, due to the retrospective type of the study.

Conclusions. Sarcopenia, a frequent comorbidity in patients with advanced liver disease, can be objectively assessed by CT-based measurements. Even if the skeletal mass index is partially influenced by the viral clearance, some improvements were recorded in patients with baseline sarcopenia. Low creatinine serum level correlates with sarcopenia. Lower BMI and ALT serum levels at baseline were predictive for no benefit in terms of muscle mass dynamics. Understanding all the mechanisms involved in sarcopenia and identifying the most vulnerable cirrhotic patients could ensure optimal adapted care strategies.

I.2.3. NONALCOHOLIC FATTY LIVER DISEASE – THE NEW PANDEMIC

I.2.3.1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is one of the most important non-neoplastic pathologies in contemporary medicine, being the most common cause of chronic liver disease worldwide. It is characterized by the accumulation of fat in the liver, in patients without significant alcohol consumption (Lonardo et al., 2019).

The clinical importance of NAFLD is related to its prevalence of up to 25-30% in the general population, thus exceeding that of viral hepatitis and alcoholic liver disease (Younossi et al., 2019). The global burden of NAFLD is rising in parallel with increasing rates of obesity, type 2 diabetes mellitus (T2DM), and metabolic syndrome (Takalkar et al, 2018).

It is worrying that the disease includes both a form considered benign (hepatic steatosis) and progressive forms (steatohepatitis with or without fibrosis) with the possibility of transformation to liver cirrhosis and in some cases, hepatocellular carcinoma (Asfari et al., 2020). However, the pathogenic mechanisms of progression from the simple form to the aggressive forms are not completely elucidated due to their complexity, through the involvement of multiple mechanisms and metabolic, immunological and genetic imbalances (Stanciu et al., 2019). In the coming years this pathology is expected to become the first indication for liver transplantation, surpassing viral liver cirrhosis of any etiology (Wong et al., 2020).

The NAFLD is emerging as a global health issue of upmost importance, and due to its continuously increasing prevalence, it is already regarded as a global pandemic (Portincasa et al., 2020).

The data on prevalence of NAFLD varies greatly depending on the definition used, the population and the diagnostic method; while the world median in the general population is considered 20%, in Europe the median prevalence is 25-26% (Bellentani et al., 2017), with an estimated prevalence of NASH ranging between 3 and 5% (Vernon et al., 2011). In the specific risk groups, the prevalence has been recorded to be much higher: in type 2 diabetes patients NAFLD prevalence is around 65%, in obesity 80%, and in patients with dyslipidemia around 50% (Assy et al., 2000).

In Romania, the largest published study evaluated the presence of NAFLD in 3005 hospitalized patients, without known liver diseases, using US examination and reported it as 20%, which was similar to the reported prevalence for the European general population (Radu et al., 2008). Another Romanian study, analyzed the prevalence and the predictive factors of NAFLD defined by the fatty liver index in T2DM patients and reported the presence of NAFLD in 79% (Silaghi et al., 2016) According to the CAP evaluation of steatosis, another study found in patients with type 2 diabetes a prevalence of NAFLD of 76%, with 60% of patients having severe steatosis (Sporea et al., 2020). In patients with morbid obesity ongoing bariatric surgery, a small histological study showed the presence of NAFLD in 100% of patients, and of NASH in 58% of cases (Livadariu et al., 2015). Another study on the same category of patients, which I co-authored, showed correlations between vitamin D-deficiency and biopsy proven NAFLD (Livadariu et al., 2018).

As personal contribution in the area of NAFLD epidemiology, I will present a study in which we evaluated the prevalence of liver steatosis and fibrosis in apparently healthy Romanian medical students (Nastasa et al., 2021). Our findings showed that the prevalence of steatosis and significant fibrosis among a cohort of apparently healthy medical students is low; in 426 students analyzed, we found that 352 (82.6%) had no steatosis (S0), 32 (7.5%) had mild steatosis (S1), 13 (3.1%) had a moderate degree of steatosis (S2), and 29 (6.8%) had severe steatosis (S3); based on liver stiffness measurements (LSM), 277 (65%) medical students did not have any fibrosis (F0), 136 (31.9%) had mild fibrosis (F1), 10 (2.4%) participants were

identified with significant fibrosis (F2), 3 (0.7%) with advanced fibrosis (F3), and none with cirrhosis (F4). In addition, we identified that overweight and obesity were not very common, but high BMI, WtHR, and WC values are associated risk factors for liver steatosis, as well as fibrosis. Therefore, the growing obesity epidemic can be avoided by a multidisciplinary approach to include lifestyle changes with special attention to regular physical exercise, and furthermore, individualized screening strategies should be established for significant LF and steatosis according to anthropometric indices.

Lately, the term metabolic dysfunction-associated fatty liver disease (MAFLD) was proposed instead NAFLD, due to its overarching character and comprehensive significance (Eslam et al., 2020). The proposed criteria for diagnosis are based on histology, imaging or biomarkers to sustain hepatic steatosis in addition to one of the following three criteria: overweight/obesity, type 2 diabetes, or metabolic dysregulation – defined by the presence of at least two metabolic risk factors. Thus, MAFLD could be defined in non-overweight/obese individuals. Lean persons are not exempted from the development of MAFLD and it is recognized that 2-60% of MAFLD patients are neither obese nor overweight (Chen et al., 2020).

As personal contribution in the area of ***"lean NAFLD"*,** I will present the results of our study evaluating the clinical features and associated risk factors of lean-NAFLD in comparison with obese-NAFLD patients. Analyzing 331 patients, mostly obese-NAFLD, we found that the proportion of subjects with at least significant fibrosis (_F2) was approximately twofold higher among obese-NAFLD (43.81%) in comparison with lean-NAFLD patients (23.29%). Moreover, obese individuals had a higher risk for liver fibrosis (OR = 2.6, 95%, CI 1.5–4.42, $p < 0.001$) than lean individuals. Although associated metabolic conditions and at least significant liver fibrosis were present in approximately one-quarter of the patients, these were more frequent among obese-NAFLD patients (Trifan et al., 2022). Our findings are consistent with other results published so far (Portincasa et al., 2020), showing the significance of the risk factors in the approach of NAFLD.

Recently, it has been generally accepted that NAFLD is no longer a condition to be ignored, with a high risk of progression to end stage liver disease, but also that there are identifiable risk factors, some of them preventable.

Therefore, individualized screening strategies for NAFLD should be established, according to the specific clinical profile of the patients, including BMI.

PERSONAL CONTRIBUTIONS IN THE DOMAIN OF NONALCOHOLIC FATTY LIVER DISEASE

	ARTICLES
1.	Nastasa R, Stanciu C, Zenovia S, **Singeap AM**, Cojocariu C, Sfarti C, Girleanu I, Chiriac S, Cuciureanu T, Huiban L, Muzica CM, Trifan A. The Prevalence of Liver Steatosis and Fibrosis Assessed by Vibration-Controlled Transient Elastography and Controlled Attenuation Parameter in Apparently Healthy Romanian Medical Students. *Diagnostics (Basel).* 2021 Dec 13;11(12):2341. doi: 10.3390/diagnostics11122341. PMID: 34943578; PMCID: PMC8700151 **IF = 3.992.**
2.	**Singeap AM**, Stanciu C, Huiban L, Muzica CM, Cuciureanu T, Girleanu I, Chiriac S, Zenovia S, Nastasa R, Sfarti C, Cojocariu C, Trifan A. Association between Nonalcoholic Fatty Liver Disease and Endocrinopathies: Clinical Implications. *Can J Gastroenterol Hepatol.* 2021 Jan 11;2021:6678142. **IF = 2.605**
3.	Trifan A, Stratina E, Rotaru A, Stafie R, Zenovia S, Nastasa R, Huiban L, Sfarti C, Cojocariu C, Cuciureanu T, Muzica C, Chiriac S, Girleanu I, **Singeap AM**, Stanciu C. Changes in Liver Steatosis Using Controlled Attenuation Parameter among Patients with Chronic Hepatitis C Infection Treated with Direct-Acting Antivirals Therapy Who Achieved Sustained Virological Response. *Diagnostics*. 2022; 12(3):702. **IF = 3.992**

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I.2.3.2. The prevalence of liver steatosis and fibrosis in apparently healthy Romanian medical students

Background and aim. There are several studies regarding the increasing prevalence of NAFLD in young adults, which parallels the high rates of obesity and metabolic syndrome in this age group (Mrad et al., 2016; Doycheva et al., 2017).

The term "young adult" is very familiar to oncologists and refers to a population of patients starting from the age of 20 years without being able to set the upper age limit in clinical practice (Barr et al., 2016). Among these patients, some present a number of risk factors for developing NAFLD, such as: obesity, T2DM, unhealthy lifestyle, smoking habits, and male sex. The interplay between these factors on a background of genetic predisposition may contribute to the installation of NASH (Doycheva et al., 2017).

Vibration-Controlled Transient Elastography (VCTE) is considered the optimal non-invasive method for assessing LF, recommended by guidelines over the years for fibrosis evaluation, especially in chronic viral hepatitis (The European Association for the Study of the Liver -EASL 2015, WHO 2016).

In addition, in recent years, the implementation of Controlled Attenuation Parameter (CAP - that reflects the fat impedance in the liver) in FibroScan® (Echosens, Paris, France) devices has allowed the concomitant evaluation of hepatic fibrosis and steatosis (Karlas et al., 2017; Chalasani et al., 2018).

Herein, we aimed to evaluate the prevalence of steatosis and fibrosis in apparently healthy Romanian medical students by VCTE and CAP score. We also researched risk factors associated with hepatic steatosis and fibrosis in this population group.

Materials and methods. This study population consisted of apparently healthy 3rd and 5th-year medical students, with a high level of education, from "Grigore T. Popa" University of Medicine and Pharmacy Iasi, evaluated between February and June 2021.

Demographic data, personal history, clinical examination, and data obtained from their general practitioner were recorded along with anthropometric and FibroScan assessments. Eligibility criteria were the absence of significant alcohol consumption (<20 g/day in women, <30 g/day in men) and of a history of chronic liver disease. Participants with unreliable transient elastography examination (<10 valid measurements with an interquartile range/median (IRQ/M) ratio >30%) were excluded. For subjects with a liver stiffness measurement (LSM value) ≥7.2 kPa on VCTE examination, laboratory data were collected.

This study was approved by the Ethics Committee of our university and was conducted according to the principles of the Declaration of Helsinki. Each student signed a written informed consent.

LSM and CAP assessment. All students were evaluated using FibroScan® 502 Touch (Echosens, Paris, France) by one experienced physician with more than 1000 explorations performed before, using one single examination on each subject, following procedure instructions. LSM were expressed in kilopascals (kPa), with the following cut-offs for LF: ± 5.5 kPa—F0 (without fibrosis) 5.6 kPa—F1 (mild), 7.2 kPa—F2 (significant), 9.5 kPa—F3 (advanced), and 12.5 kPa—F4 (cirrhosis). Liver steatosis measured by CAP was expressed in decibels/meter (dB/m), and steatosis degrees were S1 (mild)—248 dB/m, S2 (moderate)—268 dB/m, and S3 (severe)—280 dB/m.

Anthropometric measurements. Height and weight measurements were performed using a height meter and the weight scale. Overweight (± 25 kg/m2) and obesity (>30 kg/m2) were established using cut-off values defined by the WHO, while waist-to-height ratio (WtHR) is defined by dividing waist circumference (cm) to height (cm), with a settled value ± 0.50 (McCarthy et al. 2006).

Statistical analyses were performed using SPSS software version 22.0 (IBM SPP Inc., Chicago, IL, USA). Qualitative data were expressed as numbers (percentage), while quantitative variables were expressed as means ± standard deviation (SD). The Kolmogorov–Smirnov test was used for distribution analysis, continuing with the Student's t-test, Mann–Whitney U, or chi-square test that was considered appropriate for comparing group variables. The association between two variables was made by utilizing the Pearson correlation coefficient (r). Two-tailed p-values of <0.05 were considered statistically significant.

Results. Participants characteristics. A total of 505 subjects were invited to participate in this study, 439 of which were evaluated by VCTE and CAP. 13 participants were excluded due to unreliable measurements (10 cases) and examination failure without any measurements (3 cases). Four hundred and twenty-six medical students who met the admission standards were included in the final analysis. All baseline characteristics are summarized in Table 2.XII.

The prevalence of overweight, obesity, and abdominal obesity was 14.8%, 3.5%, and 7.5%, respectively. Most of the participants were in the 21-year-old group, with a predominance of female gender (67.8% females, mean age 22.22 ± 1.7 years, and body mass index (BMI) 22.59 ± 3.34 kg/m^2).

Table 2.XII. The characteristics of the overall participants included in study

	Overall cohort n, 426	**Men n, 137**	**Women n, 289**	**p-value**
Age (years)	22.22 ± 1.7	22.45 ± 1.8	22.11 ± 1.6	0.144
Females, n (%)	289 (67.8)	-	-	
Weight (kg)	65.84 ± 13.37	74.09 ± 13.29	61.95 ± 11.56	<0.001
Height (cm)	170 ± 8.56	176 ± 10.2	167 ± 10.7	<0.001
Body mass index (kg/m2)	22.59 ± 3.34	23.71 ± 3.33	22.07 ± 3.22	<0.001
Waist circumference (cm)	73.7 ± 10.29	78.79 ± 11.35	71.31 ± 8.82	<0.001
Abdominal obesity, n (%)	32 (7.5%)	17 (12.4%)	13 (4.5%)	<0.001
Waist-to-height ratio	0.427 ± 0.06	0.442 ± 0.06	0.42 ± 0.05	0.159
Non-overweight, n (%)	348 (81.7)	102 (74.5)	246 (85.1)	0.046
Overweight, n (%)	63 (14.8)	27 (19.7)	36 (12.5)	0.004
Obese, n (%)	15 (3.5)	8 (5.8)	7 (2.4)	0.037
Liver steatosis, n (%)	74 (17.4)	39 (28.5)	35 (12.1)	0.011
Steatosis degree, n (%)				0.026
0	352 (82.6)	98 (71.5)	254 (87.9)	
1	32 (7.5)	18 (13.1)	14 (4.8)	
2	13 (3.1)	5 (3.7)	8 (2.8)	
3	29 (6.8)	16 (11.7)	13 (4.5)	
Fibrosis stage, n (%)				0.186
0	277 (65)	79 (57.6)	198 (68.5)	
1	136 (31.9)	50 (36.5)	86 (29.8)	
2	10 (2.4)	6 (4.4)	4 (1.4)	
3	3 (0.7)	2 (1.5)	1 (0.3)	
CAP, dB/m	215.76 ± 48.38	234.49 ± 47.38	206.95 ± 46.42	<0.001
LSM, kPa	5.29 ± 1.35	5.36 ± 1.2	5.26 ± 1.42	0.582
M-probe, n (%)	402 (94.4)	128 (93.4)	274 (94.8)	0.410
XL-probe, n (%)	24 (5.6)	9 (6.7)	15 (5.2)	0.372

n-number of subjects; CAP, controlled attenuation parameter; LSM, liver stiffness measurement

Men were heavier (74.09 ± 13.29 kg vs. 61.95 ± 11.56 kg, $p < 0.001$), taller (176 ± 10.2 cm vs. 167 ± 10.7 cm, $p < 0.001$), with a greater proportion of overweight (19.7% vs. 12.5%, $p = 0.004$), obesity (5.8%vs. 2.4%, $p = 0.037$), and abdominal obesity (12.4%vs 4.5%, $p < 0.001$) than women.

The prevalence of hepatic steatosis among all students was 17.4%, with a mean CAP of 215.76 ± 48.38 dB/m; 32 (43.2%) of them had S1, and 42 (56.8%) had significant steatosis (S2-S3) with a CAP score above 268 dB/m. The proportion of male students among steatosis degrees was higher compared to women ($p = 0.026$), with an increased CAP value (234.49 ± 47.38 dB/m vs. 206.95 ± 46.42 dB/m, $p < 0.001$). Regarding the prevalence of LF, the majority (277 students, 65%) had no LF, while 136 (31.9%) participants had F1, 10 (2.4%) had F2, 3 (0.7%) had F3, and no one was found with F4 LF; the mean LSM of 5.29 kPa ± 1.35. Subjects with a LF ± F2 were predominantly males (61.5%) with a mean BMI of 24.58 ± 3.41 kg/m2 and a WtHR 0.462 ± 0.07. The characteristics of patients with LF ≥ F2 are presented in Table 2.XIII.

Participants characteristics according to absence or presence of liver steatosis. The participants included in the study that were diagnosed with liver steatosis were predominantly males ($p = 0.031$), with an increased weight ($p < 0.001$), BMI ($p < 0.001$), WC ($p < 0.001$), and WtHR ($p < 0.001$) (Table 2.XIV).

Table 2.XIII. Increased clinical and laboratory parameters in patients with liver fibrosis ≥ F2

	Subjects, n = 13	**Increased, n (%)**
Age (years)	22.7 ± 1.5	-
Males, n (%)	8 (61.5)	-
Body mass index (kg/m2)	24.58 ± 3.41	8 (61.5)
Waist-to-height-ratio	0.462 ± 0.07	5 (38.5)
Platelet count (G/L)	287 ± 72.45	0 (0)
ALT (IU/L)	24.7 ± 14.9	3 (23.1)
AST (IU/L)	26.3 ± 11.4	4 (30.7)
GGT (IU/L)	25.1 ± 16.6	2 (15.3)
ALP (IU/L)	62.7 ± 20.2	0 (0)
Total bilirubin (mg/dL)	0.68 ± 0.25	0 (0)
Fasting glucose (mg/dL)	88.3 ± 17.1	6 (46.1)
Total cholesterol (mg/dL)	208.5 ± 38.3	5 (38.5)
Triglycerides (mg/dL)	131.6 ± 52.9	7 (53.8)
LDL-c (mg/dL)	112.1 ± 26.6	3 (23.1)

ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; ALP, alkaline phosphatase; LDL-c low density lipoprotein cholesterol. Increased values: BMI > 25 kg/m2; WtHR > 0.5; ALT > 35 IU/L; AST > 35 IU/L; GGT > 40 IU/L; ALP > 140 IU/L; Total bilirubin > 1 mg/dL; Fasting glucose > 100 mg/dL; Total cholesterol > 200 mg/dL; Triglycerides > 150 mg/dL; LDL-c > 130 mg/dL

However, the proportion of overweight (40.5% vs. 9.4%, $p < 0.001$) and obese (9.5% vs. 2.3%, $p < 0.001$) students was significantly higher among the liver steatosis group, of whom 18.9% had abdominal obesity. Regarding LF stages, 32 (43.2%) of students had mild fibrosis (F1), 5 (6.8%) had significant fibrosis (F2), and 3 (4.1%) had advanced LF (F3), with an increased LSM value ($p = 0.027$) compared with those without hepatic steatosis, consisting of 104 (29.6%) with F1, 5 (1.4%) with F2, and none with advanced LF ($p = 0.024$).

Table 2.XIV. Baseline characteristics of participants according to the presence of liver steatosis

	No Hepatic Steatosis n, 352	**Hepatic Steatosis n, 74**	**p-Value**
Age (years)	22.18 ± 1.61	22.36 ± 1.73	0.565
Males, n (%)	98 (27.8)	39 (52.7)	0.031
Weight (kg)	63.14 ± 11.37	75.09 ± 16.06	<0.001
Height (cm)	170 ± 10.5	171 ± 10.8	0.061
Body mass index (kg/m2)	22.14 ± 3.04	24.89 ± 3.91	<0.001
Waist circumference (cm)	71.9 ± 8.82	81.23 ± 12.94	<0.001
Abdominal obesity, n (%)	18 (5.1%)	14 (18.9%)	<0.001
Waist-to-height ratio	0.418 ± 0.05	0.482 ± 0.09	<0.001
Non-overweight, n (%)	311 (88.3)	37 (50)	0.029
Overweight, n (%)	33 (9.4)	30 (40.5)	<0.001
Obese, n (%)	8 (2.3)	7 (9.5)	<0.001
Fibrosis stage, n (%)			0.024
0	243 (69)	34 (45.9)	
1	104 (29.6)	32 (43.2)	
2	5 (1.4)	5 (6.8)	
3	0 (0)	3 (4.1)	
CAP, dB/m	199.16 ± 35.39	280.41 ± 38.95	<0.001
LSM, kPa	5.23 ± 1.35	5.61 ± 1.28	0.027
M-probe, n (%)	341 (96.9)	61 (82.4)	0.244
XL-probe, n (%)	11 (3.1)	13 (17.6)	<0.001

CAP, controlled attenuation parameter; LSM, liver stiffness measurement

Correlation between anthropometric parameters, CAP and LSM. Overall, we found a significant correlation between CAP and WtHR ($r = 0.36$, $p < 0.001$) BMI ($r = 0.34$, $p < 0.001$), weight ($r = 0.34$, $p < 0.001$), and waist circumference ($r = 0.33$, $p < 0.001$).Regarding LF expressed by LSM, only WtHR ($r = 0.13$, $p = 0.040$), BMI ($r = 0.21$, $p = 0.001$), and waist circumference ($r = 0.14$, $p = 0.024$) maintained a significant correlation.

Discussion. NAFLD is a very frequent cause of chronic liver disease, affecting approximately 25% of the world population; NASH and related significant fibrosis are the greatest predictors of high mortality, liver cirrhosis, and HCC (Younossi et al., 2018).

The most important study that analyses the prevalence of NAFLD in young adults was conducted by Mrad et al. in the United States of America, on a population aged from 18 to 35 years, which showed that the prevalence of NAFLD has risen 2.5 times in the last three decades, affecting 25% of the young adults nowadays (Mrad et al., 2016). Moreover, the authors concluded that the implementation of a screening program is needed in this age group to prevent the development of cirrhosis and its complications.

To the best of our knowledge, this is the first study on the prevalence of NAFLD and LF among Romanian medical students.

In our study, approximately one in five students, who were apparently healthy, had hepatic steatosis, and one in thirty-three had significant LF (≥F2). In addition, in line with the current literature, we have demonstrated that male gender, BMI, waist circumference, and waist-to-height ratio were the main risk factors associated with hepatic fat accumulation.

Most of the students included in our study had no hepatic steatosis and our results are quite similar to those reported by recent studies. In similar research, Kaya et al. reported a 23.2% NAFLD prevalence in a group of 112 medical students with a mean CAP value of 205.6 ± 43.8 dB/m (Kaya et al, 2016).

Moreover, Abeysekara et al. conducted a study in Great Britain, which included only apparently healthy young adults, and found that the prevalence of hepatic steatosis was 20.7%, with significant steatosis approximately two-thirds of the patients diagnosed with steatosis based on VCTE (Abeysekara et al., 2020).

In our group of subjects we found that the male sex, high BMI, waist circumference, WtHR, and an increased LSM value ($p = 0.027$) are independently risk factors associated with high values of CAP score and our data are in accordance with other recent studies (Doycheva et al., 2017; Abeysekara et al., 2020). Most of participants had no LF or had only a mild form. Shaheen et al. found a high prevalence of NAFLD among Egyptian young adults (47.5% had variable stages of steatosis) and 56.7% had fibrosis (Shaheen et al., 2019). When compared to our findings, these results seem to be significantly different, and a possible reason could be the demographic contrast between the studied cohorts, considering the increased incidence of obesity and metabolic syndrome in the Egyptian population.

Nevertheless, the prospective design of this study countervails these drawbacks, as it includes a large series of asymptomatic patients with a high level of education. An advanced imaging technique, such as VCTE with CAP, was used for establishing the diagnosis of LF and steatosis, methods validated and correlated with histological findings based on LB in NAFLD patients (Vuppalanchi et al., 2018).

Conclusions. In summary, our findings show that the prevalence of steatosis and significant fibrosis among our cohort of apparently healthy medical students is low. In addition, we identified that overweight and obesity were not very common, but high BMI, WtHR, and WC values are associated risk factors for liver steatosis, as well as fibrosis.

Therefore, the growing obesity epidemic can be avoided by a multidisciplinary approach to include lifestyle changes with special attention to regular physical exercise.

Furthermore, individualized screening strategies should be established for significant LF and steatosis according to anthropometric indices.

I.2.3.3. Clinical and laboratory characteristics of normal weight and obese individuals with non-alcoholic fatty liver disease

Background and aim. NAFLD is strongly linked to the obesity pandemic and overweight status, however this disorder is also present in lean subjects with body mass index (BMI) < 25kg/m², with no known evident risk factors (Feldman et al., 2017). The term of lean-NAFLD was first introduced by Vos et al. in 2011 and since then, the prevalence of this disorder seems to be much higher than it was previously thought, mainly due to the availability and accessibility of new non-invasive diagnostic methods nowadays, thus an early diagnosis is essential for a more effective management (Vos et al., 2011; Cigrovski Berkovic et al., 2021).

Lean-NAFLD is considered to be a major clinical and diagnosis challenge, because when obesity, which is known as a clinical landmark for steatosis, is absent, the diagnosis of liver steatosis or liver damage is, most of the times, delayed or even failed to be noticed at all. As a result, the required medical intervention and treatment is most likely initiated too late. In the diagnosis process, helpful hints could be provided by the fact that lean-NAFLD and overweight/obese-NAFLD patients do share a common metabolic profile, closely associated with the components of the metabolic syndrome (MeS), such as hypertension, low high-density lipoprotein level (HDL-c), hypertriglyceridemia, type 2 diabetes mellitus (T2DM) or increased fasting plasma glucose and increased waist circumference (Sookoian et al., 2017; Bale et al., 2019).

The pathophysiology of NAFLD is yet to be completely understood, especially in individuals with normal BMI. Even in the presence of normal subcutaneous fat and a low BMI, abdominal fat or central obesity alongside insulin resistance could play an important role in the development of NAFLD in lean subjects (Youness et al., 2019). Consequently, body composition measurements, particularly body fat percentage (BF%), could provide additional helpful information in order to better understand this disease. The most accurate methods for determining BF% are magnetic resonance imaging (MRI), computed tomography (CT) scans and dual-energy X-ray absorptiometry (DXA) (Kaul et al., 2012).

The gold standard method for the diagnosis and staging the severity of the disease in patients with NAFLD is still considered to be the liver biopsy (LB). However, due to its invasive nature and possible complications, this method cannot be used as a screening option, giving way to an increasing number of non-invasive cost-efficient methods, such as biochemical tests or imaging techniques (Wong et al., 2018). Among them, Vibration-Controlled Transient Elastography (VCTE) with Controlled Attenuation Parameter (CAP) is considered to be the non-invasive method of choice, allowing simultaneous assessment of both hepatic steatosis and fibrosis, with high rates of sensitivity and specificity for staging chronic liver diseases and a low failure rate (less than 5% since the XL-probe was introduced in the machine equipment) (Karlas et al., 2010).

Therefore, this paper aims to characterize the clinical features and associated risk factors of lean-NAFLD in comparison with obese-NAFLD patients.

Materials and methods

Participants. This is a prospective study from a tertiary referral hospital in North-Eastern Romania that included consecutively lean and obese patients with clinically suspected (elevated liver enzymes) or diagnosed NAFLD by abdominal ultrasonography (US), referred to our clinic by general practitioners and colleagues in other specialties, evaluated between November 2019 and October 2021. Demographic data, anthropometric measurements, clinical examination, personal medical history and VCTE with CAP assessment were recorded for all patients. The inclusion criteria were: (1) adults over 18 years, (2) without any history of significant alcohol consumption (<20 g/day in women, <30 g/day in men), (3) no known history of malignancy diagnosed in the past year, (4) without any secondary causes of chronic liver disease and (5) patients with reliable transient elastography examination [>10 valid

measurements with an interquartile range/median (IQR/M) ratio <30%]. Blood tests were collected (anti-HCV Ab, HBsAg, total cholesterol, low-density lipoprotein cholesterol (LDL-c), HDL-c, triglycerides, total bilirubin, gamma-glutamyl transpeptidase (GGT), alanine and aspartate aminotransferase (ALT, AST), fasting plasma glucose, platelets count, serum uric acid (SUA), serum urea, creatinine, C-reactive protein (CRP), alkaline phosphatase (ALP), International Normalized Ratio (INR), albumin). In regard with their BMI, patients were divided in two sub-groups, namely lean and obese cohorts. This study was approved by the Ethics Committee of our University and was conducted according to the principles of the Declaration of Helsinki. Each participant signed a written informed consent.

Liver stiffness measurements (LSM) and CAP Assessment. The examinations were performed by two experienced physicians using FibroScan® 502 Touch (EchoSens, Paris, France) following procedure instructions.

The examination was performed starting with the M probe on the right hepatic lobe through the 9th to 11th intercostal spaces on the midaxillary line after overnight fasting, with the participant lying in the dorsal decubitus position and his right arm in maximal abduction, while the XL probe was used according to the machine instructions in obese patients. LSM were expressed in kilopascals (kPa) with the following cut-off values for liver fibrosis: ≥5.6 kPa - mild (F1), ≥7.2 kPa - significant (F2), ≥9.5 kPa - advanced (F3), and ≥12.5 kPa – liver cirrhosis (F4) (Wong et al., 2010).

In addition, for liver steatosis, the results were measured in decibels/meter (dB/m) and the cut-off values were: 248 dB/m, 268 dB/m and 280 dB/m for mild (S1), moderate (S2), respectively severe steatosis (S3) (Karlas et al., 2017).

Anthropometric measurements. Anthropometric indexes, such as height and weight were recorded for every patient in optimal conditions (no shoes and light clothes). Cut-off values for BMI established by WHO were used in order to define the two study groups (lean and obese subjects).

Statistical analysis. Descriptive statistics were computed for all factors using IBM SPSS, Version 22.0 (IBM SPSS Inc, Chicago, IL, USA). We used the Kolmogorov-Smirnov test for checking the normality of the distribution of numerical variables. Continuous variables are expressed as means and standard deviation, while categorical ones are expressed as numbers (percentage). Data were analyzed using unpaired t-test for comparison of continuous variables between groups for normally distributed data or chi-squared and Fisher's exact test depending on data type, while Mann–Whitney U test or the Kruskal–Wallis analysis of variance (ANOVA) test was used to compared skewed data as appropriate. Univariate linear regression followed by multivariate linear regression using only the significant factors was performed to identify the factors that have an highly influence on the CAP and LSM values. Pearson correlation coefficient (r) was used for establishing the association between two variables. A two-tailed p-value of < 0.05 was considered statistically significant. Only complete data sets were analyzed.

Results

Patients characteristics. In our study, 418 lean and obese patients with clinically suspected or diagnosed NAFLD were evaluated. Thirteen subjects declined the informed consent while 18 patients were excluded because of history of significant alcohol consumption. A total of 387 patients were examined using VCTE with CAP, 50 subjects being excluded afterwards (8 subjects with unreliable measurements, 3 individuals with examination failure and 39 patients with CAP values <248 dB/m). Another 6 patients were excluded because of secondary causes of chronic liver disease (hepatitis B – 3 subjects, C – 2 subjects, autoimmune hepatitis – 1 subject). 331 patients with valid measurements were included in the final analysis: 73 (22.1%) lean patients and 258 (77.9%) obese subjects. The mean age of the whole study group was 56.89 ± 13.07 years; regarding gender, 188 were men and 143 women. Hypertension

and T2DM were present in 207 (62.5%) and 101 (30.5%) respectively. All other patient's characteristics included in our analysis were summarized in Table 2.XV.

Table 2.XV. Patients characteristics

	Overall Cohort	Lean	Obese	p- value
Patients characteristics	n= 331	n, (%) = 73 (22.1)	n, (%) = 258 (77.9)	
Age (years)	56 ± 13	52 ± 15	58 ± 11	0.001
Height (cm)	170 ± 9	172 ± 8	170 ± 9	0.076
Weight (kg)	87 ± 15	68 ± 8	93 ± 12	<0.001
Gender female	143 (43.20%)	36 (49.32%)	107 (41.47%)	0.232
Gender male	188 (56.80%)	37 (50.68%)	151 (58.53%)	0.158
BMI (kg/m²)	30 ± 4	22 ± 1	32 ± 2	<0.001
LSM (kPa)	7.40 ± 4.22	6.37± 3.36	7.69 ± 4.39	0.007
CAP (dB/m)	299 ± 40	273 ± 23	307 ± 41	<0.001
Platelet count (109/L)	246 ±74	238 ± 65	248 ± 76	0.271
INR	1.02 ± 0.16	1.01 ± 0.17	1.03 ± 0.16	0.532
CRP (mg/dL)	0.52 ± 1.50	0.38 ± 0.53	0.56 ± 1.68	0.154
Fasting plasma glucose (mg/dL)	110 ± 38	96 ± 20	114 ± 41	<0.001
Serum urea (mg/dL)	38 ± 10	33 ± 7	39 ± 10	<0.001
Creatinine (mg/dL)	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.001
ALT (IU/L)	43 ± 26	31 ± 20	46 ± 27	0.003
AST (IU/L)	33 ± 19	28 ± 14	35 ± 20	0.003
GGT (IU/L)	44 ± 37	37 ± 31	46 ± 38	0.037
ALP (IU/L)	85 ± 31	73 ± 21	89 ± 33	<0.001
Total bilirubin (mg/dL)	0.7 ± 0.3	0.7 ± 0.2	0.7 ± 0.3	0.701
Total cholesterol (mg/dL)	215 ± 39	207 ± 38	217 ± 40	0.047
Triglycerides (mg/dL)	157 ± 71	153 ± 51	159 ± 75	0.437
Albumin (g/dL)	4.6 ± 2	4.5 ± 0.4	4.7 ± 2.3	0.297
LDL-c (mg/dL)	128 ± 39	117 ± 41	131 ± 38	0.013
HDL-c (mg/dL)	45 ±11	49 ± 10	43 ± 11	<0.001
Serum uric acid (mg/dL)	4.9 ± 1.4	4.2 ± 1.3	5.1 ± 1.4	<0.001
Fibrosis				
F0, n (%)	122 (38.86%)	40 (54.79%)	82 (31.78%)	<0.001
F1, n (%)	79 (23.87%)	16 (21.92%)	63 (24.42%)	0.658
F2, n (%)	63 (19.03%)	7 (9.59%)	56 (21.71%)	0.020
F3, n (%)	37 (11.18%)	6 (8.22%)	31 (12.02%)	0.363
F4, n (%)	30 (9.06%)	4 (5.48%)	26 (10.08%)	0.227
Steatosis				
S1, n (%)	90 (27.19%)	36 (49.32%)	54 (20.93%)	<0.001
S2, n (%)	62 (18.73%)	23 (31.51%)	39 (15.12%)	0.002
S3, n (%)	179 (54.08%)	14 (19.18%)	165 (63.95%)	<0.001
Hypertension, n (%)	207 (62.54%)	30 (41.10%)	177 (68.60%)	<0.001
T2DM, n (%)	101 (30.51%)	14 (19.18%)	87 (33.72%)	0.017

BMI, body mass index; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; ALP, alkaline phosphatase; LDL-c, low-density lipoprotein cholesterol; HDL-c, high-density lipoprotein cholesterol; LSM, liver stiffness measurement; CAP, controlled attenuation parameter; CRP, c-reactive protein; INR, International Normalized Ratio.

Comparison of LSM and CAP values in lean and obese-NAFLD patients. The mean value of liver fibrosis for the overall cohort was 7.4 ± 4.2 kPa, with a mean of 6.3 ± 3.3 kPa in the lean group and 7.6 ± 4.3 kPa for the obese patients (p = 0.007). The participants distribution based on LSM values in F0, F1, F2, F3 and F4 was 122 (38.86%), 79 (23.87%), 63 (19.03%), 37 (11.18%) and 30 (9.06%), with the proportion of subjects with at least

significant fibrosis (≥F2) being approximately two times-fold higher among obese-NAFLD (43.81%) in comparison with lean-NAFLD patients (23.29%). CAP values were higher according to the subjects BMI, with a mean value of 273.3 ± 23.5 dB/m in lean patients and 307.5 ± 41.3 dB/m in the obese subjects ($p < 0.001$). The distribution of lean patients in S1, S2, S3 was 36 (49.32%), 23 (31.51%) and 14 (19.18%). Data regarding the obese subjects and their CAP values shows a significant statistical difference between the two groups regarding liver steatosis, with the patient's distribution in S1, S2 and S3 being 54 (20.93%), 39 (15.2%) and 165 (63.95%) (Table 2.XV). In addition, the relative risk for liver fibrosis was considerably higher [OR 2.6, 95% CI 1.5 – 4.42, $p < 0.001$] in obese individuals, compared with lean.

Biochemical profile in lean versus obese subjects. The biochemical profiles of the two groups follow the same patterns. However, according to serological parameters, obese subjects presented a higher level of total cholesterol ($p = 0.047$), LDL-c ($p = 0.013$), SUA ($p < 0.001$), fasting plasma glucose ($p < 0.001$), ALT ($p = 0.003$), AST ($p = 0.003$), GGT ($p = 0.037$), ALP ($p < 0.001$), serum urea ($p < 0.001$), creatinine ($p = 0.001$) and lower HDL-c mean values ($p < 0.001$). Of note, there was no significant statistical difference in the mean value for the triglycerides between the two groups ($p = 0.437$) (Table 2.XVI).

Table 2.XVI. Parameters associated with the presence of fibrosis

Parameter	Obese		Lean	
	r	P value	*r*	P value
Age	0.043	0.492	0.130	0.273
Height	0.140	0.024	-0.107	0.369
Weight	0.178	0.004	-0.103	0.388
BMI	0.100	0.109	-0.034	0.776
Platelet count (G/L)	-0.287	<0.001	-0.034	0.776
INR	0.384	<0.001	0.595	<0.001
CRP (mg/dL)	0.168	0.026	0.455	<0.001
Fasting plasma glucose (mg/dL)	0.157	0.011	-0.003	0.979
Serum urea (mg/dL)	0.120	0.054	-0.128	0.281
Creatinine (mg/dL)	0.104	0.095	0.162	0.172
ALT (IU/L)	0.348	<0.001	0.015	0.900
AST (IU/L)	0.370	<0.001	0.273	0.019
GGT (IU/L)	0.426	<0.001	0.109	0.361
ALP (IU/L)	0.238	<0.001	0.055	0.644
Total bilirubin (mg/dL)	0.240	<0.001	0.106	0.370
Total cholesterol (mg/dL)	0.120	0.054	0.508	<0.001
Tryglicerides (mg/dL)	0.007	0.910	0.381	0.001
Albumin (g/dL)	0.082	0.193	-0.294	0.012
LDL-c (mg/dL)	0.164	0.008	0.263	0.024
HDL-c (mg/dL)	-0.136	0.028	-0.016	0.896
Serum uric acid (mg/dL)	0.216	<0.001	0.134	0.257

BMI, body mass index; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; ALP, alkaline phosphatase; LDL-c, low-density lipoprotein cholesterol; HDL-c, high-density lipoprotein cholesterol; CRP, c-reactive protein; INR, International Normalized Ratio.

Factors associated with advanced fibrosis in lean and obese patients. Different parameters were found to correlate with the presence of fibrosis, both in lean and obese individuals: INR ($r = 0.384$, $p < 0.001$; $r = 0.595$, $p < 0.001$), CRP ($r = 0.168$, $p = 0.026$; $r = 0.455$, $p < 0.001$), AST ($r = 0.370$, $p < 0.001$; $r = 0.273$, $p = 0.019$) and LDL-c ($r = 0.164$ $p = 0.008$; $r = 0.263$, $p = 0.024$) (Table 2.XVI). There was a strong positive correlation in the lean group between the presence of fibrosis and total cholesterol ($r = 0.508$, $p < 0.001$) and

triglycerides (r = 0.381, p = 0.001). An important feature of our study cohort is the fact that the LSM value was directly proportional with the degrees of liver steatosis (Fig. 2.15).

We conducted a univariate followed by multivariate linear regression analysis with the aim to identify the clinico-biochemical parameters associated with advanced liver fibrosis and cirrhosis (Table3). In the lean group, the multivariate analysis showed that triglycerides (β = 0.256, p = 0.013) and INR (β = 0.389, p = 0.001) were strongly associated with advanced fibrosis. While AST and CRP did not have a significant value in the multivariate analysis, they were still strongly associated, in the univariate analysis, with important degrees of liver fibrosis. Lean-NAFLD with AST higher than normal limit had a higher risk for advanced fibrosis compared with those with normal levels of liver enzymes [OR 5.3, 95% CI 1.29 - 21.74, p = 0.013]. On the other hand, in the obese group, INR (β = 0.235, p = 0.039), serum urea (β = 0.123, p = 0.048) and ALT (β = 0.182, p = 0.042) levels were strongly associated with advanced fibrosis and cirrhosis in the multivariate analysis. Moreover, obese-NAFLD subjects with ALT higher than normal limit had increased risk for advanced fibrosis [OR 5.4, 95% CI 2.9 - 10.29, p < 0.001]. The presence of both elevated liver aminotransferases in lean-NAFLD individuals had a higher risk for ≥ F3 degree [OR 2.22, 95% CI 0.2 - 23.75, p = 0.499], while obese-NAFLD subjects had a more important risk [OR 5.90, 95% CI 3.08 - 11.28, p < 0.001] for advanced liver fibrosis. We also found a significant, negative association between LSM values and platelet count in both lean (β = -0.204, p = 0.042) and obese (β = -0.109, p =0.031) groups (Table 2.XVII).

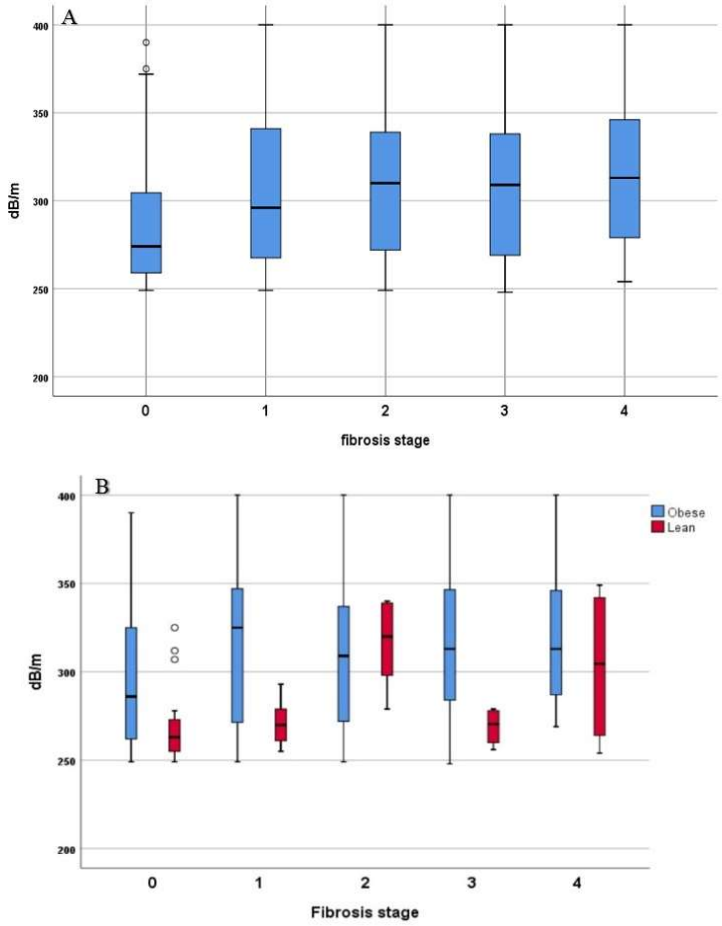

Figure 2.15. A) Distribution of CAP values according to fibrosis stage; B) Distribution of CAP values among lean and obese patients according to fibrosis stage

Table 2.XVII. Factors associated with advanced liver fibrosis using univariate and multivariate linear regression analysis

Variable	Lean				Obese			
	Univariate		Multivariate		Univariate		Multivariate	
	β	*p*	β	*p*	β	*p*	β	*p*
Age	0.126	0.287			-0.045	0.473		
Gender	0.085	0.474			-0.164	0.008	-0.014	0.878
Height	-0.101	0.393			0.214	0.001	0.173	0.150
Weight	-0.055	0.643			0.196	0.002	0.039	0.690
BMI	0.031	0.796			0.029	.640		
Platelet count (G/L)	-0.372	0.001	-0.204	0.042	-0.230	<0.001	-0.109	0.031
INR	0.518	<0.001	0.389	0.001	0.338	0.001	0.235	0.039
CRP (mg/dL)	0.256	0.029	-0.025	0.813	0.008	0.900		
Fasting plasma glucose (mg/dL)	-0.139	0.239			0.050	0.424		
Serum urea (mg/dL)	-0.023	0.848			0.148	0.018	0.123	0.048
Creatinine (mg/dL)	0.132	0.265			0.072	0.248		
ALT (IU/L)	0.106	0.373			0.283	<0.001	0.182	0.042
AST (IU/L)	0.310	0.008	0.135	0.203	0.281	<0.001	-0.078	0.518
GGT (IU/L)	-0.026	0.828			0.375	<0.001	0.148	0.054
ALP (IU/L)	0.044	0.711			0.224	<0.001	0.024	0.729
Total bilirubin (mg/dL)	0.159	0.180			0.069	0.273		
Total cholesterol (mg/dL)	0.264	0.024			0.135	0.030	-0.055	0.419
Triglycerides (mg/dL)	0.268	0.022	0.256	0.013	0.026	0.677		
Albumin (g/dL)	-0.222	0.059			-0.075	0.228		
LDL-c (mg/dL)	0.073	0.541			0.151	0.015		
HDL-c (mg/dL)	0.094	0.429			-0.133	0.032	0.019	0.774
Serum uric acid (mg/dL)	0.043	0.719			0.240	<0.001	0.095	0.183
Hypertension	0.153	0.196			0.199	0.001	0.133	0.025
T2DM	-0.093	0.434			0.055	0.380		

BMI-body mass index, T2DM-diabetes

Discussion. In the past years, it has been generally accepted that NAFLD is no longer a condition to be ignored, with a high risk of progression to end stage liver disease. Because fatty liver is, most of the times, a symptom-free disease, the diagnosis and management are challenging especially in lean patients since these individuals do not fit the classic phenotype of obesity. However, they still share a common metabolic profile with obese subjects (Maier et al., 2021). In this study we provided further information regarding the differences between lean and obese patients diagnosed with NAFLD using VCTE with CAP, to better understand and characterize the clinical features and associated risk factors of each group. Despite the fact that both groups followed the same pattern of metabolic changes, the modifications in the obese individuals were more significant than in the lean patients, results similar to those reported by other studies (Kuchay et al., 2021). We found that metabolic abnormalities such as a high value of SUA, fasting plasma glucose, CRP, total cholesterol, LDL-c, triglycerides, and low levels of HDL-c were more frequent and more severe in obese subjects. This aspect was reported in the current literature as an intermediate metabolic profile in lean-NAFLD patients, between obese-NAFLD and healthy individuals (Feldman et al., 2017). Thus, it appears that lean-NAFLD and obese-NAFLD are two distinct entities, with different metabolic and fibrosis profile and therefore, with different outcomes in terms of prognosis. In this view, Chahal et al.

recently developed a predictive score for NAFLD in lean patients, with good sensibility and specificity, which could be used in screening strategies (Chahal et al., 2022). The development and progression of NAFLD is known to be strongly associated with T2DM and hypertension, comorbidities frequently found in the general population. A meta-analysis conducted by Younossi et al., found that T2DM was more often found in NAFLD patients, affecting up to 23% of them (Younossi et al., 2018). In our study we reported a higher prevalence of T2DM, affecting 101 (30.51%) of patients, 19.18% of them being lean while in the obese group, the prevalence was noticeably higher 33.72%. Similar results have been reported by Denkmayr et al., with a prevalence of 17.5% and 45.3% in lean and obese subjects (Denkmayr et al., 2018). We also found that hypertension is more frequent in the obese patients (68.6% vs. 41.4%, $p < 0.001$) than in lean individuals. Even though the prevalence of hypertension in this groups of patients is discordant, the studies in the current literature conclude that this comorbidity is in fact more prevalent in the obese patients with NAFLD (Lu et al., 2020; Semmler et al., 2021).

Dyslipidemia, a part of the MeS, is thought to play an important role in the pathophysiology of NAFLD, both in lean and obese patients (Tilg et al., 2021). The available data is heterogeneous regarding the levels of total cholesterol and triglycerides and the differences between the two groups of patients. Denkmayr et al., reported high mean values of triglycerides and total cholesterol for both lean and obese patients, but with no significant statistical difference between the two groups (Denkmayr et al., 2018). On the other hand, a study conducted by Khayyat showed a higher mean value of total cholesterol and lower values of triglycerides among lean individuals (Khayvat et al., 2021). In a systematic review and meta-analysis by Young et al., the cholesterol and triglycerides levels were higher in obese patients with NAFLD compared to lean individuals (Young et al., 2020). Following this trend, the patients in our study presented consistent high levels of triglycerides and total cholesterol. Further analysis showed a significant statistical difference between the two groups regarding the total cholesterol ($p = 0.047$), HDL-c ($p < 0.001$) and LDL-c ($p = 0.013$) values, but with no major difference concerning the levels of triglycerides ($p = 0.437$). Thus, lean and obese patients have metabolic similarities in what concerns triglycerides, sharing the common risk factors for developing hepatic fat accumulation. In regard with the levels of liver enzymes, there were significant differences between the lean and obese patients with NAFLD, with mean AST and ALT values of 28.1 ± 14.3, 31.9 ± 20.5 and 35.6 ± 20.3 ($p = 0.003$), 46.2 ± 27.7 ($p = 0.003$) kPa respectively. A strong correlation was noted between the presence of fibrosis and increased AST levels in lean subjects ($r = 0.27$, $p = 0.019$), whereas the obese individuals presented a strong correlation between both AST ($r = 0.37$, $p < 0.001$) and ALT ($r = 0.34$, $p < 0.001$) and the presence of liver fibrosis. These results were in accordance with other studies, which reported higher mean values of liver enzymes in the obese-NAFLD patients (Hagstrom et al., 2017; Sookoian et al., 2017; Denkmayr et al., 2018). On the other hand, as previously presented, the result up to this point are heterogeneous, other studies showing mean values of AST and ALT higher in lean-NAFLD patients (Zou et al., 2020). Liver enzymes are indeed usually increased in patients with NAFLD, but not all subjects have these modifications. Burgert et al., reported that higher ALT levels were correlated with hepatic fat accumulation and insulin resistance (Burgert et al., 2006), results also advocated by Francazani et al (Francazani et al., 2008). However, a recent study conducted by Ulasoglu et al. among NAFLD biopsy-proven patients, concluded that despite the similar prevalence of advanced liver fibrosis among subjects with or without normal liver enzymes, those with hepatocytolysis had more severe histological liver findings (Ulasoglu et al., 2019). Thus, our results highlight the risk of rapid progression to liver cirrhosis also among lean individuals, mostly an apparently clinically healthy population, due to liver necrotic-inflammatory activity in those with elevated liver enzymes. A more careful screening of NAFLD in the lean population, not without necessarily relying on phenotypic criteria, is required.

In this study, most of the patients had no liver fibrosis but there was an important number of them with advanced fibrosis (F3: 37, 11.18%) and liver cirrhosis (F4: 30, 9.06%). Of note was the fact that the LSM value was directly proportional with the degree of liver steatosis. We also found by univariate analysis a significant positive association between CRP ($\beta = 0.256$, $p = 0.029$), AST ($\beta = 0.310$, $p = 0.008$), triglycerides ($\beta = 0.268$, $p = 0.022$) and advanced liver fibrosis and cirrhosis in the lean group. In the multivariate analysis, platelet count was negatively associated with F3 and F4 ($\beta = -0.204$, $p = 0.042$), while triglycerides presented a positive association ($\beta = 0.256$, $p = 0.013$) for patients with a BMI <25kg/m². Regarding the obese group, the multivariate regression analysis showed a positive correlation of advanced liver fibrosis with serum urea levels and the presence of hypertension and ALT values, while the platelet count was negatively correlated ($\beta = -0.178$, $p = 0.031$), results that are in accordance with those from the current literature (Petta et al., 2018). On the other hand, in a study by Verma et al., with biopsy proven NAFLD, more than 30% of patients with normal ALT levels had evidence of advanced fibrosis, therefore important liver injury should be suspected even in patients with normal ALT levels (Verma et al., 2013). In our cohort, the risk for advanced liver fibrosis was higher among obese patients with high ALT values, while in the lean group, a more important risk for advanced fibrosis was found in individuals with high AST values. Natarajan et al., showed similar data, concluding that patients with persistent elevated ALT levels and hepatic steatosis are at high risk for developing advanced chronic liver disease and, consequently, HCC (Natarajan et al., 2020).

According to the EASL NAFLD guidelines, screening for NAFLD/NASH is not recommended in the general population, unless there are patients at high risk to develop this disease. However, we observed an important prevalence of advanced (F3 and F4) liver fibrosis (13.7%) among lean patients, which is why a screening program should be considered, even in apparently healthy subjects with metabolic changes, regardless of the severity of the modification. In our study, the main limitation was the absence of LB in the diagnosis of NAFLD, being appropriate only for patients with a high risk of NASH. The absence of the overweight group could be considered another limitation. However, this brings more relevant data regarding the importance of obesity in the development of NAFLD, and also, by disregarding the ‘‘middle’’/‘‘grey’’ group, we emphasized the metabolic similarities in the analyzed patients. Another limitation was the absence of BF% evaluation by MRI, CT scans or DXA. Even if these methods have been validated, they have important drawbacks like the lack of accessibility for the sole purpose of BF% assessment, time consuming, radiation exposure, lack of cost efficiency, manual image analysis, which can interfere with daily clinical practice. Despite these limitations, our study has considerable strengths. Firstly, all included subjects were evaluated using VCTE with CAP for the diagnosis and staging of liver fibrosis and steatosis. This is a quantitative method leading to a low rate of bias regarding underdiagnosed liver steatosis in our cohort. Secondly, the prospective design of this study including carefully selected patients based on strict criteria highlights the risk of advanced liver fibrosis both in lean and obese subjects. Further studies are mandatory for establishing the resemblances between these two groups, dichotomizing between metabolically healthy and unhealthy populations regardless of the clinical appearance.

Conclusions. NAFLD is most likely a multicausal disease and its development and progression are yet to be fully understood. It is commonly associated with obese individuals, but lean-NAFLD patients are no longer an extraordinary situation, having an important risk of developing advanced hepatic fibrosis, cirrhosis and hepatocellular carcinoma. However, patients with lean-NAFLD usually have a milder clinical and biochemical phenotype, with lower values at the LSM evaluation when compared to obese subjects.

Chapter 3
APPROACH OF PATIENTS WITH GASTROINTESTINAL DISEASES IN PARTICULAR SETTINGS

BACKGROUND

The domain of gastroenterology is a complex one, and patients may experience simultaneous pathologies, or may associate a digestive disease to an existent condition or comorbidity. Integrated and adapted approach is therefore mandatory in many cases, where complications, advanced age or associate diseases are present.

Inflammatory bowel diseases are an example of pathology that needs attentive and tailored care. As a particular supplementary burden, extraintestinal manifestations occurrence may complicate the evolution, imposing special management. Describing a clinical profile for the IBD patient at risk for developing EIM was the main objective of the first study presented in this chapter.

The recent ***COVID-19 pandemic*** context brought many challenges and modifications in the current practice. *Clostridioides difficile* infection overhelmed the gastroenterology units – and we will present the liaisons between the two infections; an adapted approach was noted in the field of digestive endoscopy, as showed by the analysis of the endoscopic activity in our center, and, finally, SARS-CoV-2 infection was proved to be one of the precipitating factors of hepatic encephalopathy in cirrhotic patients.

The last part of this chapter, but of equal importance, is dedicated to ***elderly***. Taking care of old patients involves more than a simple routine; it is necessary to adapt the management according to patient's particular age, status and concomitant pathologies.

I.3.1.THE EXTRA BURDEN OF EXTRAINTESTINAL MANIFESTATIONS OF INFLAMMATORY BOWEL DISEASES – A "DOUBLE TROUBLE"

I.3.1.1. Introduction

Although inflammatory bowel diseases (IBDs) have been known for more than a century, there are still several pending issues, regarding comprehension of the pathogenesis, clinical presentation and evolution, as well as the optimal and timely treatment choice. The clinical picture of ulcerative colitis (UC), Crohn's disease (CD) and IBD unclassified (IBDU) is heterogeneous, with particular aspects concerning disease extension and behavior, miscellaneous evolution patterns, and, last but not least, with variable potential for extraintestinal involvement.

Globally, approximately one-third of patients with IBD develop extraintestinal manifestations (EIMs) (Larsen et al., 2010). Regardless of their severity, if present, EIMs may affect the patients' quality of life. Furthermore, according to their type and impact on the patient's medical condition, EIMs may involve the use of additional healthcare resources.

There may be certain categories of patients who are more likely to develop EIMs. Therefore, a complete characterization of patients' susceptibility profile and the identification of risk factors for developing EIMs may contribute to early recognition, as well as to defining and improving the therapeutic strategy.

Our Institute of Gastroenterology and Hepatology participates actively by enrolling IBD patients and collecting their data in the National Registry of IBD. Gathering data from patients all over the country is important for an ampler perspective and for understanding better the particularities of disease evolution.

PERSONAL CONTRIBUTIONS IN THE AREA OF EXTRAINTESTINAL MANIFESTATIONS OF INFLAMMATORY BOWEL DISEASES

ARTICLE
Singeap AM, Girleanu I, Diculescu M, Gheorghe L, Ciocîrlan M, Gheorghe C, Costache A,Tanțău A, Zaharie R, Goldis A, Gheonea D, Dobru D, Dumitru E, Prelipcean CC, Gîlcă Blanariu GE, Moscalu M, Stanciu C, Trifan A. Risk Factors for Extraintestinal Manifestations in Inflammatory Bowel Diseases: Data from the Romanian National Registry. *J Gastrointestin Liver Dis*. 2021;30(3):346-357. **IF = 2.142**

I.3.1.2. Risk factors for extraintestinal manifestations in inflammatory bowel diseases – data from the Romanian National Registry

Background and aim. Studies performed so far have shown that 6%-47% of IBD patients experience EIMs, reporting the different frequencies related to the study population, study type, length of follow-up, and definition criteria (Danese et al., 2005; Larsen et al., 2010; Yang et al., 2018). There appears to be an increase of cumulative risk for EIMs from the time of diagnosis, especially for CD patients (Vavricka et al., 2011). Throughout the disease course, around a quarter of patients may experience multiple EIMs (Isene et al., 2015).

The clinical spectrum and severity of EIMs are variable, ranging from mild evanescent signs or symptoms to truly debilitating complications. EIMs are classified into two types: immune-mediated conditions, which share presumed immunological pathogenesis with the bowel disease – joint involvement, skin manifestations, ophthalmologic disorders, amyloidosis, and sclerosing cholangitis, and nonimmune-mediated conditions, determined by metabolic or other structural processes secondary to the bowel disease – cholelithiasis, nephrolithiasis, obstructive uropathy, osteopathy. Anemia can also be considered an EIM, but in most cases, it has complex pathogenesis, involving inflammation, malabsorption, bleeding.

The aim of our study was to assess the frequency and types of EIMs in Romanian patients with IBD. The secondary objective was to identify demographical and clinical risk factors for developing EIMs.

Materials and methods

Patients. Our study included all the patients recorded in the Romanian "IBD Prospect" National Registry database. IBD Prospect database has been established more than a decade ago and has been continuously updated, including patients from thirteen Romanian tertiary centers, covering areas all across the country, belonging to eight university medical centers. These tertiary centers have been enrolling IBD patients with a confirmed diagnosis, relying on clinical, laboratory, endoscopic, imaging, and histological criteria. All demographic, history, clinical, and paraclinical parameters were recorded at baseline and during follow-up and every new event was noted in a dynamic, real-time manner. All patients have signed the informed consent before registration. The study protocol followed the principles outlined in the Declaration of Helsinki, regarding research involving human subjects and was approved by the local Ethics Committee.

Methods. We performed a descriptive cross-sectional study, assessing the point prevalence of EIMs. The global prevalence of EIMs was calculated, as well as the prevalence of EIMs among different categories of patients, according to IBD phenotype, demographic features, disease pattern, extension, and severity. Consecutively, the risk factors for EIMs were assessed. EIMs were noted as "present" or "absent" for each patient. When "present", the specific type of EIM was recorded: peripheral arthropathy, axial arthropathies - ankylosing spondylitis/sacroiliitis, erythema nodosum, pyoderma gangrenosum, uveitis, episcleritis, pericholangitis, primary sclerosing cholangitis, nephrolithiasis, obstructive uropathy, recurrent urinary tract infections (UTI), and amyloidosis. Among demographic characteristics, patient

gender, age, and place of origin were recorded. Tobacco use was also recorded, the patient being labeled as "smoker", "non-smoker" or "ex-smoker". IBD family history was assessed. IBD phenotype was classified into three categories: UC, CD, and IBDU. Crohn's disease pattern was classified according to Montreal classification, comprising: age at onset (A1: below 16 years, A2: between 17 and 40 years, A3: above 40 years), disease location (L1: ileal, L2: colonic, L3: ileocolonic, L4: upper gastrointestinal tract) and disease behavior (B1: nonstricturing, nonpenetrating, B2: stricturing, B3: penetrating, "p": perianal disease) (Silverberg et al., 2005). The extent of UC was defined as proctitis (E1), left-sided colitis (E2), or extended colitis, including pancolitis (E3) (Magro et al., 2017). Crohn's disease severity was assessed by calculating Crohn's Disease Activity Index (CDAI) (Best et al., 1976), while UC severity was graded using Mayo score (Schroeder et al., 1987). IBDU were classified similarly to UC cases.

Statistical analysis. The statistical analysis was performed using STATA 16.1 (StataCorp LLC, Texas, USA). Depending on the variables' distribution, the data were presented as mean and standard deviation or median and quartile. Pearson Chi-square test was used to test the EIMs associations based on diagnosis (CD, UC, IBDU). Possible predictors for EIMs were searched based on the results of the univariate and multivariate logistic regression analysis. The statistical significance level of $p<0.05$ was considered as the reference threshold.

Results

Characteristics of the study population. In total, 2626 IBD patients were included, namely 1099 CD patients (41.9%), 1484 UC patients (56.5%), and 43 IBDU (1.6%). The demographic and clinical characteristics of the study patients are shown in Table 3.I. There was a slight predominance for the male gender in UC, and for the female gender in CD. Mean age was slightly higher in UC patients than in CD or IBDU patients. Patients' place of origin was predominantly rural for all IBD phenotypes, with a rural/urban ratio of 2.8 in all IBD patients. Few patients (2.6%) had a familial history of IBD. The predominant location of CD was ileocolonic (L3) (43.7%), followed by colonic and ileal sites; upper gastrointestinal tract was associated in 5.1% of cases; no isolated upper gastrointestinal disease was present. The predominant behavior of CD was inflammatory (nonstricturing, nonpenetrating), in more than 50% of cases, while approximately 11% of cases presented with associated perianal disease. The extent of UC was predominantly E2. Regarding IBD severity, at the time of the analysis, moderate activity was predominant for CD and UC, followed by mild activity, remission, and severe activity.

Prevalence and types of EIMs. In total, 429 patients presented at least one EIM at the time of the study, resulting in a point prevalence of 16.3%. Phenotype analysis showed a significantly higher frequency of EIM in CD compared to UC and IBDU (23.2% vs 11.3% and 16.3%, respectively, $p<0.001$).

Table 3. II shows the overall prevalence of EIMs as well as according to IBD phenotype point prevalence of each type of EIM.

The most frequent EIM was peripheral arthropathy (8.3%), significantly associated with CD (12.4% of cases, $p<0.001$). Axial arthropathies were encountered in 3.9% of IBD patients, being significantly more frequent in CD compared to UC (5.8% vs 2.4%, respectively, $p<0.001$). Among dermatological manifestations, *erythema nodosum* had a significantly increased frequency in CD and IBDU compared to UC (2.7% and 2.3%, respectively, vs 0.4%, $p<0.001$), while *pyoderma gangrenosum* was less frequent and did not show a particular predilection for one IBD phenotype. Low prevalence and no significant association with IBD phenotype were found for pericholangitis (0.08%), primary sclerosing cholangitis (0.8%), renal stones (1.9%), recurrent UTI (1.5%), and amyloidosis (0.04%).

Table 3.I. Patients' characteristics

	All IBD patients	**CD**	**UC**	**IBDU**
	n=2626	n=1099 (41.9%)	n=1484 (56.5%)	n=43 (1.6%)
Sex ratio, n (%)				
Women/men	1245/1381 (47.4%/52.6%)	563/536 (51.2%/48.8%)	657/827 (44.3%/55.7%)	25/18 (58.1%/41.9%)
Age, years				
Mean±SD	43.8±15.4	41.6±14.9	45.5±15.4	43.8±17.7
Median (Q1;Q3)	42 (31;56)	39 (29;53)	44 (33;57)	39 (28;62)
Patient's place of origin, n (%)				
Urban/rural, n (%)	691/1935 (26.3%/73.7%)	245/854 (22.3%/77.7%)	428/1056 (28.8%/71.2%)	18/25 (41.9%/58.1%)
Smoking status, n (%)				
Non-smoker/ Ex-smoker/ Smoker	1477/688/461 (56.2%/ 26.2%/ 17.6%)	571/273/255 (52%/ 24.8%/ 23.2%)	883/406/195 (59.5%/ 27.4%/ 13.1%)	23/9/11 (53.5%/ 20.9%/ 25.6%)
Familial history of IBD, n (%)	69 (2.6%)	33 (3%)	34 (2.3%)	2 (4.6%)
Age group at diagnosis for CD, n (%)	-		-	-
A1<16 years		39 (3.6%)		
A2=17-40 years		708 (64.4%)		
A3>40 years		352 (32%)		
Location of CD, n (%)	-		-	-
L1		222 (20.2%)		
L2		397 (36.1%)		
L3		480 (43.7%)		
Concomitant L4		56 (5.1%)		
Behavior of CD, n (%)	-		-	-
B1		711 (64.7%)		
B2		250 (22.8%)		
B3		138 (12.6%)		
Perianal disease, n (%)	-	124 (11.3%)	-	-
Extent of UC, n (%)	-	-		-
E1			241 (16.2%)	
E2			760 (51.2%)	
E3			483 (32.6%)	
Activity of IBD, n (%)				
Remission		228 (20.8%)	263 (17.7%)	3 (7%)
Mild activity		306 (27.8%)	435 (29.3%)	18 (41.8%)
Moderate activity		415 (37.8%)	565 (38.1%)	15 (34.9%)
Severe activity		150 (13.6%)	221 (14.9%)	7 (16.3%)
Extraintestinal manifestations, n (%)	429 (16.3%)	255 (23.2%)	167 (11.2%)	7 (16.3%)

However, certain EIMs with low prevalence were significantly associated with IBD phenotype. Thus, ophthalmological EIMs (uveitis and episcleritis) had a global frequency of 1.03%, significantly higher in IBDU compared to CD or UC ($p<0.001$), while ureterohydronephrosis (overall frequency 0.3%) had an increased prevalence in CD patients (0.6%) compared to UC (0.07%) ($p = 0.025$).

Multiple EIMs were noted in 2.9% of IBD patients. The number of concomitant EIMs differed significantly according to IBD phenotype (Table 3.II). Thus, one single EIM occurred significantly more frequently in CD patients (18.3%) compared to UC (9.9%) and IBDU (6.9%) patients ($p<0.001$), while the concomitance of two distinct EIMs was recorded with significantly higher frequency in IBDU patients (9.3%, $p<0.001$). We identified nine cases with three simultaneous EIMs, corresponding to 0.34% of all IBD patients and two cases with four

concomitant EIMs, corresponding to 0.08% of all IBD patients. The occurrence of more than two EIMs was identified especially among CD patients.

Table 3. II. Prevalence of EIMs according to IBD phenotype

EIM	All IBD† patients	CD (n,%)	UC (n,%)	IBDU (n,%)	Statistic Test	p-value†
All EIMs	429 (16.3%)	255 (23.2%)	167 (11.3%)	7 (16.3%)	65.96	<0.001*
Peripheral arthropathies	218 (8.3%)	136 (12.4%)	78 (5.3%)	4 (9.3%)	42.09	<0.001*
Axial arthropathies	101 (3.9%)	64 (5.8%)	35 (2.4%)	2 (4.7%)	20.57	<0.001*
Erythema nodosum	37 (1.4%)	30 (2.7%)	6 (0.4%)	1 (2.3%)	24.84	<0.001*
Pyoderma gangrenosum	17 (0.7%)	10 (0.9%)	7 (0.5%)	0 (0%)	2.17	0.338
Uveitis/Episcleritis	27 (1.03%)	19 (1.7%)	6 (0.4%)	2 (4.7%)	16.52	<0.001*
Pericholangitis	2 (0.08%)	2 (0.2%)	0 (0%)	0 (0%)	3.48	0.175
Primary sclerosing cholangitis	21 (0.8%)	8 (0.7%)	13 (0.9%)	0 (0%)	0.52	0.768
Renal stones	50 (1.9%)	24 (2.2%)	25 (1.7%)	1 (2.3%)	0.83	0.643
Ureterohydronephrosis	8 (0.3%)	7 (0.6%)	1 (0.07%)	0 (0%)	7.32	0.025*
Recurrent UTI	38 (1.5%)	19 (1.7%)	18 (1.2%)	1 (2.3%)	1.41	0.492
Amyloidosis	1 (0.04%)	1 (0.1%)	0 (0%)	0 (0%)	1.74	0.418
Multiple extraintestinal manifestations						
None, n (%)	2197 (83.7%)	844 (76.8%)	1317 (88.8%)	36 (83.7%)		
One EIM	351 (13.4%)	201 (18.3%)	147 (9.9%)	3 (6.9%)		
Two EIMs	67 (2.6%)	44 (4%)	19 (1.3%)	4 (9.3%)	84.51	<0.001*
Three EIMs	9 (0.3%)	9 (0.8%)	0 (0%)	0 (0%)		
Four EIMs	2 (0.08%)	1 (0.09%)	1 (0.07%)	0 (0%)		

† Pearson Chi-square test; * Marked effects are significant at p<0.05; UTI: urinary tract infection

Risk factors for EIMs. Logistic regression univariate and multivariate analysis were performed, according to disease phenotype, in order to identify predictive factors for EIMs in IBD **patients.**

Univariate analysis (Table 3.III). Regarding CD patients, we found a significant correlation between female gender and the presence of EIMs (OR=1.56, 95%CI 1.18-2.07, p=0.002). The patient's urban area of origin was also significantly correlated with the development of EIMs (OR=1.49, 95%CI 1.1-2.04, p=0.042). Among biochemical markers, elevated C-reactive protein (CRP) (OR=1.37, 95%CI 1.13-2.19, p=0.035), low hemoglobin (OR=1.41, 95%CI 1.22-2.48, p=0.021) and low serum albumin levels (OR=1.53, 95%CI 1.39-3.99, p=0.016) were predictive factors for EIMs. According to CD location, L3 (OR=2.87, 95%CI 1.59-4.55, p=0.032), and L4 significantly increased the risk for EIMs development (OR=3.54, 95%CI 2.87-5.64, p=0.021). Though no significant correlation was found regarding age at diagnosis and the presence of EIMs, a predilection for EIMs appearance was identified for patients with age at diagnosis between 17 and 40 years (frequency of EIMs- 64.4%), compared to those aged more than 40 years at the time of diagnosis (frequency of EIMs 32%). CD behavior or the presence of perianal involvement did not appear to influence the risk of EIMs. There was no correlation between family history of IBD, smoking status, or CD activity with the presence of EIMs.

For UC patients, the predictive factors for developing EIMs were female gender (OR=1.56, 95%CI 1.13-2.15, p=0.006), patient's urban area of origin (OR=1.52, 95%CI 1.03-2.23, p=0.031), non-smoker status (OR=1.7, 95%CI 1.11-2.43, p=0.027) as well as elevated CRP (OR=2.27, 95%CI 1.91-5.74, P=0.022), low hemoglobin (OR=1.34, 95%CI:1.09-1.89, p=0.029) and low serum albumin (OR=1.42, 95%CI 1.29-2.57, p=0.045) levels. Both moderate (OR=1.65, 95%CI 1.41-3.01, p=0.032) and severe (OR=2.3, 95%CI 1.81-3.13, p=0.025) disease activity was found to be significant risk factors for EIMs development compared to remission; in severe UC patients the frequency of EIMs was 18.1%. Extensive colitis (E3

extension type) was found to significantly increase the risk of EIMs development compared to proctitis (E1 extension type) (EIM's frequency 15.9% and 7.05%, respectively, p=0.001).

In IBDU, male gender, patient's urban area of origin, as well as high CRP, low hemoglobin, and low albumin levels were predictors for EIMs.

Table 3. III. Predictive factors for EIMs in IBD patients - univariate analysis

Variable	Odd ratio Exp (β)	95% CI for Exp (B)		SE	p-value
		Lower	Upper		
CD					
Gender					
Male	Reference				
Female	1.57	1.18	2.07	0.042	0.002*
Age	1.0	0.99	1.01	0.005	0.726
Age group at diagnosis (for CD)					
A1<16 years	1.19	0.56	2.55	0.188	0.652
A2=17–40 years	1.19	0.87	1.60	0.154	0.266
A3>40 years	Reference				
Family history of IBD	1.21	0.52	2.83	0.032	0.650
Place of origin					
Rural	Reference				
Urban	1.49	1.11	2.04	0.178	0.042*
Smoking					0.417
Non-smoker	Reference				
Ex-smoker	1.25	0.89	1.73	0.168	0.188
Smoker	1.06	0.74	1.49	0.176	0.750
Active disease					
Remission	Reference				
Mild activity	1.41	0.93	2.14	0.212	0.101
Moderate activity	1.45	0.97	2.14	0.200	0.064
Severe activity	1.33	0.81	2.18	0.252	0.256
Elevated CRP	1.38	1.13	2.19	0.117	0.035*
Hb	1.41	1.22	2.48	0.149	0.021*
Serum albumin level	1.53	1.39	3.99	0.205	0.016*
Location of CD					
L1	Reference				
L2	0.98	0.65	2.87	0.213	0.890
L3	2.87	1.59	4.55	0.202	0.032*
Concomitant L4	3.54	2.87	5.64	0.183	0.021*
Behavior of CD					
B1	Reference				
B2	1.20	0.86	1.67	0.168	0.274
B3	1.20	0.79	1.82	0.212	0.383
Perianal disease	1.02	0.66	1.58	0.022	0.918
UC					
Gender					
Male	Reference				
Female	1.56	1.13	2.15	0.164	0.006*
Age	1.01	0.99	1.01	0.005	0.928
Family history of IBD	1.34	0.51	3.51	0.191	0.549
Place of origin					
Rural	Reference				
Urban	1.52	1.03	2.23	0.195	0.031*
Smoking					
Smoker	Reference				
Ex-smoker	1.52	0.98	3.14	0.196	0.058
Non-smoker	1.71	1.11	2.43	0.267	0.027*
Disease activity					

Variable	Odd ratio Exp (β)	95% CI for Exp (B)		SE	p-value
		Lower	Upper		
Remission	Reference				
Mild activity	0.53	0.33	0.86	0.247	0.011*
Moderate activity	1.65	1.42	3.01	0.225	0.032*
Severe activity	2.31	1.81	3.12	0.048	0.025*
Elevated CRP	2.27	1.91	5.73	0.272	0.022*
Hb	1.34	1.09	1.89	0.175	0.029*
Serum albumin level	1.43	1.29	2.57	0.145	0.045*
Extent					
E1	Reference				
E2	1.46	0.85	2.53	0.279	0.172
E3	2.50	1.44	4.33	0.281	0.001*
IBDU					
Gender					
Female	Reference				
Male	2.09	1.40	5.80	0.237	0.037*
Age	1.01	0.96	1.05	0.024	0.726
Family history of IBD	1.10	0.97	1.98	0.722	0.939
Place of origin					
Rural	Reference				
Urban	5.37	1.58	9.22	0.131	0.013*
Smoking					
Non-smoker	Reference				
Ex-smoker	0.59	0.05	6.17	0.195	0.663
Smoker	1.06	0.16	6.87	0.256	0.955
Disease activity					
Remission	Reference				
Mild activity	1.15	0.68	2.67	0.101	0.921
Moderate activity	1.38	0.35	3.88	0.032	0.864
Severe activity	1.01	0.21	4.97	0.211	0.986
Elevated CRP	1.44	1.13	1.78	0.229	0.036*
Hb	1.31	1.22	7.56	0.296	0.005*
Serum albumin level	1.37	1.19	9.64	0.257	0.014*

* Marked effects are significant at p<0.05

Multivariate analysis. For CD patients, female gender (OR=3.62, 95%CI 1.2-2.3, p=0.002), moderate (OR=1.84, 95%CI 1.57-2.13, p=0.041) or severe (OR=1.95, 95%CI 1.62-2.24, p=0.037) disease activity and any location other than L1 (p<0.05) acted as associated risk factors for EIMs (Table 3.IV), while for UC patients the risk was significantly increased by the association between female gender (OR=2.64, 95%CI 1.37-5.08, p=0.004), moderate (OR=2.9, 95%CI 1.3–2.8, p=0.011) or severe (OR=2.54; 95%CI 1.34–2.85, p=0.008) disease activity and E3 extension type (pancolitis) (OR=3.3, 95%CI 1.28-4.13, p=0.005), as shown in Table 3.V. Regarding IBDU patients, due to the low number of cases, only five variables could be included in the multivariate analysis; among them, patient's urban area of origin (OR=4.81, 95%CI 1.48–7.52, p=0.018) and high CRP levels (OR=2.99, 95%CI 1.93-5.68, p=0.018) were significantly associated with the risk of developing EIMs (Table 3.VI).

Discussion. We analyzed a large Romanian cohort of IBD patients, included in the multicenter electronic database "IBD Prospect" Registry, where data and events are registered in a real-time manner. We performed a descriptive cross-sectional study, assessing point prevalence of all EIMs, the frequency of different types of EIMs, as well as evaluating risk factors for developing EIMs using univariate and multivariate analysis.

Table 3. IV. Predictive factors for EIMs in CD patients - multivariate analysis

Variable	Odd ratio Exp (β)	95% CI for Exp (B)		SE	p-value
		Lower	Upper		
Gender					
Male	Reference				
Female	2.56	1.57	3.07	0.145	0.002*
Age	1.01	0.98	1.02	0.008	0.673
Place of origin					
Rural	Reference				
Urban	1.06	0.58	1.93	0.307	0.847
Familial history of IBD	1.69	0.46	6.19	0.160	0.423
Smoking					
Non-smoker	Reference				
Ex-smoker	1.73	0.97	3.57	0.154	0.083
Smoker	1.42	0.76	2.64	0.317	0.271
Disease activity					
Remission	Reference				
Mild activity	1.38	0.90	2.09	0.213	0.130
Moderate activity	1.84	1.57	2.14	0.202	0.041*
Severe activity	1.95	1.62	2.24	0.254	0.037*
Elevated CRP	1.13	0.51	2.52	0.408	0.759
Hb	1.04	0.65	1.67	0.240	0.847
Serum albumin level	1.11	0.66	1.87	0.264	0.683
Location					
L1	Reference				
L2	2.53	2.10	5.80	0.224	0.029*
L3	3.83	2.71	5.53	0.209	0.001*
Concomitant L4	4.90	2.27	5.96	0.290	0.021*

Table 3.V. Predictive factors for EIMs in UC patients – multivariate analysis

Variable	Odd ratio Exp (β)	95% CI for Exp (B)		SE	p-value
		Lower	Upper		
Gender					
Male	Reference				
Female	3.62	1.2	2.31	0.002*	0.167
Age	1.01	0.99	1.02	0.412	0.005
Familial history of IBD	1.32	0.50	3.49	0.570	0.494
Place of origin					
Rural	Reference				
Urban	1.17	0.57	2.37	0.174	0.362
Smoking					
Non-smoker	Reference			0.256	
Smoker	0.98	0.37	2.59	0.268	0.495
Ex-smoker	1.15	0.39	3.35	0.103	0.548
Active disease					
Remission	Reference				
Mild activity	1.11	1.14	1.53	0.727	0.262
Moderate activity	2.90	1.30	2.85	0.011*	0.265
Severe activity	2.54	1.34	2.85	0.008*	0.232
Elevated CRP	1.49	0.74	3.02	0.262	0.358
Hb	1.27	0.67	2.41	0.257	0.325
Serum albumin level	1.92	0.57	6.48	0.293	0.621
Extent					
E1	Reference				
E2	1.43	0.818	2.51	0.209	0.286
E3	3.31	1.286	4.136	0.005*	0.298

* Marked effects are significant at $p<0.05$

From the total of 2.626 patients, 429 presented at least one EIM at the moment of the study, resulting in a global point prevalence of 16.3%. Regarding phenotype, CD patients had a significantly higher prevalence of EIMs, compared to UC and IBDU patients ($p<0.001$). Previous data highlighted that IBD patients may experience EIMs in a largely variable proportion, between 6%-47%, depending on the study population, study type, criteria of definition, and duration of follow-up (Danese et al., 2005; Yang et al., 2018). Overall, it is appreciated that one third of IBD patients develop EIMs (Larsen et al., 2010). In our study, we identified a lower rate of EIMs compared to the global estimation, partly attributable to the study type, which assessed the presence of the EIMs at a certain time point, and also to the fact that many EIMs are transient and the absence of one particular EIM at a certain moment in time does not exclude its previous or subsequent development. Our study showed a higher rate of EIMs among CD patients, compared to UC patients, similarly to data published so far (Ott et al., 2013).

Table 3.VI. Predictive factors for EIMs in IBDU patients - multivariate analysis

Variable	Odd ratio Exp (β)	95% CI for Exp (B)		SE	p-value
		Lower	Upper		
Gender					
Female	Reference				
Male	1.38	0.24	7.85	0.385	0.713
Place of origin					
Rural	Reference				
Urban	4.81	1.48	7.52	0.168	0.018*
Hb	1.26	0.21	7.66	0.320	0.799
Serum albumin level	1.33	0.09	19.38	0.365	0.832
CRP	2.99	1.93	5.68	0.029	0.018*

* Marked effects are significant at $p<0.05$

The most frequent EIMs, both globally and for each IBD subtype, were the rheumatological manifestations (12.1% of all IBD patients), namely peripheral arthritis (8.3% IBD patients) and, less frequently, axial arthropathy (3.8% IBD patients). Even if they both refer to joint involvement and are immune-mediated manifestations, they differ concerning their relationship with disease activity. Peripheral arthritis is associated with intestinal disease activity, it follows the clinical course of the IBD and usually improves with IBD treatment, while axial arthropathy evolves independent from intestinal disease activity. In our study, we found that both peripheral arthritis (frequency in CD, 12.4% *vs* UC, 5.3%) and axial spondyloarthritis (frequency in CD, 5.8% *vs* UC, 2.4%) were significantly associated to CD ($p<0.001$). Our data regarding the frequency of these manifestations were in accordance with the results of other studies so far. Thus, classically, peripheral arthralgia/arthritis affects 5% to 10% of patients with UC and 10% to 20% of patients with CD (Orchard et al., 1998). Axial spondyloarthritis is less frequent than peripheral arthralgia/arthritis in patients with IBD, occurring in 3% to 5% of patients, although frequencies of up to 25% have been reported (Monsen et al., 1990; Smale et al., 2001). Moreover, in many studies, rheumatological manifestations are indeed the most frequent EIMs of IBD patients, but no unanimous data concerning differences in prevalence between CD and UC patients were found. In a retrospective cohort study including 626 IBD patients, Malaty et al. found an overall prevalence of joint manifestations of 17%, with 7% prevalence of peripheral arthritis, slightly higher in CD than in UC patients (Malaty et al., 2017). A similar high prevalence of peripheral arthritis among CD patients had been previously reported in a Swiss study (Vavricka et al., 2011), contrasting with another European study revealing a higher prevalence of peripheral arthritis in UC patients (6.1%) compared to CD patients (1.7%) (Salvarani et al., 2001). A Southern European study, published in 2016, analyzing 1860 Greek IBD patients showed that joint

manifestations were the most frequent, followed by dermatological EIMs (Karmiris et al., 2016). The analysis of a large Taiwanese database (3153 IBD patients) found peripheral arthropathy as the most common EIM, followed by ankylosing spondylitis (Hsu et al., 2017). A prospective Norwegian population-based study showed a cumulative prevalence of peripheral arthritis of 12% during a follow-up period of 6.1 years ((Palm et al., 2001). A recently published pediatric study also ranked joint complaints first (20% of all children), followed by aphthous stomatitis and dermatologic involvement (Cohen et al., 2020).

A higher prevalence of joint involvement in CD patients draws attention to this IBD phenotype. Defining a profile for the patient at risk for developing such type of joint manifestations is important either for treatment enhancing or early specific management, in conjunction with the rheumatologist whenever necessary.

Dermatological manifestations may occur in up to 15% of patients (Farhi et al., 2008). They are mainly represented by erythema nodosum with a frequency of 1.4% among IBD patients in our study, and pyoderma gangrenosum with a frequency of 0.7%. Other types of skin involvement are much less frequent, and we did not find any cases in our analysis (e.g., erythema multiforme, epidermolysis bulossa acquista, or acute febrile neutrophilic dermatosis, also known as Sweet syndrome). The prevalence of skin manifestations in our analysis was lower than in usual reports, probably due to their transient character. Erythema nodosum is generally more frequent and is described more commonly in CD than in UC, with variable reported prevalence rates of 2% to 7.5% in CD and 0.9 to 4% for UC (Lindgren et al., 1996; Lakatos et al., 2003). This predilection for CD was also shown in our analysis; erythema nodosum had a significantly higher point prevalence in CD compared to UC patients (2.7% vs 0.4%, respectively, $p<0.001$). Several studies showed other predictive factors for erythema nodosum: female gender, young age at diagnosis, and coexistence of another EIM (Veloso et al., 2011). This dermatological manifestation correlates with IBD activity and usually responds to treatment of the underlying IBD. Pyoderma gangrenosum is less common but more dramatic and potentially debilitating. It is classically reported in 0.5 to 5% of patients with IBD, more common in UC than CD (Farhi et al., 2008). A very recent systematic review and meta-analysis published by States et al. in 2020 showed that the incidence of pyoderma gangrenosum in individual studies ranged from 0.4 to 2.6%, being associated with female gender, CD, erythema nodosum, and ocular EIM (States et al., 2020). Pyoderma gangrenosum does not appear to be correlated with intestinal activity; it may develop independently of the IBD stage or evolution, even before intestinal symptoms arise, during remission, or even after colectomy (Jose et al., 2008). Nevertheless, even if it is usually successfully managed with combined topical therapy and general treatment of the underlying IBD, in some cases it may have a more severe course. Pyoderma gangrenosum was previously reported as progressing from erythema nodosum lesions (Gellert et al., 1983). which could partially account for the increased frequency of erythema nodosum compared to pyoderma gangrenosum.

Ophthalmological manifestations are other type of well-recognized immune-related extraintestinal manifestations of IBD, with a reported prevalence of up to 13%, more common in CD than in UC patients (Evans et al., 2007). Uveitis and episcleritis, the most common complications, were found with a point prevalence of 1.03% in our analysis, which is lower than generally reported in other studies. This finding can be explained by their short-term evolution, similarly to dermatological manifestations. While previous studies had showed that episcleritis was more frequent, recent ones seem to counterbalance this tendency in favor of uveitis (Ysene et al., 2015; Yang et al., 2018) Previous studies revealed that among CD patients, those with colonic involvement are considered to be more at risk to experience ocular complications compared to those with extracolonic location (Jose et al., 2008). Eye involvement is considered to be mainly related to UC; however, in our study ophthalmological manifestations were significantly more frequent in IBDU cases ($p<0.001$). Ocular pathology is

usually managed and solved using topical treatment, in addition to the treatment of the underlying IBD. In some cases, ocular manifestations may precede intestinal symptoms, and may be nonspecific at presentation. Besides, some ophthalmological issues may occur as complications of steroid therapy. If left untreated, ocular complications may progress, with irreversible invalidating consequences; therefore, a special attention must be paid to all eye-related symptoms, at any time of the disease course.

Among hepatobiliary EIMs, primary sclerosing cholangitis has a reported prevalence of 5% and 2% in UC and, respectively, CD patients (Lee and Kaplan, 1995). Yet, there are studies reporting a lower prevalence in UC - around 1.1% (Ye et al., 2011). We found a point prevalence of 0.8% in our analysis, slightly higher for UC compared to CD patients. Even though it is a much less common EIM than others, it has a special significance if diagnosed early, considering the very high prevalence of UC in patients with primary sclerosing cholangitis, while male patients are at higher risk for developing primary sclerosing cholangitis (Broome et al., 2006). Pericholangitis, which has historically comprised distinct cholestatic biochemical features, is nowadays more properly referred to as small-duct primary sclerosing cholangitis, and is part of the primary sclerosing cholangitis spectrum.

Renourinary manifestations may consist in nephrolithiasis (either calcium oxalate stones due to hyperoxaluria, or uric acid stones whose formation mechanism implies dehydration), obstructive uropathy with hydronephrosis, fistulae involving urinary tract, and recurrent urinary tract infections. They are non-immune mediated EIMs, expressing either metabolic abnormalities (in the care of stones) or the loco-regional anatomical extension of the inflammatory and fibrotic process (as the fistulous and obstructive complications). The renal stones were the most frequent renourinary manifestation (1.9%), followed by recurrent UTI; ureterohydronephrosis was the least frequent renal EIM, with significant understandable predilection for CD compared to UC patients (0.64% *vs* 0.07%, respectively, p=0.025).

Amyloidosis is an extremely rare systemic but severe complication, reported so far with a 0.5% prevalence in IBD patients, with a predilection for CD, as highlighted by a recent systematic review (Tosca et al., 2016). The prevalence of amyloidosis in our study was even lower (0.04%), with only one reported case associated with CD.

Multiple concomitant EIMs may appear in IBD patients, with rates reported so far between 0.3 and 4.5% (Bernstein et al., 2001). In our study, multiple EIMs were noted in 2.9% of all IBD patients, the most common situation being the association of two EIMs, significantly more frequent in IBDU patients compared to CD and UC patients (9.3% *vs* 4% and 1.3%, respectively, $p<0.001$). In the study population, a single EIM was more frequently met among CD patients-18.3% of all CD patients- compared to significantly lower proportions in UC and IBDU ($p<0.001$). The combination of more than two EIMs was much less frequently identified, with a predilection for CD patients.

In our study, distinct independent or associated risk factors were identified using univariate and multivariate logistic regression analysis and, shaping potential susceptibility profiles for EIMs.

For CD patients, univariate analysis highlighted that female gender, urban place of origin, elevated CRP, low hemoglobin, low albumin, ileocolonic location and upper gastrointestinal tract involvement were significant independent risk factors for EIMs. For UC, the significant independent risk factors for developing EIMs were: female gender, patients' urban area of origin, non-smoker status, elevated CRP, low hemoglobin and serum albumin levels, moderate and severe disease activity, and extensive colitis. Prior available data have shown that, overall, EIMs are more frequent in female IBD patients (Greuter et al., 2020), who seem to be prone to develop the majority of the most common EIMs, such as peripheral arthropathy, skin and ocular involvement. Male patients are more susceptible to less frequent EIMs, such as axial spondyloarthritis and primary sclerosing cholangitis (Lakatos et al., 2003).

Moreover, female gender is associated with more side-effects to biologic treatment, which explains lower adherence rate to biologics among female patients (Severs et al., 2018). This aspect may play a role of female gender in EIMs' occurrence, at least for those correlated with intestinal activity. The significant correlation found in our study between patients' urban place of origin and presence of EIMs may be partly justified by the fact that patients from urban areas are usually taken care of territorially, and our data comes from tertiary care centers, where generally selected difficult cases are managed. Nonetheless, urban living was identified as a significant environmental risk factor for CD and IBD in general, as proved by several studies as well as by a recent review of meta-analyses (Piovani et al., 2019). Furthermore, in our study, biochemical markers reflecting the disease activity and/or its consequences were found to be significant risk factors for EIMs. This may be explained by the inflammatory markers signaling either disease activity, the presence of other extraintestinal inflammatory manifestation, or both. It is known that many frequent immune-mediated EIMs evolve in parallel with intestinal activity, while others develop independently. A marker like hypoalbuminemia may witness pathological conditions favoring complications; low serum albumin concentration and vitamin D deficiency, which has already proved to be a risk factor for IBD, seem to be entangled (Del Pinto et al., 2015). Consequently, we can also state that poor nutritional status can represent a risk factor for EIMs. Ileocolonic location, as well as the involvement of upper gastrointestinal tract, were significantly associated in our study with the presence of EIMs in CD patients, while in UC patients, extensive colitis correlated with the risk of EIMs. Certain EIMs, including peripheral arthropathies, are more frequent in colonic CD, while others are more common in small bowel CD (Levine et al., 2011). Since we performed a global analysis, we may presume that our results could reflect a cumulative risk of EIMs for CD with ileocolonic location. Moreover, upper gastrointestinal tract location involved a particular risk for EIMs among CD patients. Regarding UC, extensive colitis has been previously correlated with a higher prevalence of EIMs (Weiss et al., 1997). Moderate and severe disease activity were found to be independent risk factors for EIMs only in UC patients. This finding can be partly attributed to the parallel course of certain common EIMs with the intestinal activity (peripheral arthritis, episcleritis). Non-smoker status as an independent risk factor for EIMs in UC patients is consistent with data shown by studies so far regarding tobacco as a protective factor for UC development (Mahid et al., 2006).

Concerning IBDU patients, male gender (in contrast to the other two main IBD phenotypes), urban place of origin and biochemical markers were independent predictive factors for EIMs development.

According to multivariate analysis, female CD patients with moderate or severe disease activity, with any other location than L1 were prone to develop EIMs, while the UC patient profile at risk for developing EIMs was represented by the female gender, with moderate or severe extensive colitis. As for IBDU, urban patients with high CRP levels are at increased risk for EIMs.

Susceptibility profiles were therefore configured, combining both demographic and clinical parameters, non-modifiable or dynamic. Taking into account that IBDs are chronic diseases, occurrence of EIMs negatively affect the patient's outcomes. One particular aspect that should also be considered when managing EIMs is heterogeneity in pathophysiology. The mechanisms underlying and supporting inflammation in EIMs can be mainly seen either as an extension of immune responses arisen in the intestine (due to molecular mimicry, T-cell trafficking and ectopic expression of gut-specific chemokines) or as independent inflammatory events, consequent to systemic changes in innate immunity, changes in the microbiome and a general shift toward a proinflammatory state. However, since the diverse mechanisms underlying and perpetuating inflammation in EIMs have yet to be clearly defined, the development of specific treatment strategies is further limited. Currently, foreseeing the risk of

IBD patients for developing EIMs could represent a first step taken to avoid it or at least to be better prepared for the consequences. Moreover, considering that some EIMs are related to an inflammatory process and other are metabolic consequences, optimizing treatments is further challenging. Treatment of EIMs should be based on the severity of symptoms and their association with IBD activity, having as primary aim the control of symptoms and preservation of mobility and function, thus contributing to improvement in patients' quality of life and reducing disability.

Conclusions. IBD patients, especially CD patients, are experiencing EIMs in a significant proportion, with the most frequent EIMs in the studied population being represented by joint manifestations. Predictive factors for the development of EIMs were established, and according to IBD phenotype, risk profiles were outlined. Significant independent risk factors for EIMs found both for CD and UC were: female gender, patient's urban area of origin, anemia, hypoalbuminemia, high CRP. Significant independent IBD phenotype-related risk factors were: ileocolonic location and concomitant involvement of upper gastrointestinal tract for CD, and non-smoker status and both moderate and severe disease activity for UC. Male gender was an independent risk factor for EIMs in IBDU, as well as high CRP, low hemoglobin and low albumin levels. According to multivariate analysis, female CD patients presenting with moderate or severe disease activity with other disease location than isolated ileal involvement and female UC patients with moderate or severe extensive colitis were the most exposed IBD patients. Anticipating the vulnerability by identifying independent and associated predictive risk factors should therefore become a part of the IBD patient work-up, in order to apply the most appropriate follow-up strategy and act in a timely manner to ensure a disease course as favorable as possible.

I.3.2. CHALLENGES AND CHANGES DURING COVID-19 PANDEMIC

I.3.2.1. Introduction

Beginning with March 2020, when World Health Organization (WHO, Geneva, Switzerland) declared the coronavirus disease 2019 (COVID-19) caused by the SARS-CoV-2 infection a pandemic, medical practice was profoundly altered and multiple challenges for gastroenterologists in approaching patients with digestive diseases were introduced, especially due to the many digestive and hepatic manifestations of severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2) infection. Analysis of this context reflected into several studies.

PERSONAL CONTRIBUTIONS RELATED TO THE SARS-CoV-19 INFECTION – GASTROINTESTINAL DISORDERS OVERLAP

	ARTICLES
1.	Cojocariu C, Girleanu I, Trifan A, Olteanu A, Muzica CM, Huiban L, Chiriac S, **Singeap AM,** Cuciureanu T, Sfarti C, Stanciu C. Did the severe acute respiratory syndrome-coronavirus 2 pandemic cause an endemic *Clostridium difficile* infection? World J Clin Cases. 2021 Nov 26;9(33):10180-10188. doi: 10.12998/wjcc.v9.i33.10180. PMID: 34904088; PMCID: PMC8638041**. IF=1.534**
2.	Chiriac S, Stanciu C, Cojocariu C, Sfarti C, **Singeap AM**, Girleanu I, Cuciureanu T, Huiban L, David D, Zenovia S, Nastasa R, Balan GG, Trifan A. The Impact of the COVID-19 Pandemic on Gastrointestinal Endoscopy Activity in a Tertiary Care Center from Northeastern Romania. Healthcare (Basel). 2021 Jan 19;9(1):100. doi: 10.3390/healthcare9010100. PMID: 33477942; PMCID: PMC7833369. **IF = 3.160**
3.	**Singeap AM**, Cuciureanu T, Zenovia S, Girleanu I, Huiban L, Muzica CM, Stanciu C, Trifan A. Spectrum of precipitating factors of hepatic encephalopathy in patients with liver cirrhosis. Medical-Surgical Journal – Revista Medico-Chirurgicala, 2021; 125 (4): 492-501.

I.3.2.2. The severe acute respiratory syndrome - coronavirus 2 pandemic and endemic *Clostridium difficile* infection

Background and aim. Many digestive and hepatic manifestations of SARS-CoV-2 infection, most often residual/post-infection, may alter the course of patients with digestive disorders (especially for patients with inflammatory bowel disease, advanced liver disease, *etc*.).

Diarrhea is one of the most common gastrointestinal symptoms in patients with COVID-19, showing a prevalence ranging from 11% to 17% (Pan et al., 2020; Maltfertheiner et al., 2020). SARS-CoV-2 can actively infect and replicate in the gastrointestinal tract through the angiotensin-converting enzyme 2 receptors disrupting the normal intestinal flora, leading to gastrointestinal symptoms, including diarrhea (Chen et al., 2020). Before the COVID-19 pandemic the most common cause of diarrhea (excluding inflammation and organic intestinal lesions) was irritable bowel syndrome and functional disorders.

Patients with SARS-CoV-2 infection have numerous risk factors for CDI, including receipt of broad-spectrum antibiotic treatment, hospitalization, elderly age, and existence of multiple comorbidities or immunocompromised status. During the COVID-19 pandemic, many patients received antibiotic treatment, sometimes with no clear indication or as primary prophylaxis for pneumonia (Granata et al., 2020). One study showed that 91% of COVID-19 patients received antibiotic treatment (Laszkowska et al., 2020), but generally over 70% of COVID-19 patients were treated with broad-spectrum antibiotics (mostly respiratory quinolones) in order to treat or to prevent bacterial co-infections and super-infections (Spigaglia et al., 2020).

We hypothesized that an increase in CDI incidence and recurrence occurred during the COVID-19 pandemic. An Italian retrospective study during the COVID-19 pandemic found a significant decrease in the incidence of healthcare-associated CDI in 2020 compared to the previous 3 years - explained by increased pandemic precautions (Bentivegna et al., 2021). However, other data showed that COVID-19 departments actually had a higher incidence of CDI compared to non-COVID-19 wards, but upon statistical analysis, the difference did not reach the threshold of significance (Ponce-Alonso et al., 2019).

Considering these contradictory data, the aim of this study was to assess the impact of the COVID-19 pandemic on the characteristics of CDI patients and to analyze the factors that influenced the incidence of CDI during the COVID-19 pandemic.

Materials and methods

Study population. We performed a prospective observational study including patients with CDI between March 2020 to December 2020. We analyzed data from this period because on March 1, 2020, the Clinical Hospital for Infectious Disease Iasi was declared a COVID-19 Unit, and as a result the Institute of Gastroenterology and Hepatology was designated the clinic to hospitalize patients with CDI. The diagnosis of CDI was based on the presence of diarrhea ($\geq$ 3 watery stools within 24 h) associated with detection of *C. difficile* toxin A or B (by enzyme immunoassay) in stool samples (19). Hospital-acquired CDI was defined as a stool sample positive for *C. difficile* toxin(s) at least 72 h after hospital admission. Each patient's stool was tested only once.

We collected demographic data (sex, age, residence), clinical and laboratory parameters, use of antibiotics, information regarding previous hospitalizations, comorbidities, associated medication, previous COVID-19 infection, treatment of CDI, and discharge. CDI data (first episode/relapse and relapse number), length of hospital stay and mortality during admission were also analyzed. The treatment started with vancomycin 125 mg every 6 h, and therapeutic response was defined as the absence of diarrhea after at least 72 h of treatment. We have excluded patients with other etiologies of acute diarrhea. The study was approved by the

Local Medical Ethics Committee. All patients provided written informed consent before study inclusion or further analysis.

Statistical analysis. Categorical variables were expressed as frequency and percentage. Continuous variables were expressed as mean ± standard variation for normally distributed continuous data. All data were normally distributed. Groups were compared using the $\chi 2$ test for categorical variables and using the independent *t* test or Mann-Whitney *U* test for continuous variables (depending on data distribution). Univariate analysis was performed for each recorded data type. Variables with $P < 0.1$ in univariate analysis were included in the multivariate analysis (logistic regression). The odds ratio (OR) with 95% confidence interval (CI) was calculated for qualitative variables included in the logistic regression. A $P < 0.05$ was considered statistically significant. All statistical analyses were performed using SPSS 20.0 software (IBM Corp., Armonk, NY, United States).

Results. A total of 3562 patients were admitted to our tertiary hospital during the study period, of whom 447 (12.5%) were diagnosed with CDI. Most of the patients were male (243 patients, 54.3%). The mean age was 59.7 ± 10.8 years, and over half of the patients had previous hospitalizations (266 patients, 59.5%). Baseline characteristics of the patients included in the study are presented in Table 3.VII.

Table 3.VII. Baseline characteristics of the study groups, *n* (%)

Parameter	Past history COVID-19 *n* = 76	Non-COVID-19 *n* = 371	p-Value
Age in yr, mean ± SD	62.6 ± 14.6	56.8 ± 17.6	0.007
Male	35 (46.1)	208 (56.1)	0.110
Country side	18 (23.7)	170 (45.8)	< 0.001
Hospitalization days, mean ± SD	8 (5)	9 (7)	0.094
Alcohol consumption	33 (43.4)	109 (29.4)	0.017
AB during hospitalization	29 (38.2)	154 (41.5)	0.588
Previous AB treatment	46 (60.5)	132 (35.5)	< 0.001
Comorbidities	65 (85.5)	348 (93.8)	0.013
Liver cirrhosis	17 (22.4)	158 (42.6)	0.001
IBD	3 (3.9)	31 (8.4)	0.187
DM	0	16 (4.3)	0.065
Malignancies	8 (10.5)	50 (13.5)	0.486
CKD	5 (6.6)	30 (8.1)	0.656
Previous hospitalizations	62 (81.6)	204 (54.9)	< 0.001
Recurrence	19 (25.0)	50 (13.1)	0.011
Leukocytes, mean ± SD	11320 (8843)	11560 (6650)	0.203
CRP, mean ± SD	2.53 (10.3)	2.52 (10.4)	0.103
Death	5 (6.6)	26 (7.0)	0.893

AB: antibiotics; CKD: chronic kidney disease; CRP: C-reactive protein; DM: diabetes mellitus; IBD: inflammatory bowel disease; SD: Standard deviation

Of all the patients included in the study, 76 (17.0%) had a history of COVID-19. All of the COVID-19 patients were diagnosed with healthcare-associated CDI. Nineteen patients (25.0%) had recurrent CDI. All patients with CDI were treated with vancomycin (125 mg) every 6 h orally. In patients with a history of COVID-19, 26 (34.2%) received an increased dose of vancomycin (250 mg every 6 h for 10 d) and 28 (36.8%) received a high dose of vancomycin (500 mg every 6 h) because they did not respond to the initial dose. In addition, 14 patients (18.4%) received vancomycin enemas. Two patients in the COVID-19 group received fidaxomicin, as they were non-responders to even the maximal doses of vancomycin. Seventeen patients from the COVID-19 group with recurrent CDI received the tapering vancomycin regimen. Compared with the COVID-19 group, the majority of patients with no

history of COVID-19 and CDI (302 patients, 81.4%) responded to the conventional doses of vancomycin (125 mg every 6 h for 10 d), and none of these patients needed fidaxomicin.

There was no significant difference in gender and hospitalization days as well as for the inflammatory syndrome between patients with a past history of COVID-19 who developed CDI and those without a history of COVID-19 (Table 3.VII). However, the patients with a history of COVID-19 and CDI had a higher mean age (62.6 ± 14.6 *vs* 56.8 ± 17.6, $P = 0.007$), previous antibiotic treatment (60.5% *vs* 35.5%, $P < 0.001$), previous hospitalizations (81.6% *vs* 54.9%, $P < 0.001$), were chronic alcohol consumers (43.4% *vs* 29.4%, $P = 0.017$) and were more prone to recurrent disease (25.0% *vs* 13.1%, $P = 0.011$). Thirty-one patients (6.9%) died during hospitalization. The mortality rate was similar in both groups (6.6% *vs* 7.0%, $P = 0.893$).

The results of the univariate and multivariate regression analyses are shown in Table 3.VIII). The multivariate analysis demonstrated that age more than 60-years-old (OR = 2.59, 95%CI: 1.452-4.624, $P = 0.001$), urban area residence (OR = 2.33, 95%CI: 1.286-4.221, $P = 0.005$), previous antibiotic treatments (OR = 1.90, 95%CI: 1.083-3.365, $P = 0.025$), previous hospitalizations (OR = 2.5, 95%CI: 1.263-4.986, $P = 0.009$) and chronic alcohol consumption (OR = 2.55, 95%CI: 1.459-4.459, $P = 0.001$) were risk factors for CDI development in patients with a history of COVID-19.

Table 3.VIII. Risk factors for *Clostridium difficile* infection after coronavirus disease 2019

Parameter	Univariate analysis			Multivariate analysis		
	OR	CI	p-Value	OR	CI	p-Value
Age > 60 yr	2.321	1.455-3.703	< 0.001	2.591	1.452-4.624	0.001
Urban area	1.935	1.273-2.940	0.001	2.330	1.286-4.221	0.005
Previous AB treatments	1.632	1.223-2.178	<0.001	1.909	1.083-3.365	0.025
Previous hospitalizations	2.444	1.503-3.947	< 0.001	2.509	1.263-4.986	0.009
Alcohol consumption	1.248	1.014-1.536	0.017	2.550	1.459-4.459	0.001

AB: antibiotics; CDI: *Clostridium difficile* infection; CI: confidence interval; OR: Odds ratio

Discussion. An increase in the number of CDI cases was expected during the COVID-19 pandemic due to the numerous risk factors of patients with COVID-19 (elderly, multiple comorbidities requiring immunosuppressive treatment, prolonged hospitalization that is frequently in intensive care units, and antibiotic treatment) (Debast et al., 2014). Our results demonstrated that 12.5% of patients admitted to our tertiary hospital were diagnosed with CDI. More than half of our patients with CDI had previous hospitalizations, and 17.0% of them were previously hospitalized for COVID-19. We found that all of the COVID-19 patients were diagnosed with healthcare-associated CDI. Our results are completely different from those of an Italian retrospective study during the COVID-19 pandemic that found a significant decrease in the incidence of healthcare-associated CDI in 2020 compared to the previous 3 years (Ponce-Alonso et al., 2019). The authors explained that the decrease of CDI was due to increased pandemic precautions.

The growing number of CDI cases is only one of many causes for concern. In recent years, one of the clinical challenges in patients with CDI is recurrent infection, which is often difficult to treat. Recurrent CDI is defined as an episode of CDI occurring within 8 weeks of a previous episode, and it may be due to relapse of the previous CDI by the same strain or reinfection by a different strain. About 15%-30% of CDI patients with an initial response to antimicrobial treatment have a risk of recurrence of the infection, and it is important to note that the risk of further recurrence significantly increases (Song et al., 2019). In our cohort, 19 patients (25.0%) had recurrent CDI.

There was no significant difference in gender and hospitalization days as well as for the existence of inflammatory syndrome between patients with a history of COVID-19 that developed CDI and those without a history of COVID-19. However, the patients with a history of COVID-19 and CDI were elderly, were from an urban area, had previous antibiotic use, and were chronic alcohol consumers.

Although the majority of the literature on the epidemiologic features of CDI is based on the association of antibiotic therapy and hospitalization settings, some other potential risk factors for CDI, such as advanced age, immunosuppression, comorbidities, chemotherapy, renal insufficiency, hypoalbuminemia, organ transplantation and use of proton pump inhibitors, have been identified to explain the increased incidence of CDI (Loo et al., 2011; Trifan et al., 2018).

COVID-19 may present as acute diarrhea and abdominal pain. Even in these conditions, with symptoms suggestive for COVID-19, testing for *C. difficile* must be done every time because patients with SARS-CoV-2 infection are patients at high risk for CDI.

Although CDI can affect individuals of all ages, the elderly are recognized as high-risk for this infection (Trifan et al., 2018). Older patients represent a vulnerable population for CDI because they often have multiple comorbidities, have frequent and prolonged hospitalizations, receive broad-spectrum antibiotics, and have altered host defense against infections. At the same time, COVID-19 seems to primarily affect elderly patients, patients who usually have severe forms of the disease, and patients who were frequently treated with antibiotics.

Sandhu et al. collected data of several studies regarding concomitant antibiotic use in patients with COVID-19 in the United States. Most of these patients received empiric antibacterial therapy with either moxifloxacin, cefoperazone or azithromycin (Saandhu et al., 2020). These antibiotics are known to be strongly associated with CDI, and the authors reported that CDI was due to the overuse of antibiotics in COVID-19 patients.

We found that chronic alcohol consumption was a risk factor for CDI after COVID-19 infection. Chronic alcohol consumption influences gut microbiota by decreasing the bacterial diversity and increasing intestinal permeability and systemic inflammation (Meroni et al., 2019). We found no other studies on the increased risk of CDI in chronic alcohol users, but we have two explanations for our result. The first is based upon the fact that almost 40% of our hospitalized patients had liver cirrhosis; the main etiology of which was alcoholism. The second is based upon the numerous data showing that during the pandemic alcohol consumption increased worldwide, sometimes to a worrisome degree.

Our study has some strengths and several limitations. This is the first prospective study that characterized CDI after SARS-CoV-2 infection. The identification of risk factors for CDI after COVID-19 highlights the importance of recognizing vulnerable groups, such as the elderly population and patients who consume alcohol. The limitations of our study are represented by the small sample of cases and the fact that our data came from a single-center care unit without information on the *C. difficile* strains. We do not yet have a definite explanation for the fact that patients with CDI after COVID-19 require higher doses of vancomycin.

Conclusion. We observed that patients with a history of COVID-19 and CDI were from an urban area, had a higher mean age, had previous antibiotic treatments and hospitalizations, were chronic alcohol consumers, and were more prone to recurrent disease. Also, escalating the doses of vancomycin to obtain the therapeutic effect was another feature of the patients studied. In these patients, the antibiotic treatment for COVID-19 should be personalized in order to diminish the risk of CDI. Further large studies are needed in order to establish if it is cost-effective to start CDI treatment with higher doses of vancomycin in patients with a past history of COVID-19.

I.3.2.3. The impact of the COVID-19 pandemic on gastrointestinal endoscopy activity in a tertiary care center from North-Eastern Romania

Background and aim. During the COVID-19 pandemic, the gastrointestinal (GI) endoscopy departments worldwide were severely affected. Many elective procedures were postponed in most centers, leading to an unprecedented decrease of the endoscopic workload globally (Repici et al., 2020). The significant impact of COVID-19 has been partly attributed to the high contagious potential of the SARS CoV-2 virus as well as the long incubation time. The main transmission pathways are through exposure to air droplets or through direct contact (Fennelly, 2020). However, alternative transmission has been shown via small airborne particles as well as through the fecal-oral pathway (Gu et al., 2020). The potential for transmission is high during aerosol-generating procedures such as GI endoscopy. Thus, the prioritization of these procedures has been advocated by many international endoscopy organizations such as World Endoscopy Organization (WEO, Munich, Germany) (Guda et al., 2020), European Society of Gastrointestinal Endoscopy (ESGE, Munich, Germany), European Society of Gastroenterology and Endoscopy Nurses and Associates (ESGENA, Munich, Germany) (Gralnek et al., 2020), Asian Pacific Society for Digestive Endoscopy (APSDE, Japan) (Chiu et al., 2020), American Society for Gastrointestinal Endoscopy (ASGE, Downers Grove, USA) (Repici et al., 2020), and American Gastroenterological Association (AGA, Bethesda, USA) (Suktan et al., 2020). These societies have issued recommendations such as the mandatory use of personal protective equipment (PPE) and the requirement for negative pressure rooms, and also suggest viral testing based on polymerase chain reaction (PCR) where available, thus substantially increasing the costs of GI endoscopy and potentially leading to an even further decrease of the number of procedures (Das, 2020). They have stated that nonurgent procedures should be deferred, and only emergency GI endoscopy should be performed in order to prevent cross contamination and to reduce the risk for the endoscopy unit personnel. Moreover, the guidelines suggest reducing the onsite endoscopy staff and maintaining only the personnel essential for the procedure, creating special circuits for patients, monitoring temperature of patients and staff, and applying questionnaires for symptoms or previous exposure. All of these measures, combined with the patients' fear of contracting the SARS-CoV2 infection from hospital milieu, have resulted in the dramatic general decrease of GI endoscopies worldwide (Rutter et al., 2020). The impact of these measures is currently being evaluated, and the available data indicate a significant decrease in the GI cancer detection rate (Rutter et al., 2020) and a reduction in trainees' opportunities of participating in GI endoscopy (Pawlak et al., 2020). The measures, mainly directed toward the protection of personnel, are based on data suggesting a high risk of transmission of the SARS CoV-2 virus in the endoscopy unit. However, recent data has suggested that appropriate use of PPE and recommended measures can significantly reduce this risk (Repici et al., 2020). We aimed to assess the impact of the COVID-19 pandemic on GI endoscopy in a tertiary care center in northeastern Romania concerning the number of procedures performed, indications, complications, and results as well as trainee involvement.

Materials and Methods. The database containing GI endoscopies performed in the "St. Spiridon" Emergency Hospital, Institute of Gastroenterology and Hepatology, Iasi, Romania was analyzed, and information was retrieved in a confidential manner. Two time periods were considered for comparison, namely 1st of March–15 September 2020 during the COVID-19 pandemic, and a similar period between 1st of March–15 September 2019. Patients' age and sex, as well as the time and type of the procedure were recorded. The indication, urgency, endoscopic diagnosis, procedures performed, success rates, and complications were also noted, as well as the participation of fellows. Incomplete records were excluded from the study.

Internal Protocol. The endoscopic procedures were performed in accordance with the established COVID-19 internal protocol, which stated that no ambulatory procedures could be carried out and that endoscopy should be performed only in hospitalized patients. The decision for hospitalization was at the discretion of the on-call doctor via the hospital emergency department. The indications for endoscopy were restricted to either GI bleeding or to cases considered at high risk for cancer. The hospital followed the general international recommendations concerning the reduction of nonessential personnel in the endoscopy room, PPE use, temperature monitoring, and special circuits, as well as personal and patient questionnaires assessing the presence of symptoms of the COVID-19 disease. All patients were tested for SARS-Cov-2 infection by PCR and endoscopy was deferred until a negative test was available, with the exception of emergency procedures. Trainees were no longer included when emergency procedures were performed in order to minimize the risk of contamination and to decrease the need for PPE. The decision to include fellows in nonurgent procedures was taken on a case-by-case basis by the endoscopist.

Statistical analysis. IBM Statistical Package for Social Sciences (SPSS, Armonk, NY, USA) version 22.0 was used for the statistical analysis. The Kolmogorov-Smirnoff test was used to assess the distribution of the continuous variable (age), which was expressed as median (interquartile range (IQR)) as it presented a nonparametric distribution. Categorical variables were expressed as frequency and percentage. Absolute numbers of endoscopic procedures and percentage reductions were calculated. Chi-square or Fischer's test was used for the analysis of categorical variables accordingly. Statistical significance was considered for a *p*-value of less than 0.05.

Results

General findings. There was a general 6.2-fold decrease in the total number of procedures as a result of the COVID-19 restrictive recommendations. During the pre-COVID-19 period, 3608 endoscopic procedures were carried out, with a mean of 138 per week. During the COVID19 period, only 582 procedures were performed, approximatively 22 procedures per week. The percentage of male patients and emergency endoscopies increased during the COVID19 period. The general characteristics of endoscopies before and during COVID-19 period are described in Table 3.IX and Figure 3.1.

Table 3.IX. General characteristics of endoscopic procedures performed before and during the COVID-19 period

Characteristic	Pre COVID-19 Time 3608	COVID-19 Time 582	*p*
Sex, women:men, *n* (%)	1620 (44.9):1987 (55.1)	235 (40.4):347 (59.6)	0.041
Emergency upper/lower GI endoscopy, *n* (%)	947 (26.2)	226 (38.8)	<0.001
Fellow involvement, *n* (%)	3250 (90)	238 (40.9)	<0.001

Although the absolute number of procedures decreased, a significant increase in the relative percentage of upper GI endoscopy and endoscopic retrograde cholangiopancreatography (ERCP) was noted, together with a significant decrease in the percentage of colonoscopies and flexible sigmoidoscopies as a result of COVID-19 imposed restrictions. The most significant reduction was found in colonoscopy, from 916 to 42 procedures, $p < 0.001$; followed by flexible sigmoidoscopy, from 189 to 14 procedures, $p = 0.009$; upper gastrointestinal endoscopy, from 2269 to 401 procedures, $p = 0.006$; and ERCP, from 234 to 125 procedures, $p < 0.001$ (Figure 3.2).

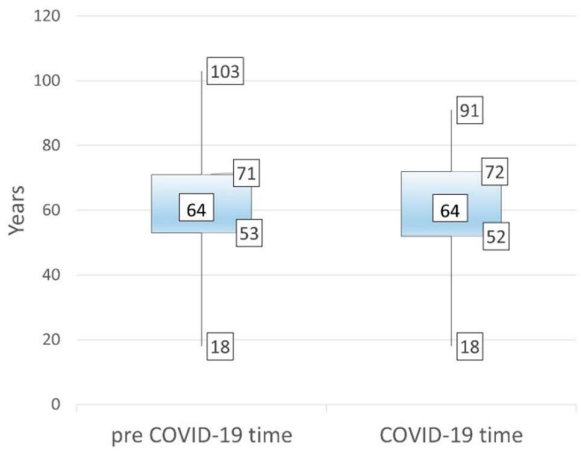

Figure 3.1. Median values of the age of the patients before and during the COVID-19 period

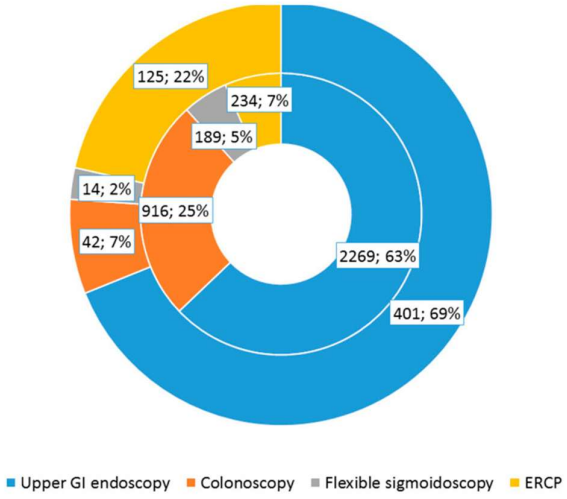

Figure 3.2. Impact of COVID-19 on the numbers of endoscopic procedures

Training Analysis. Compared to the pre-COVID-19 period, during the COVID-19 period, a significant reduction in fellows' involvement in endoscopy procedures was noted (40.9% vs. 90%, respectively, $p < 0.001$).

Upper and Lower GI Endoscopy Analysis. The detailed results of the analysis of upper and lower endoscopic procedures are presented in Table 3.X. Concerning the indications for upper and lower GI endoscopy during the COVID-19 period compared to the pre-COVID-19 period, there was a significant increase in the percent of endoscopies performed for upper GI bleeding (42.5% vs. 24.4%, respectively, $p < 0.001$), but a reduction in colonoscopies performed for lower GI bleeding (4.4% vs. 8.6%, respectively, $p = 0.002$). Moreover, endoscopies performed for several indications such as chronic abdominal pain, weight loss, control of gastric ulcer, polyps, or cancer were significantly reduced, whereas procedures indicated for anemia, change in the frequency of stools, vomiting, dysphagia, or screening for esophageal varices were not impacted by the COVID-19 restrictions. The changes in the frequency of endoscopic diagnostics were in accordance with the more restrictive indications. Thus, a significant increase in the detection of GI neoplasia was noted in the COVID-19 period (12.5% vs. 7.4%, respectively, $p < 0.001$). Moreover, gastric and duodenal ulcers were more

frequently diagnosed during the pandemic compared to the pre-COVID period, but significant differences were only noted in the case of Forrest III ulcers (8.8% vs. 5.5%, respectively, p = 0.002). The diagnostic frequency of other conditions, such as gastritis, esophagitis, achalasia, esophageal benign stenosis, inflammatory bowel disease, diverticular disease, or hemorrhoids, was not significantly modified. However, normal findings in endoscopy were significantly reduced (0.2% vs. 2%, p = 0.006). Concerning endoscopic interventions performed before and during the COVID-19 period, there was a general increase in variceal band ligation, bougie and balloon dilation of esophageal strictures, esophageal stent placement for neoplasia, and percutaneous endoscopic gastrostomy (PEG), but with no notable statistical significance with the exception of gastric or duodenal ulcer hemostasis (4.5% vs. 2.8%, respectively, p = 0.039). The COVID-19 pandemic determined an important decrease of polypectomies and endoscopic mucosal resections (EMR)s (0.2% vs. 5%, respectively, $p < 0.001$). A significant reduction of early complications was noted (7.6% vs. 19.2%, respectively $p < 0.001$). There were seven cases of delayed complications during the pre-COVID period but none during the COVID-19 period. An increased rate of therapeutic success was noted during the pandemic (94.2% vs. 90.7%, respectively, p = 0.01) compared to the pre-COVID period (Table 3.X).

ERCP Analysis. The change in ERCP indications induced by the COVID-19 restrictions was less dramatic than in the case of upper and lower GI endoscopies. Some differences, although without statistical relevance, were expressed as a relative increase in the percent of procedures carried out for neoplasia, such as pancreatic cancer, cholangiocarcinoma, lymph node, or hepatic metastasis. A reduction of ERCPs performed was found for common bile duct (CBD) stone extraction and postoperative biliary lesions. As a result of the increase in the percentage of difficult cases, the rate of success diminished during the COVID-19 period compared to pre-COVID period (76.3% vs. 87.7%, respectively, p = 0.006). However, there was a relative decrease in complications, reflected notably in a reduced immediate bleeding rate (7.3% vs. 11.6%, respectively, p = 0.196) (Table 3.XI).

Discussion. We analyzed the impact of the COVID-19 pandemic on the number, type of endoscopic procedures, and outcomes in a tertiary care unit from northeastern Romania. The information collected from our database indicated that there was a significant general decrease of the number of endoscopic procedures performed during the pandemic. This reduction in absolute numbers has also been reported in other regions, such as the United States of America (Forbes et al., 2019), United Kingdom (UK) (Rutter et al., 2020), the Netherlands (Dinmohamed et al., 2020), and China (Zhu et al., 2020). The most dramatic impact was the reduction in the number of colonoscopies and flexible sigmoidoscopies performed during the pandemic, followed by upper GI endoscopies. A lesser reduction was found in ERCPs. Similar results were reported by Rutter et al. in a national analysis of the impact of the COVID-19 pandemic on the endoscopic activity in the UK. The authors reported that colonoscopies, flexible sigmoidoscopies, and gastroscopies were reduced by 90%, 91%, and 86% respectively, but that ERCPs were reduced by only 44% (Rutter et al., 2020). In our center, we found that although the absolute numbers of endoscopic procedures were decreased, there was an increase in the percent of upper GI endoscopies as well as ERCPs, whereas the percent of colonoscopies and flexible sigmoidoscopies decreased. These changes occurred as a result of the restrictive indications adopted by our institution, limiting the procedures to either GI bleeding or to cases considered at high risk for cancer. Thus, indications such as chronic abdominal pain, weight loss, control of gastric ulcer, post polypectomy control, or postoperative cancer surveillance were considerably reduced. Our results are in accordance with data from a multicenter study carried out in Italy that indicated that most endoscopy units also limited their indications to emergency procedures or to cases presenting high risk for digestive cancer. Thus, the Italian endoscopy departments reported a subsequent total reduction in endoscopic procedures varying from no reduction to 100% (Repici et al., 2020).

Table 3.X. Analysis of upper and lower GI endoscopy before and during the COVID-19 period

Procedure Type/Result	Pre COVID-19 Time 3374	COVID-19 Time 457	*p*
GI Endoscopy Indications, *n* (%)			
Upper GI bleeding, *n* (%)	823 (24.4)	194 (42.5)	<0.001
Lower GI bleeding, *n* (%)	290 (8.6)	20 (4.4)	0.002
Anemia, *n* (%)	335 (9.9)	37 (8.1)	0.148
Chronic abdominal pain, *n* (%)	392 (11.6)	9 (2)	<0.001
Change in frequency of stool/vomiting, *n* (%)	245 (7.3)	34 (7.4)	0.895
Weight loss, *n* (%)	79 (2.3)	4 (0.9)	0.032
Control of gastric ulcer/polyps/cancer, *n* (%)	175 (5.2)	6 (1.3)	0.001
CT/MRI suspicion of neoplasia	30 (0.9)	0	0.042
Others (dysphagia, screening for esophageal varices), *n* (%)	1005 (29.8)	153 (33.5)	0.929
GI Endoscopy Results			
GI neoplasia, *n* (%)	249 (7.4)	57 (12.5)	0.001
GI polyps, *n* (%)	468 (13.9)	9 (2)	<0.001
Gastric/duodenal ulcer, *n* (%)			
Forrest 3, *n* (%)	185 (5.5)	40 (8.8)	0.002
Forrest 1-2, *n* (%)	242 (7.2)	45 (9.8)	0.69
Esophageal varix, *n* (%)	364 (10.8)	80 (17.5)	<0.001
Others (gastritis, esophagitis, diverticular disease, hemorrhoids), *n* (%)	1666 (46.4)	201 (44)	0.154
UC/Crohn's disease, *n* (%)	91 (2.7)	18 (3.9)	0.134
Normal findings, *n* (%)	69 (2)	1 (0.2)	0.006
Achalasia, *n* (%)	35 (1)	3 (0.7)	0.616
Esophageal benign stenosis, *n* (%)	5 (0.1)	3 (0.7)	0.60
Interventions Performed			
Variceal band ligation, *n* (%)	42 (1.24)	14 (3.06)	0.715
Ulcer hemostasis, *n* (%)	97 (2.87)	21 (4.59)	0.039
Bougie dilation of esophageal benign strictures, *n* (%)	14 (0.41)	4 (0.87)	0.792
Balloon dilation of esophageal benign strictures, *n* (%)	24 (0.71)	7 (1.53)	0.987
Esophageal cancer stent placement, *n* (%)	5 (0.14)	1 (0.2)	0.610
Polypectomy/EMR, *n* (%)	170 (5.03)	3 (0.2)	<0.001
PEG, *n* (%)	5 (0.14)	2 (0.43)	0.752
Pyloric balloon dilation, *n* (%)	1 (0.02)	0	0.954
Success, *n* (%)	325 (90.7)	49 (94.2)	0.01
Early complications (bleeding/perforation), *n* (%)	69 (19.2)	4 (7.6)	<0.001
Delayed complications (bleeding/perforation), *n* (%)	7 (1.9)	0 (0)	0.395

Table 3.XI. Analysis of ERCP before and during the COVID-19 period

Procedure Type/Result	Pre COVID-19 Time 233	COVID-19 Time 124	*p*
ERCP indications			
Cholangitis, *n* (%)	25 (10.7)	16 (12.9)	0.540
CBD stones, *n* (%)	164 (70.4)	81 (65.3)	0.302
Cholangiocarcinoma, *n* (%)	32 (13.7)	21 (16.9)	0.700
Pancreatic cancer, *n* (%)	22 (9.4)	15 (12.1)	0.491
Postoperative biliary lesion, *n* (%)	6 (2.6)	2 (1.6)	0.171
Others (lymph node metastasis/hepatic metastasis)	7 (3)	4 (3.2)	0.852
ERCP Results			
Complications			
Immediate bleeding	27 (11.6)	9 (7.3)	0.196
Perforation	1 (0.4)	1 (0.8)	0.649
Stent placement	50 (21.4)	30 (24.1)	0.378
Therapeutic success	199 (87.7)	90 (76.3)	0.006

In our institution, we found a significant relative increase of endoscopies performed in emergency setting. As a consequence, the upper and lower GI endoscopy findings were also different when compared to the pre-COVID-19 period. The considerable reduction of activity of the endoscopy department had a significant effect on the detection of cancers. The absolute number of cancers detected as a result of the COVID-19 pandemic decreased dramatically. This decrease is in accordance with the worldwide trend induced by the impact on cancer screening programs as well as by the more restrictive indications for endoscopic procedures (Rutter et al., 2020; Repici et al., 2020). Even though we found an absolute significant decrease in the detected GI neoplasia, the relative percentage of GI neoplasia diagnosed during the pandemic was considerably higher. Similar findings have also been reported in the UK where a general reduction of all digestive cancers was noted, with an over three-fold increase in the cancer detection rate as a result of more restrictive endoscopy indications (Rutter et al., 2020). In the Netherlands, a significant decrease in the detection of cancers was also observed as a result of the pandemic (Dinmohamed at al., 2020).

Moreover, the rates of gastric and duodenal bleeding ulcers as well as esophageal varix diagnostic were increased. As expected, the percent of GI polyps and normal endoscopic findings was considerably diminished. Concerning the endoscopic interventions, we noted an increase in the percentage of hemostasis, both for variceal and nonvariceal upper GI bleeding, but a significant decrease in polypectomy and EMR, in accordance with the considerable reduction in colonoscopies and flexible sigmoidoscopies in favor of upper digestive endoscopies. Surprisingly, the success rates were higher and there were less complications related to endoscopy during the COVID-19 pandemic. These findings could indicate a higher concern of the endoscopy personnel aiming to reduce the need for subsequent endoscopic procedures, either because of failure of the intervention or because of the development of complications.

The number of ERCPs was reduced by almost a half during the COVID-19 period. However, this decrease was less substantial when compared to the other GI procedures. Concerning the indications, a shift toward palliative treatment of cholangiocarcinoma and pancreatic cancer was noted, as well as a relative reduction of procedures carried out for common bile duct stone extraction. As a result, the success rate of ERCPs during the pandemic was relatively lower, although without statistical significance. Other studies throughout the

world have reported similar results concerning ERCPs, presenting a global reduction of procedures but to a lesser extent when compared to other upper or lower GI endoscopies (Rutter et al., 2020; Repici et al., 2020).

The fellows' involvement in the endoscopy department was considerably reduced as a result of the need for judicious use of PPE and of the recommendations imposing the minimization of personnel exposure during endoscopy. Similar results have been reported throughout the world. Forbes N et al., in a study comprising 73 institutions from North America and Canada, found that 49% of the centers included had eliminated endoscopic training altogether, and 45% had completely stopped interventional endoscopy training programs (Forbes et al., 2020). A service evaluation of the endoscopy activity in the UK found a 93% reduction in the rate of endoscopic procedures carried out by fellows (Rutter et al., 2020). An international study including 770 participants from 63 countries and 6 continents showed a reduction of trainee involvement in endoscopic procedures in 93.8% of cases. The participants evaluated that the decision to exclude the trainees from endoscopic procedures was taken in 79.9% of cases, but that a lack of cases or a shortage of PPE was noted in 58.3% and in 28.8% of cases, respectively (Pawlak et al., 2020).

Most endoscopy units throughout the world are currently working to resume the elective GI endoscopies that have been delayed during the COVID-19 imposed restrictions. This is a difficult but necessary process as the worldwide dramatic reduction in the number of procedures affects patients at risk by potentially delaying the diagnosis of cancer (Kushnir et al., 2020). Although the global health crisis is far from over, the need to gradually restart the endoscopy units has led several international societies to publish recommendations regarding the resumption of elective endoscopic procedures (Hennessy et al., 2020). These recommendations include the thorough preparation of patients, health care providers, equipment, and infrastructure in order to minimize the risk of exposure and assure a constant on-duty endoscopy personnel in the case that part of the staff becomes infected and needs to be quarantined (Soetikno et al., 2020). However, these measures could be difficult to implement in certain settings where the available infrastructure is not adequate or where there are shortages of available PPE. Clearly, the new road ahead is challenging, and the recommendations need to be tailored to the existing local possibilities in order to balance the risk of COVID-19 dissemination with the risk associated with the lack of timely endoscopic interventions (Thompson et al., 2020). In our institution the recommendations of PPE use, as well as the introduction of specific circuits and patient hygiene, has been implemented since the beginning of the pandemic. PCR viral testing was offered to all of the patients before endoscopy. These measures, combined with the judicious use of the PPE, led to the reduced number of nonurgent procedures performed. However, no urgent endoscopy was postponed. The endoscopists prioritized the procedures in accordance with the individual risks of the patients, and sometimes even performed endoscopies in disregard of their own personal safety when deferring the procedure would have put the patient in danger. Although several members of the endoscopy staff have contracted the COVID-19 disease, it was difficult to firmly establish a direct link between the infected patients and these cases as community transmission was also possible. A more in-depth analysis concerning the epidemiological investigation regarding the cases is necessary in order to be able to confidently present those results. In the light of the ongoing vaccination program, the resumption of elective endoscopy should be prioritized. The prioritization of cases should be continued, and previously postponed endoscopies should be gradually performed. Efforts should be made to achieve a good communication with the patients. This is particularly important in order to assure them that measures are being taken to assure their safety during and after the procedure.

The COVID-19 pandemic is the fifth pandemic since the Spanish Flu from 1918 and it will most probably not be the last. Thus, the experience that we gained during this difficult

time will provide the basis for future action against other potential pandemics. The judicious use of PPE and the detailed instructions for patient and personnel hygiene, as well as the development of specific circuits and the more rigorous implementation of good practice protocols, are here to stay and represent a solid foundation in the fight against future pandemics. Our study is the first to assess the impact of COVID-19 on the endoscopy practice in Romania. However, it presents some limitations. First, the study was based on a single-center analysis. Also, the database did not contain information on the rate of ERCP-related delayed complications. Thus, it could not be evaluated.

Conclusion. The COVID-19 pandemic has had a profound impact on the endoscopy department, characterized by a consistent reduction of upper and lower GI procedures, as well as a more modest reduction of ERCPs. The overall cancer detection rate was significantly reduced, indicating a risk for missing the opportunity of potentially curative treatment due to the pandemic. Endoscopic procedures were mostly carried out in emergency setting, but therapeutic procedures were associated with less complications and higher success rates. Only in time we would be fully able to understand the real impact of the COVID-19 pandemic on the well-being of our patients. However, the results suggest that this impact would result in an increased number of advanced and potentially unresectable cancers, as well as a high risk of morbidity and mortality. Thus, efforts should be made in order to safely reopen the endoscopy departments.

I.3.2.4. The spectrum of precipitating factors of hepatic encephalopathy in patients with liver cirrhosis hospitalized during a pandemic year

Background and aim. Hepatic encephalopathy (HE) is one of the most frequent complications of liver cirrhosis. It negatively affects patients' quality of life and has unfavorable short- and long-term impact on their clinical outcome. Moreover, because of complex therapeutic measures and frequent hospitalizations, HE may associate a significant burden in term of healthcare resources. The frequency of HE is related to the functional status of the liver and to the degree of portal hypertension, with direct consequence on the portosystemic shunt formation. According to the clinical presentation, HE is classified in covert HE, which includes minimal HE (detectable only by special psychometric tests) and grade 1 HE, and overt HE, which includes grade 2-4 HE according to West-Haven criteria (Vilstrup et al., 2014). In terms of prevalence, 10-15% of patients present overt HE at the moment of the diagnosis of cirrhosis (Saunders et al., 1981), and 16-21% of patients with decompensated cirrhosis associate overt HE (D'Amico et al., 1986). During the first 5 years after the diagnosis of cirrhosis, the risk for HE is up to 25% (Bustamante et al., 1999), while in evolution, up to 40% of cirrhotic patients will present with HE, mostly in a recurrent manner (Prasad et al., 2007). There are multiple well-defined precipitating factors, which can cause, by various mechanisms, episodic or recurrent episodes of HE. Identification of precipitating factor by thorough work-up is mandatory in order to apply the optimal management strategy. The recognition and, subsequently, the correction of the precipitating factor is the first step in treating HE episodes. About 90% of patients with HE requires only the correction of the precipitating factor as therapeutic approach (Strauss et al., 1992), therefore identifying and controlling precipitating factors is essential.

Precipitating factors are represented mainly by infections, gastrointestinal bleeding, constipation, electrolyte disorders, renal disfunction, hepatotoxic agents, and others. Awareness and promptly recognition definitely influence favorably the individual therapeutic approach and may warrant therapeutic success. As there is a 40% cumulative risk of another recurrence during the 6 months following the previous episode of HE (Sharma et al., 2009), tight surveillance and, ideally, avoidance of precipitating factors could represent an effective prophylactic measure. The aim of our study was to assess the prevalence of various

precipitating factors for HE in patients with liver cirrhosis in COVID era, and to identify particular precipitating factors according to the etiological profile of cirrhosis, data that could help apply a tailored, individually-adapted preventive strategy.

Material and methods. We performed a retrospective, descriptive study, including all the patients with HE and liver cirrhosis, hospitalized during the COVID-19 pandemic year (April 1st, 2020 – March 31, 2021) in the Institute of Gastroenterology and Hepatology, "Saint Spiridon" Emergency Hospital, Iasi, a tertiary referral care center in North-East Romania. Among all 743 cirrhotic patients hospitalized during this period, 526 fulfilled the West Haven criteria for HE. Demographic, clinical and biological characteristics of the patients, as well as the etiology of the liver cirrhosis were recorded. Precipitating factors were identified, and their prevalence was calculated, both overall and according to the main etiologies of cirrhosis. Statistical analysis was performed using SPSS version 24.0. Categorical data was described using frequencies and percentages. Continuous data were expressed as means and standard deviation (SD). Results were considered with statistical signification for a p-value <0.05.

Results

Overall prevalence of HE. Out of all 743 cirrhotic patients hospitalized during one pandemic year in our care center, 526 patients with HE were identified, resulting an overall HE prevalence of 71%.

Patients' characteristics. Demographic characteristic, etiology, Child-Pugh and MELD scores, and grades of HE according to West-Haven criteria are presented in the Table 3.XII. The most common causes for cirrhosis were alcohol, with almost three quarters of cases (393 patients, 74.7%) and viral, with less than a quarter (118 patients, 22.4%), while other causes were much less frequent (metabolic 1.5%, primary biliary cirrhosis 0.8%, Wilson disease 0.6%). 99% of the patients had decompensated cirrhosis, corresponding to class Child-Pugh B and C. Mean values of MELD scores were above the cut-off for liver transplantation. The most patients (40.3%) had grade 2 HE according to West-Haven criteria, followed by grade 1 (30.8%), and grade 3 (19.4%); 9.5% of patients had hepatic coma, corresponding to grade 4.

Precipitating factors of HE are presented in the table 3.XIII. Results of comparative analysis of precipitating factors frequencies according to main etiology of cirrhosis (alcoholic and virus-related) are presented in the Table 3.XIV.

Discussion. HE is a frequent complication of liver cirrhosis, and its occurrence is usually determined by another concomitant pathological condition or acute event which act as trigger factors. Mechanisms involved are complex, hyperammonemia being the leading path towards the alteration of the brain functioning. The spectrum of the clinical manifestations of HE is heterogenous, and the severity of this complication can vary from mild, subtle symptoms to life-threatening conditions as hepatic coma. Depending on the methods of assessment and the type of study population, prevalence of HE varies. While among all cirrhotic patients, HE prevalence is usually up to 20% (Vilstrup et al., 2014), some studies so far showed more increased prevalence in hospitalized cirrhotic patients (Duah et al., 2020). However, patients with advanced, decompensated and/or complicated cirrhosis are the most prone to develop HE. This explains why our analysis showed a much higher prevalence (71%); during pandemic, the hospitalized population consisted almost exclusively in severe cirrhotic patients, usually associating serious specific complications. Current classification distinguish covert HE (minimal HE and grade I according to West Haven criteria) from overt HE (grades II-IV according to West Haven criteria). In our study, 162 patients (31%) had covert HE (grade I), while the majority (364 patients, 69%) presented overt HE. The majority of cirrhotic patients which present with HE manifestations need hospitalization. Treatment may involve advanced support measures, and furthermore, identification and correction of precipitating factors is mandatory. Precipitating factors are often multiple, either concomitant or consecutive to each other, because one complication of cirrhosis frequently causes another.

Table 3.XII. Characteristics of the study patients

Parameters	Patients, n=526
Age, mean ± SD	57.37 ± 11.5
Gender ratio, n (%)	
Men	318 (60.5)
Women	208 (39.5)
Place of origin, n (%)	
Urban area	243 (46.2)
Rural area	283 (53.8)
Etiology of cirrhosis, n (%)	
Alcoholic	393 (74.7)
Virus-related	118 (22.4)
Hepatitis C virus infection	49 (9.3)
Hepatitis B and D virus infection	32 (6.1)
Hepatitis B virus infection	28 (5.3)
Hepatitis B and C virus infection	9 (1.7)
Nonalcoholic, nonviral	15 (2.9)
Metabolic	8 (1.5)
Primary biliary cirrhosis	4 (0.8)
Wilson disease	3 (0.6)
Child-Pugh classification	
Class (A/B/C), n (%)	5/128/393 (1/24.4/74.7)
Score, mean ± SD	10.91 ± 2.15
MELD score, mean ± SD	21.05 ± 7.95
MELD-Na score, mean ± SD	23.07 ± 8.06
West-Haven hepatic encephalopathy grading, n (%)	
1	162 (30.8)
2	212 (40.3)
3	102 (19.4)
4	50 (9.5)

Table 3.XIII. Precipitating factors of HE in the study group

	Precipitating factors	Patients, n (%)
Nitrogen overload	Gastrointestinal bleeding	166 (31.6)
	Variceal bleeding	129 (23)
	Non-variceal bleeding	37 (7)
	Constipation	52 (9.9)
	Renal disfunction	152 (28.9)
Metabolic alteration	Dyselectrolytemia	138 (26.2)
Systemic stress	Infections	230 (43.7)
	Spontaneous bacterial peritonitis	73 (13.9)
	Urinary tract infection	41 (7.8)
	Clostridioides difficile colitis	34 (6.5)
	COVID-19	31 (5.9)
	Sepsis	25 (4.8)
	Respiratory tract infection (other than COVID-19)	21 (4)
	Cellulitis	5 (1)
	Surgery	8 (1.5)
Hepatotoxic agents	Active alcohol consumption	65 (12.4)
Others	HCC	44 (8.4)
	TIPS	4 (0.8)
Non-adherence to therapy		72 (13.7)
Number of precipitating factors		
1		258 (49.1)
>2		207 (39.3)
Unidentified		61 (11.6)

Table 3.XIV. Comparative analysis of precipitating factors frequencies according to main etiology of cirrhosis

Precipitating factors, n (%)	Alcoholic N = 393	Viral N = 133	*P*
Gastrointestinal bleeding	124 (31.6)	42 (31.6)	0.965
Variceal bleeding	92 (23.4)	37 (27.8)	0.308
Non-variceal bleeding	32 (8.1)	5 (3.8)	0.088
Constipation	42 (10.7)	10 (7.5)	0.291
Renal failure	106 (27)	46 (34.6)	0.098
Dyselectrolytemia	103 (26.2)	35 (26.3)	0.981
Infections	181 (46.1)	49 (36.8)	0.064
Spontaneous bacterial peritonitis	58 (14.8)	15 (11.3)	0.317
Urinary tract infection	32 (8.1)	9 (6.8)	0.610
Clostridioides difficile colitis	24 (6.1)	10 (7.5)	0.568
COVID-19	20 (5.1)	11 (8.3)	0.179
Sepsis	24 (6.1)	1 (0.8)	**0.012**
Pulmonary tract infection	19 (4.8)	2 (1.5)	0.090
Cellulitis	4 (1)	1 (0.8)	0.785
Surgery	6 (1.5)	2 (1.5)	0.985
Active alcohol consumption	65 (16.5)	-	-
Non-adherence to therapy	61 (15.5)	11 (8.3)	**0.026**
HCC	25 (6.4)	19 (14.3)	**0.004**
TIPS	2 (0.5)	2 (1.5)	0.256
Number of precipitating factors			
1	189 (48.1)	69 (51.9)	0.451
≥2	160 (40.7)	47 (35.3)	0.274
Unidentified	44 (11.2)	17 (12.8)	0.622

In our study, infections were the most frequent precipitating factors (43.7%), the commonest being spontaneous bacterial peritonitis, followed by urinary tract infection, *Clostridioides difficile* colitis, COVID-19 pneumonia, sepsis, respiratory infections (other than SARS-COV-2) and cellulitis. Universal data so far show that infections are recognized among the main precipitating factors for both episodic and recurrent HE. There seem to be, however, certain differences according to the region reported: infections appear as the most frequent precipitating factors (responsible for up to 65% of cases of HE) in the developing countries (Mumtaz et al., 2010; Onyekwere et al., 2011; Duah et al., 2020), while studies from western world report infections with much lower frequency as precipitating factors for HE (Lim and Kim, 2008; Poudyal et al., 2019). Adherence, good nutritional and immunologic condition and functionality of health system might explain lower frequency of infections.

In relation to cirrhosis etiology, we found a significant higher frequency of sepsis as trigger factor for HE in patients with alcoholic cirrhosis compared to those with virus-related cirrhosis (6.1% and 0.8%, respectively, P=0.012). Alcoholic patients have poorer immune status and generally negative health outcome (Hansen et al., 2016), which could explain why they are the most exposed to septic complications. Respiratory tract infections were found to be more frequent in alcohol-related cirrhosis, reflecting the higher risk of pulmonary complications in this group of patients, involving issues as self-neglecting or aspiration. The second most frequent precipitating factor of HE was gastrointestinal bleeding. Nearly one third of all patients (31.6%) presented bleeding as major risk factor; among them, variceal bleeding was more prevalent, with approximately three quarters of cases, while the rest had non-variceal

bleeding. Cirrhotic patients usually bleed from esophageal or gastric varices (60-65%) (Lu et al., 2020), but non-variceal sources are also recorded (mainly peptic ulcers, erosive gastritis, Mallory-Weiss syndrome) (González-González et al., 2011). In our study, non-variceal bleeding tended to be more frequent in patients with alcoholic cirrhosis compared to patients with virus-related cirrhosis. Indeed, alcohol consumption can directly cause hemorrhagic upper gastrointestinal lesions, such as acute erosive gastritis, esophageal ulcers, Mallory-Weiss syndrome, and, in the same time, it has been associated *per se* with an increased risk of upper gastrointestinal bleeding, especially secondary to peptic ulcer (Kärkkäinen et al., 2015; Strate et al., 2016). While variceal bleeding is classically seen as the most dramatical and potentially lethal complication, with 6-week mortality up to 20% (Garcia-Tsao and Bosch, 2010), the non-variceal bleeding in patients with cirrhosis is as well of upmost importance, since clinical outcomes are more severely influenced, in terms of morbidity and even mortality, compared to non-cirrhotic patients (Morsy et al., 2014). Rapid adapted treatment is mandatory in all cases of gastrointestinal bleeding, to avoid high rate of mortality.

Renal failure was the third most common precipitating factor (28.9% of all patients). There are many mechanisms of renal impairment in liver cirrhosis, leading directly to nitrogen overload and consequent brain disfunction. According to etiology of cirrhosis, renal disfunction was more frequent (34.6%) in virus-related cirrhosis (the second most common, after infections and before gastrointestinal bleeding). The spectrum of renal impairment in patients with liver cirrhosis covers several main entities, as acute kidney injury with or without features of hepatorenal syndrome, acute kidney disease and chronic kidney disease (Angeli et al., 2015). In our analysis, virus-related cirrhosis was found to be more exposed to renal complications, probably because both hepatitis B virus infection and hepatitis C were reported as causes of chronic renal disease, mostly due to membranous glomerulonephritis (Hong et al., 2018), or cryoglobulinemia and focal segmental glomerulosclerosis, respectively (Angeletti et al., 2019). Dyselectrolytemia was the fourth most frequent precipitating factor (26.2%), with similar prevalence in relation to the etiology of cirrhosis. Hyponatremia, hypokalemia or hyperkalemia and alkalosis are the main electrolyte imbalances that can trigger HE, independently or associated to renal disfunction (Younas et al., 2021). Diuretics and consequent hypovolemia or dehydration are risk factors for both electrolyte disorders and acute renal failure, and tight surveillance is mandatory to avoid harmful overlap.

Active alcohol consumption was unfortunately frequent as trigger factor for HE among patients with alcoholic cirrhosis (16.5%). This underlines the urge need to actively combat alcohol consumption, which is not only a main cause for chronic liver disease, but also a factor for further progression and recurrent complications. Conversely, in the viral cirrhosis group, no cases of active alcohol consumption were identified. As listing for liver transplantation requires at least 6 months abstinence, the significance of the matter is tremendous. A particular issue appears to be non-adherence to therapy, more significant in patients with alcoholic cirrhosis compared to viral cirrhosis (15.5% and 8.3%, respectively, P=0.026). Neglecting the treatment of a potential precipitating factor or lack of proper prophylaxis measures put the patients at risk for developing HE. Again, more efforts for a better compliance seem to be needed in alcoholic patients.

An important severe, potentially fatal complication of liver cirrhosis is hepatocellular carcinoma (HCC), which develops in one third of cirrhotic patients overall during their lifetime, with annual risk of up to 8% (Sangiovanni and Colombo, 2011). HE may be induced by hepatic failure associated to HCC. All patients with liver cirrhosis, regardless of etiology, are at risk for HCC, but hepatitis B and C virus-related cirrhosis are particularly exposed. In our analysis, prevalence of HCC as causal factor of HE was significantly higher in virus-related cirrhotic patients compared to alcoholic cirrhosis (14.3% and 6.4%, respectively, P=0.004). Surveillance is mandatory in all cirrhotic patients, to allow diagnosis in curable stages. Seen as a relatively

common precipitant factor of HE, especially for recurrent episodes (Bajaj et al., 2020), constipation was found to be in our study responsible for less than 10% of cases. Our study included only hospitalized patients during pandemic, while patients with non-severe forms of HE, having constipation as trigger factor, were managed as outpatients. Surgery is a stress condition that may decompensate and induce HE (Onyekwere et al., 2011). A few patients in our study (1.5%) had surgery as responsible factor for HE, as interventions in cirrhotic patients should be reserved only for critical and life-menacing situations, especially if already decompensated. A less frequent but equally important causal factor for HE was transjugular intrahepatic portosystemic shunt (TIPS). TIPS may complicate with HE in various proportions (18-50%), depending mostly on the type of stent (diameter, bare or covered) (Peter et al., 2013). In our analysis, four cirrhotic patients presented with HE as a consequence of the TIPS. Time of insertion and the type of TIPS, used as treatment of recurrent bleeding or refractory ascites, must be very well weighted, in order to assure a favorable risk-benefit ratio.

Identifying and correction of precipitating factors are essential, as in many cases, up to 90%, controlling the cause assures the effective treatment of HE episode (Strauss et al., 1992). However, some cases can be challenging, when no factor is finally found or when, by contrary, multiple factors overlap. We found a relatively low proportion (11%) of patients without identifiable precipitating factor. Exhaustive work-up must be always performed in order to find the potential trigger, but clinicians must be aware of the existence of the so-called "non-precipitated" HE, mainly caused by spontaneous porto-systemic shunts (Nardelli et al., 2020). HE may have more than one precipitant factor, situation found in our study in a high proportion (39%), similar results being reported by other several studies (Raphael et al., 2016; Vinoth et al., 2019). Independent or related to each other, various concomitant events or complications of cirrhosis can act synergically towards HE development, and their prompt recognition influence patient's outcome.

Conclusions. HE is a frequent complication of liver cirrhosis, with increased prevalence in hospitalized cirrhotic patients. Infections (SARS-CoV-2 included), gastrointestinal bleeding, renal disfunction and dyselectrolytemia are the most common overall precipitating factors, and almost 40% of patients have multiple precipitating factors. According to cirrhosis etiology, a significant higher prevalence was found for sepsis and non-adherence in alcoholic cirrhosis, while HCC was significantly more frequent as precipitating factor in virus-related cirrhosis. Non-variceal bleeding was more frequent in alcohol-induced cirrhosis. Active exhaustive search and promptly control of the precipitating factors are mandatory, and being aware of the risk factors could assure early identification and treatment.

I.3.3. CARING FOR THE ELDERLY – MORE THAN ROUTINARY PRACTICE

I.3.3.1. Introduction

The challenges of providing care to aged patients have the tendency to become more frequent nowadays, in gastroenterology and hepatology. The physiological effects of aging on general behavior, on structural and functional mechanisms contribute to an increasing prevalence of several common pathologies, as constipation, gastroesophageal reflux disease or malnutrition (Lucak et al., 2019). Moreover, older adults are more susceptible to drug interactions, to deleterious effects of medication (gastrointestinal bleeding being the most dramatical) and are more prone to develop drug-induced liver injury (Kochar et al., 2021). Elderly has higher baseline risks for adverse events such as malignancies and infections (Singh et al., 2017; Greuter et al., 2020). Comorbidities with concomitant polymedication, frailty and ageing-related impairments may complicate both evolution and the decision-making process, especially in the sphere of liver cirrhosis and inflammatory bowel disease.

Thus, we found that overall mortality was increased in elderly both in non-variceal and variceal acute bleeding despite successful endoscopic therapy (Cojocariu et al., 2019, Girleanu et al., 2019), antiplatelet therapy is a risk factor for gastrointestinal bleeding in elderly, having duodenal ulcer as the most frequent cause (Huiban et al., 2019), octogenarians with previous hospitalizations or recent antibiotic treatment are at risk for *Clostridium difficile* infection (Trifan et al., 2018) when associate arterial hypertension and chronic cardiac failure elderly, but also that elderly onset IBD is associated with a less aggressive phenotype (Trifan et al., 2018), and that antiviral treatment is highly effective and safe in patients aged 70 years or older with HCV compensated cirrhosis, adding new evidence that advanced age should not be a barrier *per se* in treating this subgroup of HCV patients (Trifan et al., 2017).

Optimizing the elderly care signifies adapting the management according to patient's particular age, status and concomitant pathologies. Keeping the right balance during maximizing the therapeutic effect simultaneously with minimizing the risks is a difficult and provocative, but essential issue in elderly.

PERSONAL CONTRIBUTIONS RELATED TO THE APPROACH OF ELDERLY PATIENTS IN HEPATO-GASTROENTEROLOGY

ARTICLES	
1.	Trifan A, Girleanu I, Stanciu C, Miftode E, Cojocariu C, **Singeap AM**, Sfarti C, Chiriac S, Cuciureanu T, Stoica O. Clostridium difficile infection in hospitalized octogenarian patients. Geriatr Gerontol Int. 2018;18(2):315-320. **IF = 2.118**
2.	Trifan A, Stanciu C, Gheorghe L, Iacob S, Curescu M, Cijevschi Prelipcean C,Stefanescu G, Girleanu I, Chiriac S, Mihai C, Brisc C, Goldis A, Sporea I, Miftode E, Bataga S, Rogoveanu I, Preda C, Caruntu FA**, 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 (Baltimore).* 2017; 96(50):e9271. **IF = 2.028**
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4.	Cojocariu C, **Singeap AM**, Sahawneh DM, Girleanu I, Petrea OC, Stanciu Cl, Trifan A. Non-Variceal Acute Upper Gastrointestinal Bleeding in Elderly Patients. Proceedings of the Central European Gastroenterology Meeting CEURGEM Timisoara, Romania, 13-15 February 2019. Filodiritto International Proceedings 2019, ISBN 978-88 85813-53-3.
5.	Girleanu I, Stanciu C, Teodorescu DS, Petrea OC, Cojocariu C, **Singeap AM**, Sfarti C, Teodorescu A, Nemțeanu R, Chiriac S, Huiban L, Muzică C, Cuciureanu T, Trifan A. Predictors of In-hospital Mortality in a Cohort of Elderly Cirrhotic Patients with Variceal Bleeding. Proceedings of the Central European Gastroenterology Meeting CEURGEM Timisoara, Romania, 13-15 February 2019. Filodiritto International Proceedings 2019, ISBN 978-88 85813-53-3.
6.	Huiban L, Stanciu C, Muzica CM, Cuciureanu T, Chiriac S, **Singeap AM**, Girleanu I, Petrea OC, Trifan A. The Impact of Long-Term Antiplatelet Therapy on the Upper Gastro-Intestinal Tract to Elderly Patients. Proceedings of the Central European Gastroenterology Meeting CEURGEM Timisoara, Romania, 13-15 February 2019. Filodiritto International Proceedings 2019, ISBN 978-88 85813-53-3.
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I.3.3.2. Non-variceal acute upper gastrointestinal bleeding in elderly patients

Background and aim. Acute upper gastrointestinal bleeding (AUGIB) is one of the most serious and dramatic emergency hospitalizations in gastroenterology. Often AUGIB represents a potentially life-threatening medical emergency that requires hospital admission and attentive monitoring of the patients. AUGIB may be caused by different lesions with variable prognostic; one of the most prognostic factors is the variceal or nonvariceal bleeding cause. The main cause of AUGIB are peptic disease and esophageal varices and more than 40% of the admissions for AUGIB are secondary to peptic disease (Asma and Stanley, 2012). Despite advances in endoscopic and pharmacological treatment for peptic ulcer bleeding (PUB), mortality remains high at 5-10% worldwide with a substantial cost for healthcare systems (Sung et al., 2010). Must often patients have died not because to the bleeding and in successful hemostasis but because to the multiorgan failure or cardiopulmonary complications (Sung et al., 2010). In the last years we observed an increasing number of elderly patients admitted in our unit for AUGIB, data similar to that reported in the Western countries. The main reason of high incidence of AUGIB in elderly patients is the increase in life expectancy worldwide (Teocharis et al., 2008). Previous studies have reported that the age represents an independent significant risk factor for poor clinical outcome in patients with AUGIB, with mortality rates ranging from 12% to 35% in patients over 60 years, less than 10% in patients younger than 60 years and few data regarding patients over 80 years (Rockall et al., 1996; Christensen et al., 2007; Theocharis et al., 2008). Bleeding incidence and mortality are higher in elderly patients, especially in those with co-morbidities (Zullo et al., 2007). Most often elderly patients have significant co-morbidity (the most frequent cardio and pulmonary disease) and significant concomitant medication, sometimes with gastrointestinal adverse events. The use of aspirin, non-steroidal anti-inflammatory drugs (NSAIDs), antiaggregant/anticoagulant agents, drugs with proved data regarding bleeding risk is higher in elderly people. There is no NSAID (including selective cyclo-oxygenase (COX)-2 inhibitors) that can be completely safe for gastrointestinal tract. Many reports highlights that even low-dose aspirin (acetylsalicylic acid) (less than 325 mg/day) or standard dose of antiplatelet medication are associated with risk of gastrointestinal bleeding (Lanas et al., 2006; Lanas et al., 2011). The aim of our study was to evaluate the etiology, demographic characteristics, clinical outcome and factors related to mortality in patients presented with non-variceal acute upper gastrointestinal bleeding. One of our purposes was also to determine if there were differences between the causes and the clinical outcome of non-variceal upper gastrointestinal bleeding (NVUGIB) in octogenarians, in the patients between 65-80 years and those younger than 65 years old. We analyzed the factors related to mortality in octogenarians with AUGIB.

Materials and Methods. Our study is a prospective, descriptive analysis one; we have studied data of consecutive patients with NVUGIB admitted in "St. Spiridon" Emergency Hospital between 1st January 2018 and 31th December 2018. Both newly admitted patients and the ones with bleeding episodes during the initial hospitalization for other causes than NVUGIB were considered appropriated and included in our study. All patients with signs of gastrointestinal bleeding (hematemesis, melena, bloody nasogastric aspiration, etc.) were evaluated by emergency upper digestive endoscopy. The hemodynamic stability was the mandatory condition before performing upper digestive endoscopy and all patients have been closely monitored first in the emergency reception unit of the hospital. The upper endoscopy was performed in the 1st 24 hours of admission in most cases, only after complete clinic and laboratory evaluation and the balancing of hemodynamic parameters. The procedure was

performed after topical pharyngeal anesthesia with 10% lidocaine spray and only in few and selected cases sedation was given, according to local protocol. Specific treatment of non-variceal bleeding was made according guidelines of the Romanian Society of Gastroenterology and Hepatology: association between medical (high dose of Proton Pomp Inhibitors–PPIs – 80 mg bolus injection followed by continuously infusion of 8mg/h for 5 days) and endoscopic treatment; injection of adrenaline diluted at 1:10.000 was the performed in almost all cases depending on the Forrest classification and the risk of rebleeding. Other hemostatic techniques (hemoclips, argon plasma coagulation (APC), hemostatic powder etc.) were added to adrenaline injection in selected cases. Endoscopic hemostasis was performed in all cases with high risk of rebleeding: Forrest Ia-b, Forrest IIa. The stigmata of active or recent bleeding were assessed according to the Forrest classification: Forrest Ia: active spurting bleeding; Ib: active oozing bleeding; IIa: nonbleeding visible vessel (NBVV); IIb: adherent clot; IIc: spots; III: ulcer with a clean base. We distributed each of the three groups of patients (us we mentioned later) into two subgroups according to the risk of rebleeding: patients with high risk of rebleeding (Forrest Ia-b and Forrest IIa) and patients with low risk (Forrest IIb, IIc and Forrest III). After performing upper digestive endoscopy and confirmation of non-variceal bleeding we divided the patients into three groups according to their age: Group A included patients younger than 65 years old, Group B included patients between 65 and 79 years old, and Group C the patients older than 80 years. The patients with variceal bleeding or patients without any determined bleeding causes during endoscopy were not admitted to the study. We recorded all data regarding the demographic characteristics (age, sex), hemodynamic and laboratory parameters on admission, endoscopic features, smoking and alcohol habits, associated comorbidities, Rockall score, medical history, consumption of non-steroidal anti-inflammatory drugs (NSAIDs), anti-platelets or anticoagulants. Hemodynamics instability was defined as systolic blood pressure less than 100 mmHg and a pulse rate of more than 100 beats/min. The status of Helicobacter pylori infection was evaluated and treatment has begun during hospitalization in all cases where the infection has been confirmed. In this study, we also assessed the prevalence of possible risk factors of NVGIB and their age group specific trend among the general population, and compared the prevalence between patients over 80 years older and the others. The clinical outcome was analyzed according to the duration of hospitalization, the complications during hospitalization, the number of transfused blood units per patient, the rate of rebleeding, the need for emergency surgical hemostasis and mortality. Clinical and endoscopic factors that might have contributed to the mortality were evaluated. All these parameters were correlated with in hospital mortality initially by using univariate analysis. Variables found to be significant in the univariate analysis ($P<0.05$) were included in a multivariate stepwise logistic regression model. All analyses were conducted by using statistical software (SPSS, version 10.0).

Results. 463 patients with NVUGIB were hospitalized in our hospital between 1st January 2018 and 31th December 2018. Among them, 293 patients (212 males and 81 females) were younger than 65 years (63.3%-Group A), 119 patients (74 males and 45 females) were between 65 and 80 years (25.7%-Group B) and 51 patients were over 80 years (11%-Group C) (Table 3.XV).

The most patients with NVUGIB were male (32.4% vs. 67.6%) ($p=0.0027$) and the increased male prevalence is maintained for patients from A and B group; in patients over 80 years the sex distribution of NVUGIB is similar 52.9% vs. 47.1%. The location of lesions was approximately similar across all three-study group ($p=0.126$): gastric lesions (peptic ulcer,

erosive gastritis, angiodysplasia, gastric cancer) in 44.1% of cases, followed by duodenal causes (peptic ulcer, duodenitis) 36.9% and esophageal lesions (Mallory-Weiss, esophagitis, esophageal tumors) in 19.0% of cases (Table 3.XVI). There was no statistically significant difference in the history of previous ulcer between the three groups.

Table 3.XV. Clinical and epidemiological characteristics of patients with non-variceal upper gastrointestinal bleeding

Characteristics	**All patients n=463**	**Group A (18-64 y) n=293**	**Group B (65-79 y) n=119**	**Group C (>80 y) n=51**	**p**
Mean age	59.2±15.7	49.8±11.2	72.1±4.4	83.2±3.1	
Female vs. Male n, %	150/313 32.4/67.6%	81/212 27.6/72.4%	45/74 37.8%/62.2%	24/27 52.9%/47.1%	0.052
Heart rate on admission	86±18.5	86.7±16.3	87.1±15.8	88±19.2	0.623
Blood pressure on admission	119±22.3	101±19.4	120±21.1	123±23	0.542
Anticoagulants	13 (2.8%)	5 (1.7%)	7 (5.8%)	1 (1.9%)	0.062
Anti-platelets	19 (4.1%)	8 (2.7%)	6 (5.04%)	5 (9.8%)	**0.043**
NSAIDs	28 (6%)	15 (5.11%)	9 (7.56%)	4 (7.84%)	0.545
Surgery	28 (6%)	17 (5.8%)	7 (5.88%)	4 (7.84%)	0.850
Death	32 (6.9%)	19 (6.48%)	8 (6.72%)	5 (9.8%)	0.686
Bleeding source: oesophageal/gastric/duodenal	88/204/171 19/44.1/36.9%	66/127/100 22.5/43.3/34.2%	15/53/51 12.6/44.5/42.9%	7/24/20 13.7/47/39.3%	0.126
Active bleeding	211 (45.6%)	113 938.5%)	70 (58.8%)	28 (54.9%)	**<0.0001**

Table 3.XVI. Causes of acute upper gastrointestinal bleeding according to age

	All patients % (n)	**Group A n/293 (%)**	**Group N n/119 (%)**	**Group C n/51 (%)**
Mallory-Weiss	13.67% (63)	**48/293 (16.38%)**	10/119 (8.4%)	5/51 (9.8%)
Esophagitis	2.59% (12)	6/293 (2.04%)	3/119 (2.52%)	**3/51 (5.88%)**
Erosive gastroduodenitis	8.87% (41)	20/293 (6.8%)	**17/119 (14.28%)**	4/51 (7.84%)
Gastric ulcer	31.7% (147)	94/293 (32.1%)	37/119 (31.1%)	16/51 (31.4%)
Duodenal ulcer	36.3% (168)	95/293 (32.4%)	45/119 (37.9%)	17/51 (33.3%)
Neoplasia	4.5% (21)	12/293 (4.09%)	5/119 (4.2%)	**4/51 (7.8%)**
Other	2.38% (11)	4/293 (1.37%)	3/119 (2.52%)	**4/51 (7.8%)**

Relative to the three age groups in the group A 32.1% of NVUGIB have been a complication of gastric ulcers (18.27% Forrest Ib, with high risk of rebleeding); quite frequently (the highest incidence) in this group of age was Mallory-Weiss syndrome (16.38%) and bleeding from duodenal ulcer in 32.4% of cases. In group B the most common cause of bleeding was peptic duodenal (37.9%) and gastric ulcer (31.1%). In patients over 80 years esophagitis was more common than in the other two groups (2.04% vs. 2.52% vs. 5.88%) (p=0.042). Also, in patients over 80 years the NVUGIB due to esophageal/gastric tumors (7.8%) or to other causes (angiodysplasia, polyps, etc.) (7.8%) are more frequently than in two other groups of patients. On upper endoscopy active bleeding was significantly high in group B patients (p=0.0001), statistically significant. There was no incidence or complication directly related to diagnostic or therapeutic endoscopy. The percentage of patients taking NSAIDs before the bleeding episode was greater in the Groups B and C, but not statistically significant between the last two groups. The antiplatelet therapy before the bleeding episode was significant greater in the octogenarian group (p=0,043), while the use of anticoagulants was increased in the patients between 65 and 80 years old (5.8% in Group B vs. 1.9% in Group C (Table 1). Although NSAIDs was the most frequently used predisposing drug in all patients

(6%), anti-platelets drugs had the highest prevalence in elderly patients (9.8%). The prevalence of almost each comorbid condition and concomitant drug usage were also higher in the last group. Rebleeding rates after endoscopic hemostasis showed insignificant differences between the three groups, as well as the need for surgery; emergency surgical intervention for continuing or recurrent bleeding was required only in 6% of patients: 5.8% vs. 5.88% vs. 7.84% (p=0.850). Co-morbidities were more common in Group B and C patients (p=0.04); only 5.2% of patients over 80 years have had no comorbidity. The most associated diseases were hypertension, cardiovascular diseases, diabetes mellitus, chronic renal disease and neurologic disease.

In multivariate analysis, the presence of severe co-morbidity, age over 80 years (p=0.012) and esophageal bleeding p=0.029 (Mallory-Weiss syndrome) were independently related to mortality (Table 3.XVII, Fig. 1). Although in-hospital complications were more common in patients over 80 years it was no significant differences (28.9% vs 20s.7%); the most common complications were infection (cutaneous, urinary, pulmonary, etc.), ischemic episode (cerebral or coronarian), mental status deterioration. Mortality rate was 6.9%, greater, in elderly patients (9.8%), but this difference did not reach statistical significance (Table 3.XVII).

Table 3.XVII. Risk factors for mortality

Parameter	OR	IC	p
Female gender	1.31	0.71-2.43	0.344
Anticoagulants	0.89	0.12-6.63	0.910
Anti-platelet drugs	1.02	0.93-1.12	0.526
NSAIDs	1.03	0.92-1.16	0.413
Active bleeding	1.17	0.80-1.71	0.674
Esophageal lesions	2.86	1.15-4.55	0.029
Age > 80 yrs	2.55	1.14-3.37	0.012

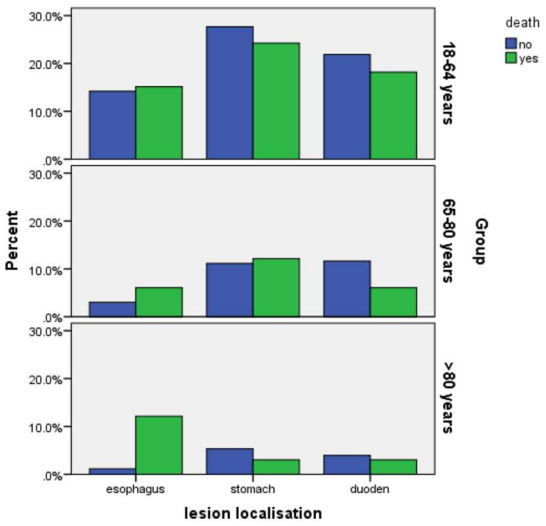

Fig. 3.3. Mortality risk according to the lesion localization

Discussion. AUGIB is associated with higher rates of hospitalization and mortality in the elderly patients comparing with patients, most likely due to multiple comorbidities. The incidence of AUIGB is decreasing in the last year's most likely due to reduced incidence of non-NSAIDs related peptic disease, better management of the chronic peptic ulcer disease, eradication of Helicobacter pylori, etc. On the other hand, more than 10 years some studies reported the increase in the proportion of older patients admitted with AUGIB (Di Fiore et al.,

2005; Theocharis et al., 2008). In our study more than half (63.3%) patients were under 65 years and only 36.7% of them were over 65 years. Our results are similar with some old data from literature (Hernandez-Diaz et al., 2002), but this data shows a lower incidence of AUIGB in patients over 80 years (11%) than in other studies varying between 25-41% (Koziel et al., 2011; Asma and Stanley, 2012). In UK the most patients with AUIGB are elderly: 63% of all patients were over 60 years and 28% were over 80 years old (Hearnshaw et al., 2011). We don't have a clear explanation for our result; it may be due to a lower life expectancy in our population, the excessive use of PPIs, etc. The most studies state that AUGIB occurs more frequently in the male sex and advancing age (Theocharis et al., 2008). In our evaluation the most patients with NVUGIB were male (32.4% vs. 67.6%) (p=0.0027) and the increased male prevalence was maintained for patients under 80 years. Theocharis G et al., find that percentage of female patients in the octogenarians' group is significantly higher than that in youngers. We found that after 80 years the incidence of NVUGIB sex distribution is comparable 52.9% vs. 47.1%.

The most causes of bleeding in our patients were peptic ulcer (gastric or duodenal) and the location of lesions was approximately similar across all three-study group, data according to other studies. In our analysis's esophagitis had an increase incidence (5.88%) in patients over 80 years; this endoscopic finding it is not unusual considering esophageal reflux factors in elderly persons. A systematic review including 93 studies found that relative risk of AUGIB in NSAID users ranged from 2.7 to 33.9 (Lau et al., 2011) and in older patients the risk of serious adverse events, such as peptic ulcer bleeding while taking NSAIDs, is 5.5 times that of controls, whereas in younger patients it is only 1.5 times (Theocharis et al., 2008). Moreover, concurrent use of NSAIDs and antiplatelet drugs or oral anticoagulants, often used for thromboembolic prophylaxis in the geriatric population, increases the risk of bleeding. About 13% of our patients had taken NSAID and/or oral-anticoagulant or antiplatelet drugs. The high mortality and economic burden of AUGIB have raised concerns regarding risk factors for the disease. Additionally, many underlying diseases, drugs, and unhealthy lifestyle (e.g., smoking) are proven risk factors of AUGIB. Also, the increasing trend of NSAIDS-induced AUGIB has also led to research focusing on elderly patients, who are potential usual consumers of these predisposing drugs. On the other side, indications of aspirin or non-aspirin non-steroidal anti-inflammatory drugs have been increasing during the last years and this has had detrimental consequences, mainly to the elderly. Age is an independent risk factor for NSAID related GI tract toxicity and ulcer formation. Over half of the cases of AUGIB are due to peptic ulcer bleeding irrespective of the patients' age. Despite the preventive effects of proton pump inhibitors on gastrointestinal toxicity from NSAIDs, it has been found that 70%-80% of users at risk for gastroduodenal complication does not receive gastroprotection. Severity of bleeding does not seem to be higher in the octogenarian's group. Prognostic factors of rebleeding, rates or need for emergency surgical hemostasis in our study were not more frequent in the very elderly. Blood loss and emergency surgery is poorly tolerated by elderly patients with increased comorbidity. Successful endoscopic therapy reduces the rebleeding rates and the need for emergency surgical hemostasis, which is a known risk factor for mortality especially in the elderly, due to high rate of co-morbidity in these patients. Only 4 out of 51 patients over 80 years of age required emergency surgical intervention in our study. Despite this, overall mortality was increased in octogenarians in comparison to younger patients; this is due to the higher co-morbidity in these patients. The difference may have not been very significant because in our study were included only patients with AUGIB subjected to endoscopy. The

percentage of deaths related directly to bleeding was generally low even in the very elderly, and the majority of elderly patients with AUGIB died of unpreventable causes. In our practice, very few patients over 80 years of age are treated with anticoagulants (mostly because of their increased costs, low pensions and multiple comorbidities). Also, in Romania our octogenarians have not yet reached the standard of living and quality of life of most Europeans. The same thing cannot be said about the 65-79-year-old population, where the addressability is higher, so the incidence's growth trend is obvious and similar with the one in Western Europe.

Conclusions. Although in Western Europe the incidence of gastrointestinal hemorrhage has been increased significantly in the elderly population due to the consumption of anticoagulant medication, NSAIDs- and antiplatelet-therapy, and also because of the increase in the life expectancy, the data that we obtain regarding the octogenarians in our study are below our expectations.

I.3.3.3. Particularities of elderly inflammatory bowel disease patients in a tertiary referral center

Background and aim. Approximately 10-20% of IBD cases including ulcerative colitis (UC) and Crohn's disease (CD) are diagnosed after 60 years of age (Ananthakrishnan et al., 2016). Population aging along with IBD increasing incidence will contribute to a rise in the size of older patients diagnosed with IBD in the near future. Thus, data regarding management of elderly patients with IBD are scarce, mostly due to the fact that this category of patients is normally excluded from clinical trials (Arnott et al., 2018). Recent studies have underlined the fact that elderly patients with IBD are characterized by a milder disease activity, predominantly with colonic involvement compared to younger IBD patients (Tran et al., 2019). Furthermore, older-onset IBD typically is not associated with disease progression (Arnott et al., 2018). Determining the most appropriate therapeutic choice in the elderly is challenging due to multiple factors like comorbidities, polypharmacy, drug interaction and the risk of non-compliance (Juneja et al., 2012). Most of the data indicate that aminosalicylates are frequently used in elderly IBD because of their excellent tolerability. Likewise, almost 63% of older CD patients and 58% of older UC patients received corticosteroids within 5-10 years after diagnosis (Ha and Katz, 2013). Regarding immunosuppressive therapy, thiopurines are frequently used in elderly IBD patients with up to 60% in CD, and 25% in UC respectively (Jorissen et al., 2021). Because of multiple comorbidities and greater risk of infections, TNF inhibitors are rarely recommended in elderly IBD patients (Lowenberg, 2020). The prognosis of elderly patients with IBD is controversial. Some studies have demonstrated a decreased need for steroids, immunomodulators, and surgery and less hospital admissions for flares in cases of elderly IBD (Choi et al., 2015). However, other studies have also shown that IBD-related hospitalizations were associated with higher morbidity and mortality in older patients than in younger ones (Ananthakrishnan et al., 2016). Therefore, in the present study we aimed to evaluate the clinical features and particularities of elderly patients with IBD admitted in a tertiary referral center.

Patients and Methods. This was a retrospective study of prospectively collected data from IBD patients admitted and followed in a single tertiary referral center between January 2011 and December 2016. Demographic data and clinical characteristics like age at diagnosis, IBD phenotype, disease extent and severity, type of medication used, need for surgery, length of hospital stay were recorded. Likewise, comorbidities such as cardiovascular disease, pulmonary disease, metabolic, neurological and neoplastic disorders were also carefully documented from the patients' medical charts. Data from elderly patients (≥65 years) with IBD

(study group) were compared with those from the younger patients hospitalized during the study period (control group). *Statistical analysis* - continuous variables with normal distribution are expressed as mean ± SD, whereas categorical variables are expressed as percentages and absolute values. The $\chi 2$-test was used for categorical data. Quantitative variables with normal distribution were compared using the Student's t-test, whereas for those with non-normal distribution, the Mann-Whitney test was used. Univariate and multivariate analysis were carried out on a number of recorded variables, and odds ratio (OR) with 95% confidence interval (CI) was calculated for quantitative variables included in the logistic regression. Statistical analysis was carried out using the SPSS 20.0 software (SPSS, Chicago, IL, USA). Statistical significance was considered $P<0.05$.

Results. During the study period there were 332 patients admitted with IBD in our institution, and among them 40 (12.04%) were elderly (≥65 years). Demographics and clinical characteristics of both study groups are shown in Table 3.XVIII.

The elderly group consisted of predominantly UC patients (85%), with only 6 (15%) patients diagnosed with CD. IBD patients from the elderly group had predominantly a mild form of disease activity compared with younger patients (48.5% vs 39.1%, p=0.550). Likewise, elderly patients had more comorbidities than the younger ones (85% vs 33.2%, p=0.0001). On univariate analysis comorbidities remained significant associated with elderly IBD patients (OR=0.258, CI=0.171- 0.345; p=0.001). Older UC patients presented with predominantly proctitis and left-sided colitis compared to younger ones. Furthermore, late-onset CD more likely manifests as colonic disease and less likely as ileocolonic (Table 3.XVIII). In what concerns the therapeutic approaches, almost 97.5% of elderly patients were treated with amino salicylates.

Table 3.XVIII. Characteristics of study groups

Variable	**Elderly n=40**	**Non-elderly n=292**	**p-value**
Age, years (mean ± SD)	70.8±3.74	40.2±12.6	0.0001
Gender, male n (%)	25 (62.5)	170 (58.6)	0.606
UC/CD	34/6	196/96	**0.022**
Comorbidities, n(%)	34 (85)	97 (33.2)	**0.0001**
Disease activity, n(%)			
Mild	17 (42.5)	106 (36.3)	0.55
Moderate	13 (32.5)	115 (39.3)	
Severe	5 (12.5)	50 (17.1)	
CD distribution, n(%)			
Ileal	(0)	29 (9.9)	0.419
Colonic	4 (10)	41 (14)	
Ileo-colonic	2 (5)	24 (8.2)	
Upper gastrointestinal tract	0 (0)	2 (0.6)	
UC extension, n(%)			
Proctitis	7 (17.5)	31 (10.6)	0.078
Left-sided colitis	22 (55(	98 (33.5)	
Pancolitis	5 (12.5)	67 (22.9)	
Treatment			
Aminosalicylates, n(%)	39 (97.5)	261 (89.3)	0.112
Corticosteroids, n(%)	23 (57.5)	188 (64.3)	0.396
Immunomodulators, n(%)	3 (7.5)	76 (26.2)	**0.010**
TNF inhibitors, n(%)	1 (2.5)	70 (23.9)	**0.002**
Death, n(%)	0 (0)	1 (0.34)	0.711
Surgery, n(%)	3 (7.5)	35 (11.9)	0.403
Hospital stay, days (mean ±SD)	9.9±4.6	8.6±5.6	0.145

The elderly patients tend to receive less immunomodulatory treatment (7.5% vs 26.2%, p=0.010), biologics (2.5% vs 23.9%, p=0.002), or corticosteroids (57.5% vs 64.3%, p=0.396). As regards prognosis of elderly patients with IBD, a few proportions of these patients required surgery compared to younger study group (7.5% vs. 11.9%, p=0,403). Moreover, there were no significant differences between the two study groups regarding length of hospital stay (9.9±4.6 days vs 8.6±5.6 days, p=0.145).

Discussion. In agreement with recent data, our study shows that elderly IBD patients develop a less aggressive disease course. When compared to younger patients, elderly CD showed a greater proportion of isolated colonic disease (Norton et al., 2013). Elderly UC patients showed a predominance of left sided colitis as in previous studies (Katz and Pardi, 2011). Contrarily to past studies (Biederman et al., 2015), we found almost a similar rate of corticosteroid use among study elderly with IBD and younger IBD patients, but in agreement with previously reported data (Lang et al., 2017) we found a lesser use of immunosuppressants and anti-TNF agents in elderly patients. An explanation could be that because of multiple comorbidities and greater risk of infections, TNF inhibitors are rarely recommended in elderly IBD patients . Our findings are consistent with results anterior published demonstrating that elderly UC was associated with less extensive disease (Ha and Katz, 2013). A study of 1705 UC patients study showed that rates of proctitis and distal colitis tended to increase among patients diagnosed at age 50 years or older (p=0.02 and p=0.019, respectively) (Katz and Pardi, 2011). A notable finding of our study was that the elderly-onset group had less severe disease than the non-elderly group (P=0.001). There are some limitations in our study. First, we examined a small number of patients. Second, our study employed a retrospective, single-center design. Finally, variability in practice among the physicians may also have affected patient outcomes. In conclusion, our study confirms that elderly onset IBD is associated with a less aggressive phenotype. Clinical features were similar between the elderly and the younger patients with IBD, except the fact that elderly patients had more comorbidities. More studies on the therapeutic management of UC in elderly, balancing clinical outcomes and safety concerns, are needed in this particular setting.

I.3.3.4. 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

Introduction. The elderly population is most likely to be infected with HCV and has advanced liver disease as compared to the younger people (Thabut et al, 2006). Advanced age has been a major limitation of pegylated interferon and ribavirin therapy for chronic HCV infection because of its poor response and tolerability. Consequently, the great majority of elderly patients (if not all, in some countries), defined as those aged 65 years or older, were denied antiviral treatment solely on the basis of their advanced age (Gramenzi et al, 2009) Interferon-free regimens are safe and highly effective, allowing treatment for elderly chronic HCV-infected patients without any age limit (Saab et al, 2016, Conti et al, 2017). However, pivotal trials of all oral combinations with direct-acting antivirals (DAAs) included few elderly patients with compensated cirrhosis (Ferenci et al, 2014; Feld et al, 2016). Twelve-week treatment of HCV genotype 1 compensated cirrhosis with paritaprevir/ritonavir, ombitasvir, and dasabuvir (PrOD) with ribavirin was approved in many countries, including Romania, based on the results of a phase III trial showing an SVR response 12 weeks after the end of therapy (sustained virologic response 12 weeks after the end of treatment (SVR12)) well above 90% (Poordad et al, 2014). More recently, the HCV regimen of 12-week PrOD without ribavirin reported 100% SVR12 in HCV genotype 1b-infected patients with compensated

cirrhosis, meaning that ribavirin does not provide evidence of improving the effectiveness in such patients treated with PrOD (Feld et al, 2016).

This study aims to assess the real-world efficacy and safety of PrOD with ribavirin for the treatment of HCV genotype 1b compensated cirrhosis in patients aged 70 years and older.

Material and methods

Patients. One thousand and eight patients with HCV genotype 1b compensated cirrhosis, treatment-experienced or naïve, were prospectively followed and treated with PrOD+ribavirin for 12 weeks across 10 academic centers of gastroenterology/infectious diseases from all over Romania, between December 1, 2015 and July 31, 2016. Eligible patients were enrolled and assessed following the criteria established by the Romanian National Health Insurance House: adults 18 years of age and above with HCV genotype 1, Child–Pugh class A compensated cirrhosis defined as F4 by Fibromax Biopredictive (Fibrotest score ≥0.75). Exclusion criteria were: decompensated liver cirrhosis, severe chronic kidney disease, documented malignant neoplastic disease, active alcohol consumption, and human immunodeficiency virus coinfection. All eligible patients signed an informed consent and received treatment with PrOD+ribavirin according to the therapeutic protocol. The PrOD regimen contains paritaprevir 75 mg boosted with ritonavir 50 mg and ombitasvir 12.5 mg (Viekirax, AbbVie Deutschland Gmbh & Co Ludwigshafen, Germany) 2 tablets in a single daily dose, and dasabuvir (Exviera 250 mg AbbVie Deutschland GmbH & Co Ludwigshafen) twice-daily administration. The dose of ribavirin was 1000 mg/day in patients weighting <75 kg or 1200 mg/day in those weighting >75 kg. This study was approved by National Ethics Committee, and written informed consent was obtained from each patient in accordance with the principles of the Declaration of Helsinki.

Methods. Blood and urine samples were taken for laboratory analyses at baseline, on weeks 4, 8, 12 (end of treatment (EOT)), 12 weeks after the treatment, and whenever it was necessary. Baseline clinical data referred to gender, age, treatment history, comorbidities, and concomitant medication.

Laboratory data included HCV RNA level (at baseline, EOT, and SVR12), genotype and subgenotype, liver function tests (aspartate and alanine aminotransferases, bilirubin, alkaline phosphatase, gamaglutamyl transpeptidase, albumin, and international normalized ratio), serum creatinine and creatinine clearance, hemoglobin, platelet count, and alpha-fetoprotein. Child–Pugh and Model of End-Stage Liver Disease scores were calculated at baseline and 12 weeks after the end of therapy. Serum HCV RNA levels were measured with the COBAS TaqMan HCV Quantitative Test (Roche Molecular Systems, Inc. Branchburg, NJ) with a lower limit of quantification and detection of 15 IU/mL.

Efficacy of therapy was assessed by the percentage of patients achieving SVR12 (defined as HCV RNA below the limit of detection 12 weeks after the end of therapy) calculated based on intention-to-treat (ITT) and per-protocol (PP) analysis. ITT population was defined as all patients receiving at least 1 dose of medication while PP population included all patients who completed the 12 weeks of therapy. Safety and tolerability assessment included physical examinations, laboratory data analysis, and all adverse effects (AEs) recorded from the time of the 1st dose of treatment to the last one. Severe adverse events (SAEs), therapy discontinuation, and death rate were recorded.

Statistical analysis. Continuous variables with normal distribution were expressed as mean±SD, while categorical variables were expressed as absolute values and percentages. The Chi-square test was used to compare categorical data. Quantitative variables with normal distribution were compared using the Student *t* test. For nonnormal data, we used nonparametric methods such as the Mann–Whitney *U* test, while the Kolmogorov–Smirnov test was used to check the normality of the data distributions. The efficacy analysis examined data concerning the total patient population by age at baseline (≥70 or <70 years), whereas the

safety analysis described the number and percent of patients with adverse effects or laboratory abnormalities. *P* value less than 0.05 was considered statistically significant. Statistical analysis was carried out using the SPSS 19.0 software (SPSS Inc., Chicago, IL).

Results

Baseline characteristics. Among the 1008 patients included in our analysis (51.7% females), mean age 59.2±8.7 years (range 33–82), and 117 (11.6%) were aged ≥70 years. Most of the elderly patients were females (58.9%), mean age 73.3±2.8 years (range 70–82), and 37 of them (31.6%) were treatment-experienced. Comorbidities were reported in 60.6% of patients aged ≥70 years compared to 39.8% of those below 70 years (*P*<0.001). The most frequently met comorbidity in the patients ≥70 years was cardiovascular disease (hypertension, ischemic heart disease, and atrial fibrillation) (Table 3.XIX). At baseline, a significant number of patients aged ≥70 years had reduced estimated glomerular filtration rate and hemoglobin level than those <70 years (Table 3.XIX). Improvement in the laboratory results was noted at the EOT, while aspartate aminotransferase and alanine aminotransferase values in both age groups were normalized in most of the patients at 4 weeks of therapy. There were no differences in Child–Pugh and Model of End-Stage Liver Disease scores between patients ≥70 and those <70 years of age.

Table 3.XIX. Baseline demographics and laboratory characteristics in patients aged ≥70 and <70 years treated with paritaprevir/ritonavir, ombitasvir, and dasabuvir+ribavirin

Characteristics	**≥70 y (n=117)**	**<70 y (n=891)**	**p**
Age, y, mean±SD, range	73.3±2.8 70-82	57.4±7.5 33-69	**<0.001**
Female, n,%	69 (58.9)	452 (50.7)	0.093
Treatment experienced, n,%	37 (31.6)	503 (56.4)	**<0.001**
Comorbidities, n,%	71 (60.6)	355 (39.8)	**<0.001**
Cardiovascular	48 (41)	177 (19.9)	**<0.001**
Diabetes mellitus	13 (11.1)	123 (13.8)	0.42
Platelet count x10^9/L, mean±SD	143.54±6.1	142.63±2.3	0.94
Hemoglobin, g/dL, mean±SD	13.62±1.7	14.25±1.6	**<0.001**
Albumin, d/dL, mean±SD	4.01±0.4	4.02±0.6	0.97
eGFR, mL/min, mean±SD	72.04±23.3	101.7±30.7	**<0.001**
Total bilirubin, mg/dL, mean±SD	1.04±0.45	1.09±0.5	0.4
AST, UI/L, mean±SD	101.25±59.8	101.5±86.5	0.97
ALT, UI/L, mean±SD	98±56	100.7±69.1	0.5
INR, mean±SD	1.19±0.4	1.15±0.26	0.18
Child-Pugh score, n,%			
5	104 (88.8)	779 (87.4)	0.65
6	13 (11.2)	112 (12.6)	
MELD score, mean±SD	8.01±1.2	7.95±1.6	0.87

ALT-alanine aminotransferase, AST=aspartate aminotransferase, eGFR=glomerular filtration rate, INR=international normalized ratio, MELD=Model for End-Stage Liver Disease, SD=standard deviation

Efficacy. SVR12 rates based on ITT analysis were 97.4% in patients ≥70 years, compared to 97.8% in those <70 years of age (*P*=.82), while SVR12 rates based on PP were 100% in the older group compared to 99.6% in the younger group (*P*=.61), as shown in Table 3.XX. The SVR12 in treatment-naïve patients was 97.5% (78/80) for those ≥70 years of age and 98.2% (381/388) for those <70 years, while for treatment-experienced patients the SVR12 was 97.0% (36/37) for those ≥70 years and 99.4% for those <70 years of age, the differences not being statistically significant.

Safety. A total of 37.6% of patients aged ≥70 years and 34.6% of those <70 years of age (*P*=.51) reported at least 1 AE considered by their physicians as treatment-related.

The great majority of AEs were mild and manageable, none leading to treatment discontinuation. The most frequent reported AEs in both age groups were: asthenia, pruritus, insomnia, and headache (Table 3.XX).

Table 3.XX. Efficacy and safety of paritaprevir/ritonavir, ombitasvir, and dasabuvir+ribavirin treatment by age

Characteristics	**70 y (n=117)**	**70 y (n=891)**	**p**
Efficacy			
ITT SVR12, n, %	114 (97.4)	872 (97.8)	0.82
PP SVR12, n, %	114 (100)	872 (99.6)	0.61
Safety			
Any AE	44 (37.6)	308 (34.6)	0.51
Common AEs	13 (11.1)	92 (10.3)	0.79
Asthenia	5 (4.3)	67 (7.5)	0.20
Pruritus	4 (3.4)	33 (3.7)	0.87
Insomnia	3 (2.6)	31 (3.5)	0.60
Headache	3 (2.6)	26 (2.9)	0.82
SAEs, n, %	4 (3.4)	23 (2.6)	0.54
Decompensation of liver cirrhosis	1 (0.9)	14 (1.5)	0.74
Variceal bleeding	0	5 (0.5)	
Ascites	1 (0.9)	3 (0.3)	0.48
Hepatic encephalopathy	0	4 (0.4)	
Isolated grade 4 increase of direct bilirubin	0	2 (0.2)	
Cardiovascular			
Heart failure	1 (0.9)	1 (0.1)	**0.03**
Stroke	1 (0.9)	1 (0.1)	**0.03**
Malignant arrhytmia	0	1 (0.1)	
Acute pancreatitis	0	1 (0.1)	
Sepsis	0	1 (0.1)	
Severe depression	0	2 (0.2)	
Nonvariceal upper bleeding	0	2 (0.2)	
Acute kidney failure	1 (0.9)	0	
Treatment discontinuation, n, %	3 (2.6)	15 (1.7)	0.36
Death	1 (0.9)	6 (0.8)	0.88
Decompensation of liver cirrhosis			
Ascitis	1 (0.9)	0	
Hepatic encephalopathy	0	3 (0.3)	
Isolated grade 4 increase of direct bilirubin	0	2 (0.2)	
Cardiovascular			
Heart failure	0	1 (0.1)	
Stroke	1 (0.9)	1 (0.1)	**0.03**
Severe depression	0	2 (0.2)	
Death, n, %	1 (0.9)	6 (0.8)	0.88
Liver disease related death			
Variceal bleeding	0	2 (0.2)	
Severe liver decompensation	0	2 (o.2)	
Nonliver disease related death			
Heart failure	1 (0.9)	0	
Sepsis	0	1 (0.1)	
Malignant arrhytmia	0	1 (0.1)	
Ribavirin treatment			
Dose reduction	31 (26.5)	162 (18.2)	0.14
Discontinuation	11 (9.4)	51 (5.7)	0.13

AE=adverse events, ITT=intention-to-treat, PP=per-protocol, SAE=severe adverse events, SVR=sustained virologic response

Severe AEs were reported in 4 patients (3.4%) aged ≥70 years (1 decompensation of liver cirrhosis, 1 heart failure, 1 stroke, and 1 acute kidney failure), compared to 23 patients (2.6%) in the group <70 years of age (*P*=.54) (14 decompensation of liver cirrhosis: 5 variceal bleeding, 3 ascites, 4 hepatic encephalopathy, 2 isolated grade 4 increase of direct bilirubin). One death occurred (0.9%) in a patient aged 79 years (heart failure, not related in any way to PrOD/RBV therapy), and 6 deaths were reported (0.7%) in those under 70 years (2 variceal bleeding, 2 severe liver decompensation, 1 sepsis, and 1 malignant arrhythmia) (*P*=.88) (Table 3.XX). In the elderly group, of the 4 patients with SAEs, 3 discontinued therapy (1 death, 1 liver decompensation, and 1 stroke), and the 1 with acute kidney failure continued therapy after withdrawal of ribavirin. In the younger group, among the 23 patients with SAEs, 15 of them discontinued therapy (6 deaths, 3 hepatic encephalopathy, 2 isolated grade 4 increase of direct bilirubin, 2 severe depression, 1 stroke, and 1 heart failure). Modification of the ribavirin dose (due to anemia and/or increased bilirubin levels) was required in 31 (23.1%) of the patients aged ≥70 years and in 162 (18.2%) of those <70 years (*P*=0.14).

Discussion. The elderly patients with chronic HCV infection, defined in most studies as those aged 65 years or older, were usually denied previous pegylated interferon and ribavirin therapy because of severe adverse effects and poor response (Roeder et al, 2014). Therefore, there is a large cohort in real clinical practice setting of untreated elderly patients with chronic HCV infection and with advanced liver disease. This cohort is in great need for a treatment due to the progressive nature of their disease.

Fortunately, interferon-free HCV therapy with DAAs is highly effective and safe, allowing treatment for elderly patients in whom several studies reported similar SVR rates as those obtained in younger patients (Saab et al, 2016; Conti et al, 2017). Controlled clinical trials with PrOD+ribavirin in patients with chronic HCV genotype 1 infection have reported SVR12 rates ranging from 91.8% to 98.3%, while with PrOD without ribavirin in HCV genotype 1b noncirrhotic patients SVR12 rates varied from 96.7% to 99.5% (Poordad et al, 2014; Zeuzem et al, 2014). Poordad et al (2014) in a phase 3 clinical trial of patients with HCV genotype 1 compensated cirrhosis (Child–Pugh class A) treated with PrOD+ribavirin for 12 weeks reported SVR12 rates of 91.8% (98.5% in HCV genotype 1b patients). Based on the results of this study, PrOD+ribavirin for 12 weeks regimen has been recommended for patients with HCV genotype 1 compensated cirrhosis by international guidelines (AASLD/IDSA 2015, EASL 2015). More recently, Feld et al (2014) have demonstrated that PrOD regimen without ribavirin for 12 weeks was highly effective (100% SVR 12) and well tolerated in HCV genotype 1b patients with compensated cirrhosis, and now this regimen is recommended by both American Association for the Study of Liver Diseases/Infectious Diseases Society of America and European Association for the Study of the Liver guidelines. Eliminating ribavirin from this regimen without reducing efficacy will certainly improve the safety profile.

In our real-world cohort of HCV genotype 1b patients aged 70 years or older with compensated cirrhosis treated with PrOD+ribavirin for 12 weeks, the SVR12 rates based on ITT or PP analyses were 97.4% and 100%, respectively, compared to 97.8% and 99.6%, respectively, in cirrhotic patients aged <70 years, the differences being statistically nonsignificant. Of the patients aged ≥70 years, 37.6% reported at least 1 AE considered as treatment-related, a proportion slightly higher but with no statistical significance compared with patients under 70 years of age (34.6%). Most AEs were mild and none was leading to treatment discontinuation. Also, the percentage of SAEs was not significantly higher in patients aged ≥70 years when compared to those less than 70 years of age (3.4% vs 2.6%; *P*=.54). This safety profile is even better than one might expect, considering that all subjects included in the study were older patients with cirrhosis; the safety profile in our study was undoubtedly better than in other studies (Chamorro et al, 2016). Such high SVR 12 rates and good safety profiles obtained in our study may be partially explained by the requirements imposed by our national

regulations according to which treatment was conducted only in tertiary centers and under the close monitoring of experienced gastroenterologists and infectious diseases specialists.

Our study was carried out in a real-life setting on a homogeneous elderly population (≥70 years of age) with HCV-genotype 1b compensated cirrhosis only, which is what makes it uniquely interesting among many others of its kind. There are but few published studies regarding efficacy of PrOD±ribavirin in patients with HCV genotype 1 compensated cirrhosis in real life setting (Walker et al, 2015; Chan et al, 2017). Thus, Chamorro-de-Vega et al (2016) from Spain evaluated in a prospective study the effectiveness and safety in real clinical practice of PrOD±ribavirin for 12 weeks in patients with chronic HCV genotype 1 (82% genotype 1b) infection and reported a SVR12 rate of 93.8% in cirrhotic patients and 100% in noncirrhotic patients, while AEs occurred in 91.7% of patients (in mild forms, mostly), although none led to premature discontinuation. Of note, patients' average age was 60 years. The study of Walker et al (2015) assessed real-world effectiveness of 2 therapeutic regimens (PrOD and sofosbuvir/ledipasvir) in patients with HCV genotype 1 infection and reported similar high SVR12 rates in both regimens, consistent with results from registrations trials; however, for PrOD regimens with 100% SVR12 rates, the sample size was very low (n=15) and included only 1 cirrhotic patient and, therefore, no direct comparison with our study is possible. Another published study assessing real-world effectiveness and safety of PrOD±ribavirin comes from Poland and reported an SVR12 rate of 98.3% in patients with liver cirrhosis, and a higher rate of AEs (72% of cases) than in our study (Flisiak et al, 2016). From Asia (Hong Kong), Chan et al (2017) in a retrospective, real-life study including 41 patients with chronic HCV genotype 1 infection (85% had genotype 1b and 61% had compensated liver cirrhosis), PrOD+ribavirin regimen for 12 weeks achieved 95% SVR12 rate, results comparable to the pivotal studies from the West. Similar results have been reported by other studies which included elderly patients treated with PrOD±ribavirin or other DAAs regimens (Lubel et al, 2016; Ioannou et al, 2016).Conti et al (2017) evaluated the efficacy and safety of some DAA regimens in elderly patients, defined as those over 65 years of age with HCV-related advanced fibrosis/cirrhosis, in a real-life clinical setting, and reported that all DAAs regimens used (including PrOD±ribavirin) were effective and safe in elderly patients with genotype 1b cirrhosis, with SVR12 of 95%. Ioannou et al (2016) also reported high SVR rates in the Veteran Affair National Health System patients with HCV genotype 1 and cirrhosis, either treatment-naïve or experienced, treated with PrOD and ribavirin, similar to that obtained under sofosbuvir-based regimens. Saab et al (2016) evaluated four open-label phase 3 clinical trials and reported SVR12 of 94% in patients >65 years with HCV genotype 1 cirrhosis who had received ledipasvir/sofosbuvir for 12 weeks, and this regimen proved safe and tolerable for elderly patients.

To our knowledge, our study represents the largest one yet published on PrOD+ribavirin efficacy and safety in patients aged ≥70 years with HCV-genotype 1b compensated cirrhosis in a real-life setting. This study has some strengths such as being prospective, multicentered and including a large number of homogeneous patients ≥70 years of age with HCV genotype 1b compensated cirrhosis only, treated with PrOD+ribavirin. However, our study has also some limitations, the most important one being the absence of assessment concerning long-term impact of SVR12 on the progression of liver disease in elderly patients.

Conclusion. Our results demonstrate that a 12-week regimen of PrOD+ribavirin is highly effective, safe, and well-tolerated treatment for patients aged 70 years or older with HCV-genotype 1b compensated cirrhosis, adding new evidence that advanced age should not be a barrier anymore in treating this growing subgroup of HCV patient.

I.3.3.5. Clostridium difficile infection in hospitalized octogenarian patients

Background and aim. The most important risk factors for CDI are the use of antibiotics, hospitalizations and advanced age (Khanna et al., 2010; Huttunen and Aittoniemi, 2012). Other potential risk factors, such as immunosuppression, multiple comorbidities, chemotherapy, renal insufficiency, hypoalbuminemia, organ transplantation, use of proton pump inhibitors (PPI), and the emergence of a new hypervirulent and easily transmissible strain of the bacterium known as NAP1 (North-American Pulsed-Field Type 1) in some North American and European areas, have been identified to explain the increased incidence of CDI (Loo et al., 2011; Morfin-Otero et al., 2016). Older patients are unanimously recognized as a vulnerable, high-risk population for CDI because they often have multiple comorbidities, and are more likely to have frequent and prolonged hospitalizations and to receive broad-spectrum antibiotics, which disrupt intestinal microbiota (Calfee, 2008; Szabo et al., 2015). In addition, host defense against infections including C. difficile is altered in older patients by senescence of the immune mechanisms associated with aging (Shin et al., 2016). Older adults (aged ≥65 years) have almost all the above-mentioned risk factors for CDI. Most of the studies evaluating CDI in older adults included patients aged between 65 and 80 years, and just a few have assessed CDI in the very elderly (i.e., ≥80 years) (Lessa et al., 2015; Leibovitz et al., 2016). Life expectancy has increased over the past years in most countries, and the number of older adults with a high risk for CDI is also increasing. Several studies reported that in older adults, not only are CDI rates significantly higher than in younger age groups, but also elderly patients are at increased risk of adverse outcomes including mortality (Miller et al., 2010; Patel et al., 2016). The aim of the present study was to evaluate the risk factors, clinical course, treatment and outcome of CDI in hospitalized octogenarian patients.

Materials and methods. This was a retrospective analysis of patients aged ≥80 years diagnosed with CDI at two tertiary referral centers for northeast Romania from 1 January 2014 to 30 September 2016. The patients' medical charts were reviewed, and demographic information including age and sex, prior hospitalizations, clinical and laboratory parameters, and antibiotics and PPIs use in-hospital and 2 months before admission were carefully searched, as well as the area the patients lived in (urban, rural, care home residence). Comorbidities were recorded in all patients, and classified as cardiovascular, pulmonary, metabolic, chronic renal failure, neurological, neoplastic and digestive. The diagnosis of CDI was based on the presence of diarrhea (≥3 watery stools within 24 h) plus the presence of any or both C. difficile toxins (A or B) in stool samples. Hospital-acquired CDI was defined as a stool positive for C. difficile toxins obtained at least 72 h after hospital admission. Clinical features, treatment and outcome (length of hospital stay, surgery, mortality, recurrence of CDI) were carefully recorded. Data for patients with CDI (study group) were compared with those from the older (≥80 years) patients hospitalized during the study period (control group). Follow-up evaluation was carried out 30 days after completion of CDI therapy in the study group and 30 days after admission in the control group by reviewing medical charts and by directly contacting patients or their physicians. As this was a retrospective study and completely anonymous information was collected from existing records, no institutional approval was required. ***Statistical analysis***. Continuous variables with normal distribution are expressed as mean SD, whereas categorical variables are expressed as percentages and absolute values. The $\chi 2$-test was used for categorical data. Quantitative variables with normal distribution were compared using the Student's t-test, whereas for those with non-normal distribution, the Mann–Whitney test was used. Univariate and multivariate analysis (logistic regression) were carried out on a number of recorded variables, and odds ratio (OR) with 95% confidence interval (CI) was calculated for quantitative variables included in the logistic regression. The level of statistical significance was defined as $P < 0.05$. Statistical analysis was carried out using the SPSS 20.0 software (SPSS, Chicago, IL, USA).

Results. During the study period, there were 286 patients aged ≥80 years hospitalized in our institutions, and among them 79 (27.6%) were diagnosed with CDI. The patients with CDI had a mean age of 83.9-3.3 years (range 80–94 years) and 39 (49.3%) were men. The age and sex distribution were not significantly different between patients with and those without CDI (83.9±3.3 years vs 83.7±3.1 years, P = 0.652; men 49.3% vs 42.5%; P = 0.297, respectively). Patients' characteristics (demographics, comorbidities and laboratory parameters) are shown in Table 3.XXI.

Table 3.XXI. Characteristics of patients with and without Clostridium difficile infection

Variables	Patients with CDI (*n* = 79)	Patients without CDI (*n* = 207)	Significance (*P*-value)*
Age (years) Mean ± SD	83.9 ± 3.3	83.7 ± 3.1	0.652
Sex			
Male, *n* (%)	39 (49.3)	88 (42.5)	0.297
Comorbidities, *n* (%)	76 (96.2)	183 (88.4)	0.044*
Cardiovascular disease			
Arterial hypertension, *n* (%)	48 (60.7)	89 (42.9)	0.007*
Atrial fibrillation, *n* (%)	17 (21.5)	32 (15.4)	0.224
Chronic cardiac failure, *n* (%)	32 (40.5)	41 (19.8)	<0.0001*
Pulmonary disease			
Pneumonia, *n* (%)	6 (7.5)	22 (10.6)	0.440
Metabolic disease (DM), *n* (%)	13 (16.4)	21 (10.1)	0.130
Chronic kidney disease, *n* (%)	21 (26.5)	24 (11.5)	0.001*
Neurologic disease, *n* (%)	10 (12.6)	36 (17.3)	0.330
Neoplasia, *n* (%)	13 (16.4)	36 (17.3)	0.851
Digestive			
Liver cirrhosis, *n* (%)	7 (8.8)	17 (8.21)	0.860
Laboratory			
Neutrophils, /mm^3 (mean ± SD)	12 392.2 ± 873.1	6239.8 ± 218.5	<0.0001*
Creatinine, mg/dL (mean ± SD)	1.48 ± 1.3	1.72 ± 7.7	0.786
Hemoglobin, g/dL (mean ± SD)	11.1 ± 2.1	11.8 ± 2.2	0.007*
Hospitalizations in 2 months before admission, *n* (%)	43 (54.4)	71 (34.3)	0.002*
History of surgical interventions, *n* (%)	7 (8.8)	2 (0.9)	<0.0001*
Antibiotics use in 2 months before admission, *n* (%)	31 (39.2)	44 (21.2)	0.002*
Antibiotics use during hospitalization for infections other than CDI, *n* (%)	59 (74.6)	66 (31.8)	<0.0001*
PPI use in 2 months before admission, *n* (%)	34 (43.1)	76 (36.7)	0.326
PPI use during hospitalization, *n* (%)	45 (56.9)	105 (50.7)	0.345
Treatment			
Vancomycin, *n* (%)	8 (10.1)	0	NA
Metronidazole, *n* (%)	44 (55.6)	0	NA
Metronidazole + vancomycin, *n* (%)	27 (34.1)	0	NA
CDI outcome			
Length of hospital stay, days (mean ± SD)	15.3 ± 5.1	11.1 ± 4.3	<0.0001*
Surgery, *n* (%)	0	0	NA
Recurrence, *n* (%)	9 (11.3)	0	NA
Death during hospital stay, *n* (%)	0	0	NA

Most of the patients with and without CDI (90.6%) had at least one chronic underlying disease, and over half of them (55.6%) had two or more. The most frequent comorbidities were cardiovascular disease (47.9%) and neoplasia (17.1%). Patients diagnosed with CDI had more comorbidities than those without CDI (96.2% vs 88.4%, P = 0.044). Of the comorbidities, arterial hypertension, chronic cardiac failure and chronic kidney disease were significantly associated with CDI (Table 1). Within the 2 months before admission, 114 (39.8%) of all patients had at least one hospitalization for underlying disease during which 75 (26.2%) received antibiotics and nine (3.1%) had a history of surgical interventions. Patients with CDI had more prior hospitalizations compared with those without CDI: 43 (54.4%) versus 71 (34.3%), P = 0.002. There were no significant differences between the two groups in the use of PPI before admission or during hospitalization, whereas antibiotics use was significantly higher within 2 months before admission or during hospitalization for infections other than CDI in patients with CDI (Table 3.XXI).

The most used antibiotics were cephalosporins (3rd generation), fluoroquinolones and carbapenems. Laboratory parameters measured at the time of diagnosis for the patients with CDI and on admission for those without CDI, showed significantly higher neutrophil levels in patients with CDI (12 392.2±873.1 vs 6239.8±218.5, P < 0.0001), and lower hemoglobin levels (11.1±2.1 vs 11.8±2.2, P = 0.007). Among the stool samples evaluated for C. difficile toxins, 57 (72.1%) were positive for both toxins, 18 (22.8%) had toxin B and four (5.06%) had only toxin A. The most common symptom in patients with CDI was watery diarrhea without blood, with a variable frequency between four and 14 times a day. Moderate, cramping abdominal pain was present in all patients.

On univariate analysis, prior hospitalizations, history of surgical interventions, antibiotic use 2 months before or during hospitalization and comorbidities were associated with CDI in octogenarian patients (Table 3.XXII). On multivariate logistic regression analyses, previous 2 months hospitalizations (OR 10.231, 95% CI 1.769–58.965, P = 0.009), antibiotic use 2 months before admission (OR 12.596, 95% CI 1.024–15.494, P = 0.048), antibiotic treatment during hospitalization (OR 6.302, 95% CI 3.510–11.316, P < 0.0001), arterial hypertension (OR 11.228, 95% CI 1.917–65.783, P = 0.007), chronic kidney disease (OR 4.474, 95% CI 1.037–19.299, P = 0.045) and chronic cardiac failure (OR 7.328, 95% CI 2.068–25.967, P = 0.002) were independently associated with CDI (Table 3.XXII).Within the first 24 h after diagnosis, all of the patients received antibiotic therapy for CDI: vancomycin was used in eight (10.1%) patients, metronidazole in 44 (55.7%), and in 27 patients (34.2%) with intolerance to metronidazole or unresponsiveness in the first 2–4 days, the therapy was switched to vancomycin. In six (7.6%) patients with CDI, the antibiotic treatment for other infections (two respiratory and three urinary tract infection, one spontaneous bacterial peritonitis) was continued. The mean CDI antibiotic treatment was 8.5 _ 3.3 days. Patients with CDI had an increased length of hospital stay than those without CDI (15.3±5.1 days vs 11.1±4.3 days, P < 0.0001). None of the infected patients had surgery, and nine (11.3%) had recurrence of infection during the follow-up period. There was no mortality in patients with CDI during hospitalization. Two patients died within 30 days after the CDI diagnosis, none related to CDI (one myocardial infarction, one stroke).

Discussion. Older age is a well-recognized risk factor for CDI. It has been shown that the average age of patients with CDI hospitalizations was 20 years older than the age of those with hospitalizations for other reasons (Castrillon et al., 2013). The older population is particularly vulnerable, and studies reported CDI rates twofold higher in those aged ≥65 years, and sixfold higher in octogenarians compared with the younger age group (Keller and Surawicz, 2014). The pathophysiology of the entire gastrointestinal tract is altered in these individuals as a result of atrophic gastritis, reduced gastric acid secretion, diminished bicarbonate and mucus secretion, and decreased colonic motility and prostaglandin production

– all of which contribute to the development of CDI (Bhutto and Morley, 2008). Because of the steady rise in life expectancy, the number of older patients at risk for CDI is expected to increase.

Table 3.XXII. Univariate and multivariate regression analyses of risk factors associated with Clostridium difficile infection in hospitalized octogenarian patients

	Univariate analysis			Multivariate analysis		
	OR	CI	*P*-value	OR	CI	*P*-value*
Previous 2 months hospitalization	1.80	1.240–2.620	0.003*	10.231	1.769–58.965	0.009*
History of surgical interventions	6.18	3.637–10.518	<0.0001*	4.995	0.412–6.589	0.207
Previous 2 months antibiotic treatment	1.81	1.259–2.622	0.002*	12.596	1.024–15.494	0.048*
Antibiotic treatment during hospitalization	3.80	2.422–5.960	<0.0001*	6.302	3.510–11.316	<0.0001*
Comorbidities	1.08	1.019–1.162	0.029*	3.341	0.977–11.425	0.055
Arterial hypertension	1.41	1.115–1.791	0.011*	11.228	1.917–65.783	0.007*
Chronic kidney disease	2.35	1.393–3.972	0.002*	4.474	1.037–19.299	0.045*
Chronic cardiac failure	2.04	1.395–2.999	0.001*	7.328	2.068–25.967	0.002*
Diabetes mellitus	1.64	0.866–3.118	0.190	NA	NA	NA
Proton pump inhibitors during hospitalization	1.12	0.889–1.419	0.417	NA	NA	NA
Urinary tract infection	0.74	0.331–1.628	0.574	NA	NA	NA
Liver cirrhosis	1.07	0.465–2.502	0.860	NA	NA	NA
Chronic leukemia	2.62	0.540–12.711	0.241	NA	NA	NA
Pneumonia	0.71	0.301–1.697	0.583	NA	NA	NA

The present study evaluated the risk factors, clinical features, treatment and outcome of CDI in hospitalized octogenarian patients. Using a stepwise logistic regression model for multivariate analysis, we found that antibiotic use within 2 months before admission and during hospitalization for treatment of other infections than CDI, and comorbidities (arterial hypertension, chronic cardiac failure and chronic kidney disease) were independent risk factors for CDI. There are several factors to which patients with heart failure are predisposed to CDI: advanced age, multiple comorbidities and frequent hospitalizations, all leading to higher rates of bacterial infections, and a larger use of broad-spectrum antibiotics that alter gut microbiota determining an overgrowth of pathogenic bacteria (Mamic et al., 2016). This group of patients is at high risk for CDI because they often have multiple comorbidities, frequent and prolonged hospitalizations, and antibiotic treatments leading to altered intestinal microbiota. To our surprise, we found no increased risk of CDI in octogenarians on PPI treatment; many of these patients might have atrophic gastritis with low gastric acid output, and PPI cannot further lower gastric acid secretion, without any additional risk for CDI. All patients with CDI had a moderate disease form, none requiring surgery, and none died during hospitalization, findings in contrast to that reported in other studies, which found high mortality rates and high rates of severe forms of CDI in octogenarians (Hall et al., 2012). One explanation for our favorable outcomes might be the moderate disease, prompt diagnosis and treatment of infection. It is rather difficult to establish a real causal link between death and CDI in very old patients with multiple comorbidities. None of our octogenarians with CDI died during hospitalization, but two of them died within 30 days after the CDI diagnosis, none related to CDI (one myocardial infarction, one stroke). There are no specific therapeutic recommendations for older adults with CDI; however, some authors recommend vancomycin as first-line therapy in octogenarians with severe infection (Mizusawa wt al., 2015). As a general rule, treatment should be based on the severity of the CDI disease. In our study group, the patients had mild or moderate disease and received mostly metronidazole treatment for CDI. Recurrence of CDI occurred in nine (11.3%) of the present patients, all being relapses (manifested in <8 weeks after initial

infection; Table 3.XXI). In the present study, the length of hospital stay was significantly longer for patients with CDI than for those without, a finding that supports those reported by other studies in older patients (Castrillon et al., 2013; Keller and Surawicz, 2014).

Conclusion. Hospitalized octogenarians with previous hospitalizations or recent antibiotic treatment, arterial hypertension, chronic cardiac failure or chronic kidney disease are at risk for CDI. Clinicians should have a high index of suspicion for CDI when evaluating hospitalized octogenarians who develop diarrhea in order to rapidly diagnose and treat.

I.3.3.6. Predictors of in-hospital mortality in a cohort of elderly cirrhotic patients with variceal bleeding

Background and aim. Variceal bleeding (VB) is a frequent complication of liver cirrhosis with high rates of morbidity and mortality, especially in elderly population. Early identification and management of the factors predicting in-hospital mortality might decrease mortality. The aim of this study is to evaluate the predictive factors for in-hospital mortality in elderly cirrhotic patients with variceal bleeding.

Materials and methods. We performed a retrospective study of cirrhotic patients admitted in our tertiary department with VB between January 2017 and December 2017. Cirrhotic patients aged ≥65 years presented with VB in our hospital, were included in the study. Their clinical data were investigated retrospectively. We excluded patients with a past history of endoscopic treatment of variceal hemorrhage, extra-hepatic metastasis of hepatocellular carcinoma (HCC) and/or portal vein tumoral thrombosis of HCC. The data was collected from patients personal files, where they have signed an informed consent. Liver function was quantified by Child-Pugh (Cholongitas et al., 2005) and Model of End-Stage Liver Disease (MELD) scores (Durand and Vall, 2005). Variceal bleeding was confirmed by upper endoscopy examination performed at the admission or in the first 12 hours after admission. Patients were followed up to 30 days, discharge from hospital or death (whichever came first). Statistical analysis was performed using SPSS Software Version 19.0 for Windows (SPSS Inc.,Chicago, IL, USA). Students t test was used to compare normally distributed continuous variables and the Mann‑Whitney U test for variables without normal distribution. The Chi-square test and Fisher approximation method were used to compare categorical variables. Logistic regression models were used to identify clinical variables associated with mortality. Univariate analysis was performed for each recorded variable. Variables with P-value < 0.1 in univariate analysis were included in multivariate analysis. For adjusted effect we used the logistic regression. The level of statistical significance was defined as $P<0.05$.

Results. In this study we included 75 elderly cirrhotic patients, mean age 70.29±4.8 years (range 65-83 years), most of them females (60.0%). The first three etiologies of LC were alcoholic (62.7%), viral C infection (22.7%) and viral B infection (14.6%). The majority of the patients had mean Child-Pugh score 9.93±3.17, with a mean MELD score of 15.56±7.7. Of the 75 patients evaluated, 48 (64.0%) had other comorbidities as: diabetes mellitus, arterial hypertension, atrial fibrillation, chronic kidney disease or neurological disease. Eleven (14.6%) patients had an anticoagulant treatment. Fifty-eight patients (77.3%) were previously diagnosed with esophageal varices, and only 22.1% received beta-blockers. In the majority of cases the upper digestive endoscopy was performed at the admission (74.7%), the main source of bleeding were the esophageal varices- 67 patients (89.3%), and only 8 patients (10.7%) bled from gastric varices. Variceal active bleeding was described in 27 patients (36.0%). Variceal banding and terlipressin was the treatment in 22 patients (29.3%), the rest of the patients receiving only the vasoactive treatment. Blackmore probe was inserted in 21 patients (28.0%) with rebleeding after banding or they had a hemorrhagic shock. Twenty patients (26.7%) died during hospitalization, the causes of death being: hemorrhagic shock, hepatic coma or multiple organ failure.

The multivariate analysis demonstrated that increasing age (OR 1.59, CI 1.029-1.866, P=0.011), hemodynamic instability at presentation (OR 11.51, CI 1.078-133.41, P=0.043), comorbidities (especially diabetes mellitus and chronic kidney disease) (OR 4.918, CI 1.954-12.637, P=0.001), and failure to control bleeding (OR 2.031, CI 1.388-16.227, P=0.029) were independent risk factors, significantly associated with in-hospital mortality among cirrhotic elderly patients presented with VB (Table 3.XXIII).

Table 3.XXIII. Univariate and multivariate regression analysis of risk factors associated with death in cirrhotic patients with variceal bleeding

Parameter	Univariate analysis			Multivariate analysis		
	OR	95%CI	p-value	OR	95%CI	p-value
Age>75 years	1.937	1.32-3.23	**0.009**	1.591	1.03-1.86	**0.011**
Hemodynamic instability at presentation	8.351	2.26-16.55	**0.005**	11.512	1.07-13.33	**0.043**
Comorbidities	2.888	1.93-5.96	**0.037**	4.918	1.95-12.67	**0.001**
Failure to control bleeding	2.494	1.93-7.13	**0.003**	2.031	1.39-16.22	**0.029**
Anticoagulant treatment	1.294	0.12-4.66	0.554	1.348	0.13-15.11	0.109
Creatinine >1.2 mg/dL	1.373	0.69-2.72	0.411	-	-	-
Ascites	1.147	0.95-1.38	0.347	-	-	-

Discussion. VB has a higher rate of mortality in elderly cirrhotic patients despite the maximal treatment, and the rate of beta-blockers are less indicated in this special category of cirrhotisc. For general population the in-hospital mortality rate range between 8%-14.2% (Ismail et al., 2006; Bambha et al., 2008). The comorbidities, especially diabetes mellitus and chronic kidney failure, failure to control bleeding, hemodynamic instability at admission, and age over 75 years old were recognized as mortality risk factors. Mortality was related to the severity of bleeding. Our results are in line with previously published data (Han et al., 2014). The incidence of gastrointestinal bleeding increases with aging and accounts for 1% of hospital admissions in people aged 80 years and over (Kaplan et al., 2001). Aging per se is a risk factor, as it has been recently demonstrated in a large prospective study on people who were not taking antiplatelet agents (Tariq and Mekhjian, 2007). The in-hospital mortality rate in our study was higher than previously reported (Wang et al., 2012) maybe in relation with the number of the comorbidities and the association of failure to control bleeding. The mortality rate in elderly with non-variceal upper hemorrhages was reported up to 10% lower that the mortality rate in cirrhotic patient with VB (Selak et al., 2018). Considering LC as a systemic disease, especially in the advanced stages, it could represent a risk factor for mortality in elderly. Acute variceal bleeding is associated with a poor prognosis in old cirrhotic patients. Mortality was related to the failure to control bleeding and in-hospital complications. Our results are in line with previously published data (Wang et al., 2012) In hospital rebleeding and complications played a highly significant role for stratification of patients with high mortality. Both occurred after admission which indicates that prognosis may change from day to day. This necessitates intensive monitoring of elderly cirrhotic patients with acute VB during hospitalization and further improvement to control bleeding might still improve the prognosis of these patients. The rate of primary prophylaxis with beta-blockers was lower in our study group, many patients having contraindication for non-selective betablockers as: atrio-ventricular block, peripheral arterial disease or obstructive pulmonary disease. The different beta-blockers metabolism in elderly and often also the polymedication (Motivala et al., 2016) could be associated with increased side effects in older cirrhotic patients.

Conclusion. In-hospital mortality rate was 26.7%, and the predictive factors were: increasing age, hemodynamic instability at presentation, comorbidities and failure to control bleeding. These patients require care in more specialized units during the bleeding episode, intensive monitoring, energetic resuscitation, early endoscopy, and aggressive follow-up in the intensive care units.

SECTION II
FUTURE DIRECTIONS IN THE ACADEMIC, PROFESSIONAL, AND RESEARCH FIELD

II.1. FUTURE DEVELOPMENTS IN THE ACADEMIC ACTIVITY

I will continue to prioritize the quality of the didactic act, which depends by both the content of the didactic material, and the teaching way. The main goal will remain the success in the knowledge transfer, along with an efficient way of evaluating the students regarding the acquisition of knowledge / skills. For a good result of assimilating knowledge in the practical internships of medical semiology and gastroenterology, I propose to favor the student-centered teaching method, based on active-participatory learning. I propose to put into practice the notions learned in the course "Train-the-Trainers", and to organize in groups, work teams, with the aim of: encouraging individual work, the purpose of developing the spirit of collaboration and team, but also capacity of others' analysis and self-assessment.

I intend to get involved in writing a practice guide in medical semiology and gastroenterology for the French section students.

Concerning the residents training, I would like to implement scientific analysis sessions, to discuss studies/ articles published in the national/ international scientific flow.

My plans in the didactic activity include also:

• Coordination of at least three undergraduate theses per year, for students in the final years of the University of Medicine and Pharmacy „Gr. T. Popa" Iași, both the Romanian and French section

• Encourage and guide student participation with oral presentations in student communication sessions, symposiums and congresses

• Organize student workshops, dedicated to deepening knowledge or practical skills

• Introduction of a new optional course for the 5th year French section students

• Thematic seminars and clinical case sessions with residents

II.2. PERSPECTIVES FOR THE PROFESSIONAL AREA

I will continue exercising the profession of gastroenterologist, with as much as possible seriousness, devotion and human warmth. In order to be constantly up to date with the norms and recommendations of medical practice, I will continuously instruct myself and I will assimilate the novelties in terms of guidelines and protocols.

A desideratum of maximum global importance, assumed by the World Gastroenterology Organization, is the elimination, by 2030, of viral hepatitis C. Our country adheres to this goal, through the efforts and activities of the Romanian Society of Gastroenterology and Hepatology. I am planning to contribute through my own efforts to achieve this aim, by continuing my activity as member involved in the LIVE-RO program - population screening for chronic hepatitis B/C/D virus infections" (P.O.C.U. Project - Beneficiary Fundeni Clinical Institute, Partner: "Grigore T. Popa" U.M.F. Iasi).

I also intend to continue my involvement in the liver transplant program, through clinical activity - pre-transplant patient evaluation, post-transplant follow-up, and management of the national program "Prophylaxis of chronic hepatitis recurrence in transplant patients", as local coordinator.

At the appropriate time, given the completion of the "Training Program for Screening in Cervical, Breast and Colorectal Cancers", within the Project co-financed by the European Social Fund through the Sectoral Operational Program Human Resources Development 2007-2013, I would like to participate in the screening program for colorectal cancer.

As a particular field in which I had the chance to initiate and prepare myself through special courses, I will continue the investigations with the small bowel and colon capsule endoscopy, carried out in the Institute of Gastroenterology and Hepatology in Iasi in national premiere. Iasi remains the only center in Moldova/ the North-Eastern region of the country where CE is performed.

I am planning to strengthen the skills of therapeutic endoscopy, as well as expanding the areas of experience with: enteroscopy, echoendoscopy and ultrasound of the gastrointestinal tract.

Towards the patients, I will assume a behavior based on fairness and compassion, and I will also maintain a good professional relationship within the collective.

II.3. PROJECTS IN THE SCIENTIFIC RESEARCH

As general principle and guiding direction, I hope that all my future scientific activity will continue shining a favorable light on the "Grigore T. Popa" University, under whose auspices I obtained important results so far.

II.3.1. CAPSULE ENDOSCOPY – EXPANDING THE BOUNDARIES

II.3.1.1. NEW VALENCES OF CE IN IBD

Comprehensive activity score for Crohn's disease, predictive for sustained deep remission

The most ambitious goal of IBD treatment has shifted lately towards deep remission, defined by both clinical remission and mucosal healing. Moreover, the notion of "clearance disease" entered into the debates of gastroenterologists, better explained for ulcerative colitis, but less clearly defined for Crohn's disease (Colombel et al., 2021). There is no gold standard or widely accepted best CE scoring system in practice yet for assessing IBD severity. There are several proposed score, specifically developed for Crohn's disease (Lewis score, CECDAI), but none has high correlation with others parameters assessing disease activity, such as fecal calprotectin (Simon et al., 2019). In the same time, the Crohn's disease activity index which is currently used does not take into account endoscopic remission.

Therefore, my purpose is to create a ***comprehensive activity score*** using clinical data, biological markers and CE findings that could help fully assessing the patient's disease activity and also evaluating the response to therapy, and then to validate it for the prediction of ***long-term sustained deep remission.***

The role of "Crohn's" capsule system in IBD patients

"Crohn's" capsule is a recent version of the CE dedicated to patients with IBD. Via two camera heads, it has a 336-degree view and, in the same time, allows examination of the small bowel and colon during the same exploration. Crohn's disease may affect any portion of the GI tract, and thus this "pan-enteric" capsule may bring more valuable data. I intend to study its role in the diagnostic, the ***extent evaluation*** and activity assessment of Crohn's disease.

The impact of CE on the outcome of IBD unclassified

The utility of CE in IBD unclassified is recognized, due to its capability of exploring the entire small bowel. A patient with diagnostic uncertainty between ulcerative colitis or colonic Crohn's will be reclassified as Crohn's if specific small bowel lesions are identified by the CE. Conversely, the absence of small bowel lesions will plea for ulcerative colitis.

However, according to severity, the treatment may be similar or by contrary, might be changed. Reclassification rates Reclassification rates vary in different studies, between 16-44% (Monteiro et al., 2018, Lopes et al., 2010), according to diagnostic method and to the study population. I have already analyzed the role of CE in the evaluation of IBD unclassified, and I intend to study further the ***impact of CE reclassification*** on the outcome of IBD patients due to consequent changes in the management.

Predictive factors for obstruction in Crohn's disease assessed by Patency capsule

Patency capsule (PC) is a degradable, non-diagnostic blind capsule, with the same dimensions as the SBCE. It is recommended in patients with established or suspected Crohn's disease, before performing SBCE, in order to avoid CE retention due to stenosis. Excretion of an intact or its radiologic visualization in the large bowel is considered gastrointestinal patency. Stenosing phenotypes are not always clinically manifest, but there may be an evolutive potential. I intend to prospectively study predictive factors (clinical, biological and imagistic) associated with non-patency**,** in order to outline a ***risk profile*** for obstruction in patients with Crohn's disease.

II.3.1.2. SCREENING FOR COLORECTAL CANCER BY COLON CAPSULE ENDOSCOPY (CCE)

CCE is an attractive alternative for exploring the large bowel, due to its non-invasiveness and the lack of need for sedation, compared to the colonoscopy. There are however several drawbacks, the main one being the incapacity of taking biopsy or making therapeutic gestures, and the second being the rigorous preparation regimen. CCE is a valuable method when standard colonoscopy cannot or could not be performed, except situations when preparation cannot be completed or in the case of stenosis. Many controlled prospective studies have validated the sensitivity and specificity of 2nd generation CCE in the colorectal cancer screening (Spada et al, 2011, Eliakim et al., 2009, Van Gossum et al., 2009). CCE might be an alternative to colonoscopy and fecal immunochemical test for colorectal cancer screening. A recent meta-analysis showed that CCE is safe and effective for the detection of polyps and colorectal cancer in a screening setting, with accuracy comparable to colonoscopy and superior to computed tomographic colonography (Vuik et al, 2021). However, completion rate remains an issue to improve (57%-92%), depending on the booster used. It must be underlined that positive CCE exams require further colonoscopy, which may be reflected into the higher costs, but nonetheless with higher acceptability from the patient.

CCE as a filter test in colorectal cancer. Comparing CCE and standard colonoscopy in terms of correlation and negative predictive value could suggest if CCE may be a useful filter test for colonoscopy in screening patients for colorectal cancer. I intend to analyze the results of the CCE and of the colonoscopy performed in the same group of patients. The frequency of the polyps, significant lesions and cancer will be calculated for each procedure, as well as the results correlation between the two procedures. The effectiveness and the good performance of the CCE could endorse it as a preferable, non-invasive primary method for colorectal cancer screening.

Follow-up by CCE after colon adenoma resection. Patients with adenomas are at risk for developing new adenoma or even cancer. Metachronous lesions are detected in up to 30% during follow-up colonoscopies (Minamide et al., 2021), while interval cancer was reported during an adequate surveillance (Kim et al, 2018). Patients with colorectal adenomas have to be included in a post-polypectomy surveillance program, according to specific risk characteristics as the number, the size, and histological features of adenomas. CCE might be an alternative to colonoscopy, with better compliance, as surveillance method after polypectomy in low-risk, intermediate risk or high-risk adenomas, respectively.

II.3.1.3. ARTIFICIAL INTELLIGENCE IN CE

The development of artificial intelligence (AI) has rapidly increased in medicine in the last years, with many clinical applications. Endoscopy has become a main field of interest for applying AI. Especially CE may be expected to benefit from the AI progresses, because of the very large number of images and time-consuming interpretation process.

Blood indicators. Even from before the apparition of the AI, a "suspected blood indicator" (SBI) was imagined and incorporated into the CE reading software. The SBI helps readers identifying quickly the presence of the blood into the intestinal tract, with a sensibility of near 96%; the drawback is however the lack of specificity (20-65%). Afterwards, computer models were developed and several deep learning models were proposed for identifying the blood, with much higher sensitivity, specificity and precision (over 98%) (Xing et al., 2018, Aoki et al., 2020). However, the recognition was based on still images, with no validation on video segments. Moreover, no blood features were provided in terms of aspect (fresh, melena or clots) or quantity.

Angiectasias. They are among the most frequent lesions detected by CE. AI using a convolutional neural network (CNN) automatically detected angioectasias with high sensitivity (98.8%) and specificity, respectively (98.4) (Tsuboi et al., 2020). Prospective clinical studies are needed to validate these promising results.

Small bowel ulcers. The great variety of ulcerative lesions in terms of size, shape and cause may be a challenge for erosions and ulcer detection. Detection of SB ulcers by deep learning models based on CNN and support-vector machines (SVM) had around 88% sensitivity and 91% specificity (Aoki et al., 2020, Klang et al., 2020), with shorter reading time.

Small bowel tumors (SBT). Automatic detection of SBT was made with 91% sensitivity and 80% specificity by a deep neural network-based system, as showed by Saito et al., in 2020. The learning process was based on still images, and to the date there are no ample studies on videos. A crucial matter remains the differentiation between the real tumors and the "fake" ones or normal non-significant structures, as normal folds, bulges, phlebectasias, lymphangiectasias, lymphoid nodular hyperplasia, Brunner glands or even papilla. As a matter of fact, this is also a current and dilemma among the human CE readers, and more data and algorithms are still waited in order to elucidate it.

Assessing the quality of bowel preparation. Even if it is widely recommended, the evaluation by CE readers of the degree of cleanliness remains poorly reproducible. Recently, two deep learning models based on CNN were developed, and both achieved over 95% accuracy (Leenhardt et al., 2020, Noorda et al., 2020).

For all the above and even more, learning models may be developed and perfected. I intend to involve myself in the implementation of AI and machine learning solutions in the domain of capsule endoscopy.

Limits of the AI

Despite the progresses and the results achieved so far, many gaps and uncertainties concerning the performance of AI remain. All the studies so far are retrospective, many are based on still images and less on videos. Most current models are supported by labeled data as "gold standard", which are provided by different observers. Furthermore, external validation studies are lacking. In the same time, differences in digital resources and economic status will allow or deny countries to access the latest progresses.

Until AI will be firmly validated, and fully trustworthy, the human - meaning the physician with training and expertise in the CE reading, will remain the recognized responsible for interpreting the images.

Supplementary studies and new data are expected in the future, so that AI finds its valuable place. Reciprocally, finding the place of the investigating specialist in the field of

paraclinical explorations (currently assaulted and, perhaps, in the future, dominated by AI), is a challenge whose solution depends on us.

II.3.2. CONFOCAL LASER ENDOMICROSCOPY – NEW PIECE IN THE PUZZLE OF GASTROINTESTINAL PATHOLOGY DIAGNOSIS

Confocal microscopy is an optical imaging technique for increasing optical resolution and contrast of a photomicrograph. Confocal laser endomicroscopy (CLE) has only recently been added to the gastroenterologist's armamentarium. It allows microscopy of the gastrointestinal mucosa during ongoing endoscopy, enabling ***real time optical biopsy***. Without eliminating the need for biopsy and histo-pathology, CLE targets biopises or endoscopic interventions to regions of specific interest („smart biopsies") instead of untargeeted random biopsies. CLE has been studied in a various diseases in both the upper and the lower GI tract, and recently even in imaging of the bile duct, the pancreas and the liver (Pilonis et al., 2022; Fugazza et al., 2016). Several trials have addressed the use of CLE in neoplastic and inflammatory diseases from the upper, middle and lower gastrointestinal (GI) (Capuano et al., 2019; Kunovsky et al., 2020).

In a translational approach, CLE is a unique tool to study patho-physiology in vivo, and even enables molecular imaging, thereby widening our understanding of basic and clinical science.

CENEMED, "Multidisciplinary platform for medical research and development in the N-E region", is a unique project in Romania, co-financed by the Operational Program Competitiveness, Priority Axis: Research, Technological Development and Innovation (RDI). It has as general objective the increase the research-development and knowledge transfer capacity of the University of Medicine and Pharmacy "Grigore T. Popa" Iasi, by creating a multidisciplinary research center in various medical fields, including gastroenterohepatology, in order to research diagnostic methods and personalized therapeutic means for pathology of interest capable of increasing survival and improving the quality of life of patients in the Northeast region.

Cellvizio® (Mauna Kea Technologies**),** part of the CENEMED equipment, is a type of CLE system, the world's tiniest microscope, which can be attached to the endoscope. Cellvisio platform combines a laser scanning unit and a processor utilizing proprietary software, using a range of flexible confocal miniprobes. The simple contact between the tip of the miniprobe and the tissue generates real-time microscopic cellular images viewed directly on the Cellvizio screen. The images provide a deep observation of the mucosa and of what is happening in real time.

Cellvizio® is a minimally invasive procedure that can lead to making better decisions in patient management in a diversity of functional and organic pathologies.

Departing from the first data gathered form the implementation of the CLE system into the practice, we will study the following:

- The progression from healthy tissue to Barrett's esophagus and esophageal cancer, in patients with long-standing gastro-oesophageal reflux disease
- In situ diagnosis of H. pylori, premalignant and malignant gastric lesions
- Diagnosis of gluten enteropathy (celiac disease)
- Endoscopic surveillance of ulcerative colitis and Crohn's disease
- Early detection of colorectal cancer
- The study of lymphatic system and blood vessels in IBD
- Quantify the intestinal permeability in patients with liver cirrhosis and correlate this to the onset of spontaneous bacterial peritonitis.

II.3.3. INTESTINAL ULTRASOUND – VALUABLE NON-INVASIVE TOOL FOR INFLAMMATORY BOWEL DISEASE MONITORING

Diagnostic, monitoring and surveillance methods of IBD patients are represented by endoscopy, computed tomography, magnetic resonance imaging, and intestinal ultrasound. The reference standard for assessment of mucosal disease activity is considered the endoscopy (Spiceland et al., 2018). Nevertheless, there are difficulties and disadvantages for performing it frequently, due to its invasive feature, burden for the patient, need for sedation and high costs. CT exams are recommended generally only in the acute setting or when emergency complication is suspected, and have the irradiation drawback, while MRI is useful for evaluation of both bowel inflammation and complications, but with the limits of low accessibility and increased cost (Pouillon et al., 2018).

A suitable tool for the bowel monitoring IBD is intestinal ultrasound, a non-invasive, non-irradiant, accessible and widely available technique. The signs of inflammation are: bowel wall thickening, loss of normal bowel stratification, hyperemia with increased vascularity, inflammation of mesenteric fat, and lymphadenopathy (Bettenworth et al., 2019). Recent studies showed that intestinal ultrasound is valid and accurate for assessing the disease activity when compared to biomarkers and endoscopy (Bots et al., 2021; Smith et al., 2020), and consequently has the potential to reduce the need for endoscopy, and to modulate non-invasively the disease management (Bots et al., 2022). Moreover, intestinal ultrasound is preferred by patients over other modalities (Miles et al., 2019).

We intend to evaluate the role of intestinal ultrasound in the management of IBD patients, and to propose an ***algorithm of point-of-care ultrasound*** in ulcerative colitis and Crohn's disease patients.

II.3.4. NEW INSIGHTS ABOUT LIVER CIRRHOSIS

II.3.4.1. Stratification of prognosis in cirrhosis

Cirrhosis is traditionally seen as an irreversible stage of chronic liver disease. However, lately, stopping the progression, or even regression, seem achievable. Stratification relies on risk factors and evaluation of disease progression/regression.

Risk factors for complications and mortality

Identifying risk factors is an essential step towards optimal timely management. I propose to initiate a prospective study to evaluate the risk factors for complications and mortality in cirrhotic hospitalized patients. We already assessed the main predictors for mortality in a large cohort of hospitalized patients with alcohol-related cirrhosis, founding that infections, hepatic encephalopathy and variceal hemorrhage are the main risk factors (Horia-Octav Minea, Ana-Maria Singeap, Irina Girleanu, Stefan Chiriac, Tudor Cuciureanu, Laura Huiban, Cristina Muzica, Sebastian Zenovia, Robert Nastasa, Remus Stafie, Ermina Stratina, Adrian Rotaru, Carol Stanciu, Anca Trifan, *"Predictive evolutionary factors for the prognosis of alcoholic cirrhosis",* ***awarded oral presentation*** at the National Congress of Gastroenterology, Hepatology and Digestive Endoscopy, Bucharest, July 2022).

Non-invasive evaluation of disease progression/regression

The non-invasive tools for the assessment of fibrosis, ranging from biomarkers to elastography techniques that allows the liver stiffness measurement, were initially introduced to overcome the drawbacks of liver biopsy and invasive hepatic venous pressure gradient (HVPG) measurement. Their diagnostic accuracy and efficacy became made them useful much beyond their initial purpose, especially in the prediction of consequences of chronic liver

disease including portal hypertensive complications and the development of hepatocellular carcinoma (de Franchis et al., 2022).

Liver elastography performed either with FibroScan (transient elastography) or ultrasound shear-wave elastography (SWE) has greatly enhanced the likelihood of early diagnosis and the stratification of cirrhosis, facilitating the identification of patients with compensated disease who are at high risk of complications, prior to the occurrence of decompensation. Liver stiffness and more recently spleen stiffness, measured by various ultrasound elastography methods reflects the severity of liver disease and portal hypertension in patients with compensated cirrhosis (Maurice and Pinzani, 2020).

Apart ***liver stiffness*** measurement, an area of interest is represented by ***changes in spleen stiffness,*** who might parallel changes in HVPG and portal pressure gradient after non selective beta blockers (NSBB) or TIPS, and thus we might contribute to answering the eternal issue of ***reversibility/non/reversibility*** of cirrhosis.

II.3.4.2. Follow-up of liver transplanted patients

The Institute of Gastroenterology and Hepatology is part of the Regional Centre of Liver Transplantation. We have the chance to be able to provide to the patients the best definitive solution for liver cirrhosis, when liver transplantation is indicated and feasible.

As ***regional coordinator of the program for prophylaxis of the hepatitis B recurrence in liver transplanted patients***, I have the opportunity to implicate myself directly in the long-term of post- transplantation care of patients with hepatitis B - related cirrhosis.

Steatosis after liver transplantation

NAFLD is not only an increasing cause of liver transplantation, but it also might occur afer transplantation, as recurrence of the disease or de novo hepatic steatosis. The prevalence of hepatic steatosis afer liver transplantation ranging from 30–60% in different studies (Kappus and Abdelmalek, 2017; Eshraghian et al., 2020). Risk factors for hepatic steatosis after liver transplantation have been hypothesized, including especially traditional risk factors of NAFLD: obesity, hyperlipidemia, post-transplant diabetes and hypertension (Eshraghian et al., 2020). We are planning a prospective analysis on patients awaiting liver transplantation, to identify liver graft steatosis (measured by CAP) and correlate its occurrence to preexistent or after transplantation clinical or metabolic risk factors.

Dynamics of anti-hepatitis B antibodies following liver transplantation in patients with hepatitis B – related cirrhosis

Prophylactic administration of hepatitis B immunoglobulin (HBIG) and nucleos(t)ide analogues (NAs) is the standard treatment for controlling hepatitis B virus (HBV) recurrence after liver transplantation (LT). However, a controversy still remains regarding the optimal prophylactic protocol, particularly regarding the duration, dosage and route of HBIG administration (Volpes et al., 2020). The high cost of intravenous infusions of HBIG in large doses, along with required hospitalization and the association with frequent adverse events, the HBIG-free monoprophylaxis with a NA and complete withdrawal of antiviral prophylaxis are current interest topics.

For instance, during the last years, several single-center studies have shown the safety and efficacy of combination of low-dose HBIG and entecavir or tenofovir followed by a monoprophylaxis with ETV or TDF, which seems to be a reasonable and cost-effective approach to ensure HBsAg and HBV viral load negativity after LT (Orfanidou et al., 2021). However, more studies are needed on the long-term efficacy and safety of complete withdrawal from HBIG and even NA. As a part of this issue, I intend to study the dynamics of anti-hepatitis B antibodies following liver transplantation in patients transplanted in our center, in terms of levels, quantitative changes and their influencing factors.

SECTION III
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